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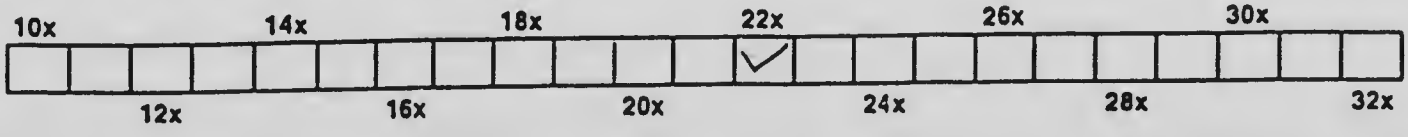

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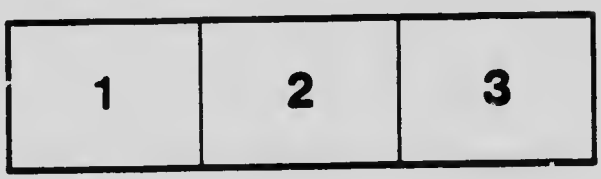

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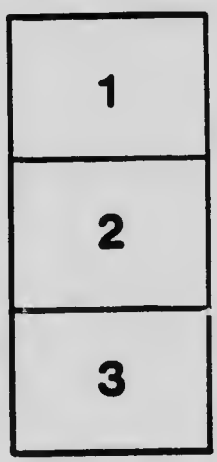

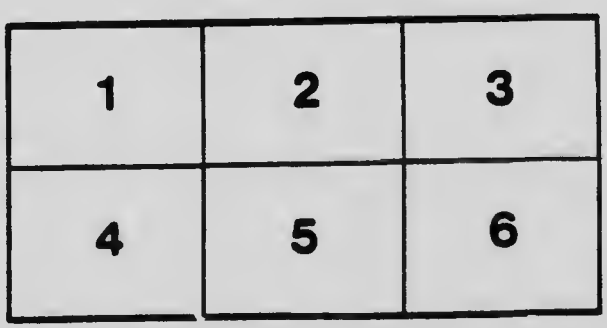

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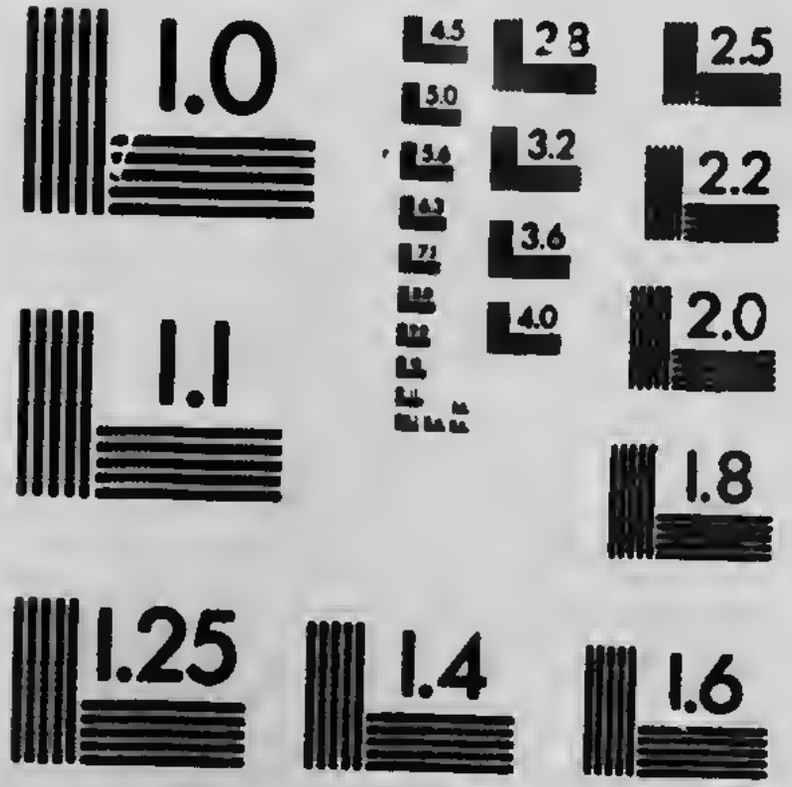

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RECENT ADVANCES IN PHYSIOLOGY AND BIO-CHEMISTRY

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EDITED BY LEONARD HILL, M.B., F.R.S.

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WITH DIAGRAMS

LONDON
EDWARD ARNOLD
41 & 43 MADDOX STREET, BOND STREET, W.
1906

PREFACE

This book was designed to set before the student of medicine the progress made in those branches of physiological study which have an immediate bearing on pathology and therapeutics, and to thereby give him an insight into the methods of research, and a training in the processes of deduction, which cannot be gained from the bare and unstimulating outlines of the text-book. In the text-book all branches of physiology are treated as of equal value; in reality many parts of the science have little bearing on practical medicine, while others are of fundamental importance and cannot be studied too deeply. Some of these latter the Editor and his co-workers have endeavoured to treat in such a manner as to make the student think out problems, rather than to learn facts in a parrot-like way.

In carrying out this idea each writer has been held responsible for his own views and interpretations of the subject which he has undertaken, and if, as occasionally happens, the writers on cognate subjects have overlapped and differed in their conceptions of the mechanisms involved, the Editor has not sought to remove such differences. It is to the advantage of the student to study such opposed views, and thereby have his powers of criticism and judgment sharpened.

The Editor hopes that the book will also be of value to the clinician, who wishes to realise the views of the chief European and American authorities on such subjects as—diabetes; uric acid metabolism; hæmolysins and immunity; mountain sickness, caisson sickness, and oxygen as a therapeutic agent; the metabolism of fat and treatment of obesity; the influence of temperature and relative dryness of the atmosphere, of work, diet,

baths, clothing, &c., on metabolism; the causation of dyspnœa, and of Cheyne-Stokes respiration; the influence of the thyroid and suprarenal glands on metabolism; the action of the digestive ferments; Catalysts, and chemical excitants; the colloidal structure of living matter and the influence of electrolytes in solution—a subject of immense importance to therapeutics; the formation and absorption of lymph; the urinary excretion, and so forth.

In the bibliography at the end of each article only such references are given as will enable the reader to find his way into the full literature of the subject dealt with.

The Editor hopes, if this volume fulfils a want which he believes exists, to edit a subsequent volume in which other subjects of equal interest and importance may be treated in a like manner.

He offers his best thanks to Drs. J. S. Haldane and J. B. Leathes and Mr. J. Craw for reading certain parts of the proofs.

OSBORNE HOUSE, LOUGHTON.
November 26, 1905.

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By BENJAMIN MOORE

By LEONARD HILL

RECENT ADVANCES IN PHYSIOLOGY

CHAPTER I

ENERGY TRANSFORMATIONS IN LIVING MATTER

Regarded from the physical standpoint, all the acts which constitute our existence are due to transformations of energy in the world outside us and in our own bodies. It is by the medium of such energy transformations occurring in our sense organs that we are made aware of the existence of the external world, and kept informed of the changes going on in it, and it is by such transformations going on in the various living cells which constitute our bodies that energy is obtained for all those operations by which the body is nourished and enabled to adapt itself to the rest of the world around it.

The same statement is true of all organised and living matte however high or low its degree of organisation may be. Whether the living matter belong to the vegetable or animal division, to micro-organism or man, it is alike a field for the display and reception of energy transformations, and its life manifests itself by the taking in of energy in one or more forms and the giving of it up in others.

In dealing with the physical aspect of the phenomena of living organisms, it may be well at the outset to discuss how closely such phenomena are associated with those observable in the inorganic world.

In the first place, the bodies of living organisms are built up from the same chemical elements as are found in inorganic structures. In the process of organisation the chemical constitution becomes so complex, and the physical organisation of the complex chemical molecules, whereby living matter is eventually formed, so intricate, that the organic chemist and physical chemist have hitherto not been able to follow the process, and hence we know

but little regarding the constitution and structure of living matter.

Although the intermediate stages in the process cannot be followed by any means at present known to us, we can learn something by watching the ends of the process, by noting the ingesta and egesta of the living matter, and studying qualitatively, and quantitatively, the energy changes displayed by living matter. As a result of such observations, it is found that the two fundamental laws of the inorganic world, namely, the conservation of matter and the conservation of energy, are obeyed throughout the whole range of organised nature. Both these laws can be as well demonstrated by using a living animal, and making a debit and credit account for the matter and energy taken in and given out, as by performing a combustion experiment or causing any other transformation of energy and matter by means of non-vital matter and non-vital energy-transformers. We see that this must be so when we consider that living matter is formed from the same material sources as non-living matter, and, further, that both its building up and its sources of energy for all its changes, when built up, arise from non-vital forms of energy; thus the same fundamental laws must apply to it as to the inorganic world, for otherwise no balance could exist between the two domains.

It by no means follows from this, however, that there is no difference except complexity of structure between living and non living matter, that there is no form of energy peculiar to living matter, and that if we only knew how to apply to living plants and animals the laws pertaining to the forms of energy found in inorganic nature, we should find nothing superadded, nothing to justify such terms as *living* or *vital*. The very existence of such words as "living" and "vital" indicate the primary conception of something essentially different in nature, and it ought to be noted that it is the presence of certain peculiar energy phenomena which gives rise to the necessity for introducing such words, and not complexity of structure or development. We call things *living* because of the energy changes they exhibit, and not because they are complex chemically or physically. Further, when these peculiar energy phenomena are gone, the objects are dead, and even during life they are more typically living the more markedly they chance to show the distinctive energy phenomena of life.

The rapid advances of physical chemistry within the past generation have fostered and encouraged in many minds the belief, which every day appears to grow stronger and more popular, that all the phenomena exhibited by living structures are capable of explanation by application of the laws governing non-living matter, that by processes of diffusion and osmosis through peculiarly constructed membranes, behaving somewhat differently indeed to those as yet fabricated by the art of man, but nevertheless membranes, all the complex changes and transformations affected by the cell are capable of explanation.

The evidence of experiment clearly shows, however, that the living cell is not a peculiarly constructed membrane obeying, even where it most directly seems to disobey, the physical laws of diffusion and osmosis; but is an *energy machine or transformer* by virtue of the operation of which a form of energy appears peculiar in its manifestations and phenomena to living matter, and producing adaptations and combinations in non-vital matter, which in many instances have not been imitated, and in others have only been imitated by obviously different processes, by the application of other forms of energy acting through non-vital transformers.

At the same time it may be pointed out that this in no way stultifies the application of physical chemistry to biological problems, or minimises the great service which an increased knowledge of physical chemistry has done and will do for biology.

In recent times, advances in physical chemistry, and in the knowledge of the properties of solutions, and of reactions occurring in solution, have pointed the path to advances in biological science, and it is in this direction that in the future most onward movement is to be expected.

A knowledge of physical chemistry, and more especially of the laws and phenomena of solutions, both of colloids and crystalloids, is indispensable to the modern biologist, taking that word in its widest sense, for it is here that we shall gain our closest approach, as far as can be at present seen, to the phenomena taking place in the living cell.

This follows because the cell in structure consists of colloids and crystalloids in common solution in water, and hence much may be gained by a knowledge of the laws of such a solution.

It is imperative to add, however, that this peculiar solution possesses something superadded, that this colloidal solution has a structure and organisation which differentiate it from all non-living colloids, such as starch, gelatine, or proteid in solution; impress upon it peculiar properties, and make it the scene of those typical energy transformations which we aggregate together under the term *life*.

It is unfortunate that the rebound from the bondage of the old view of a mysterious *vital force* or *vital energy*, possessing no connection or correlation with the forms of energy exhibited by non-living transformers of energy, should have led to the equally mischievous view of the present day, that no form of energy whatever is present in living cells save such as are seen in the case of non-living matter.

In order to avoid confusion with ancient fallacies, the writer has elsewhere suggested the use of the term "biotic energy" to represent that form of energy peculiar to living matter, and exhibited in those energy phenomena which are confined to living matter and are indeed its intrinsic property, by which it is differentiated and known to be alive.

It must be pointed out that this point of view is equally distinct on the one hand from the ancient one of vital force, which postulated something entirely distinct from the forms of energy of the non-living world, and on the other from the modern view that there exists in living matter no form of energy which is not *identical* with the forms of energy exhibited in non-living structures.

The conception, in brief, is that biotic energy is just as closely, and no more, related to the various forms of energy existing apart from life, as these are to one another, and that in presence of the proper and adapted energy-transformer, viz. the living cell, it is capable of being formed from or converted into various of these other forms of energy, the law of conservation of energy being obeyed in the process just as it would be if an exchange were taking place between any two or more of the latter forms.

We know no more or no less of the intrinsic nature of this biotic energy than we do of any of the non-vital forms; but we do know that it is confined to living matter, which acts as a transformer between it and other forms, and that the loss of this property means the death of the living matter, that the pheno-

mena are as distinctive as those of other forms of energy, and that these phenomena are studied by the same types of process as are applied in the study of other forms of energy.

It is perhaps not as commonly recognised as it ought to be that for all forms of energy the object of study of the chemist or physicist is the transformation of one form of energy into another, and the phenomena observable during such transformation.

Our advances in natural science are made by studying experimentally new transformers by which hitherto unobserved forms of energy are developed, by noting as closely as possible the nature of the instrument or transformer and adapting this to its work, and by studying the effect of each form of energy so developed upon various forms of matter, and the routes or transformers by which it is dissipated into other forms of energy.

In all cases we observe that some material agency or arrangement is necessary in order that the transference from any one specific form to any other may be effected, and that this agent differs with the forms between which exchange is going on. The machine or structure through which the change is effected may be termed the *energy-transformer*.

Observation teaches that bodies or substances which by their structure or arrangement are specially adapted for promoting certain energy exchanges are quite inert with regard to other exchanges.

In the case of some energy transformations the property of acting as transformer appears to be shared by all forms of matter, although in varying degree, while in other transformations the property is most specific, and associated with some special arrangement of matter. Thus, for example, all metals possess the property in varying degree of electrical conductivity, and in inverse proportion act as transformers for the conversion of electrical energy into heat energy. The chlorophyll of the green plant, on the other hand, has the very specific power of converting light energy into chemical energy, and here acts as a peculiar energy-transformer. Similarly, all enzymes are energy-transformers, limited and specialised in range of action, for the transformation of chemical energy. Again, iron in most marked degree (and a few other metals to a less extent), is, by some special structural arrangement, specially adapted to act as a transformer in the case of magnetic energy, effecting its conversion into electrical or

mechanical energy, or *vice versâ*. Similarly, for radiant light and heat some bodies are transparent, and transmit these forms of energy unaltered, while others are opaque and transform the energy into other forms.

It is not necessary to go on multiplying examples, but it may be urged that in exactly similar fashion the protoplasm of the living cell of plant or animal is on account of its peculiar structure and constitution a transformer of energy, specially adapted for the intermediate conversion of chemical energy, presented in certain suitable forms, into biotic energy, and for its final conversion into other forms, such as mechanical energy and heat.

A consideration of the forms of energy recognised as different in the non-living world shows us that the only criterion of difference in type is the existence for each type of energy of a set of phenomena peculiar to it, and the production by the play of the particular form of energy of results typical of it, which cannot be produced, at any rate under like conditions of operation, by any of the other forms of energy.

It is no argument against the existence of a discrete form of energy that it is only produced from other forms of energy and passes back again into other forms. In fact, it must be so produced and so pass back, or the balance of which the law of conservation of energy is the expression would be upset. Hence the facts that vital phenomena arise from the expenditure in the cell of chemical energy, and that the phenomena are accompanied by development of heat, electricity, and other forms of energy are no arguments that such vital phenomena are not characteristic of a type of energy found only in living structures.

It is only necessary to prove that a set of energy phenomena exist in living structures which are characteristic of life, that energy effects are produced and can be demonstrated in living cells which cannot be shown apart from life, in order to prove that we here have to deal with a type of energy which does not appear in non-living matter.

We may pass, accordingly, to an enumeration of some of the peculiar energy properties of living matter upon which reliance can be placed as proving that such matter is a peculiar energy-transformer in which a peculiar type of energy (biotic energy) is developed alongside of other forms which also occur in inorganic nature.

I. The mode of production of living matter is characteristic, and cannot be brought about by the action solely of inorganic forms of energy. Living matter is produced only by the action of other living matter upon the materials and forms of energy of the non-living world. In the process the matter involved is built up into substances of great chemical complexity, and it has been supposed that this is the essential portion of the process of production of a living structure; but it must be noted that even this very production of complexity of structure from simple inorganic bodies at the expense of the energy of the solar rays takes place and can only take place in a living structure itself.

The very building up of the machine or transformer in which the manifestations of biotic energy are subsequently to take place is then a cogent argument that here we are dealing with a type of energy which is not met with elsewhere. For nowhere else in Nature does a similar process appear to that of the production of living structure, and by no combination or application of the forms of energy apart from life can it be repeated or simulated.

II. The life cycle of the cell, no less than its birth or first production, yields a strong argument for the existence within it of a peculiar type of energy.

It might be argued that it was merely the complex structure of the protoplasm that was required in order that the inorganic forms of energy might be set in play; and that given this structure, osmosis and diffusion and chemical and electrical energy did the rest. But how comes it, then, that a cell perfect in structure does not remain perpetually an engine for the play of such inorganic forces, and why does not life last perpetually in the same cell in a state of equilibrium? Why does the cell divide and reproduce itself and pass through a cycle, if it is merely a structure for the play of inorganic forms of energy? No such phenomena of change and reproduction and death are seen anywhere in the inorganic world, nor can they be reproduced elsewhere than in living cells.

If, on the other hand, the living cell possesses, in addition to its peculiar and complex chemical and physical structure, the property of producing from the inorganic forms of energy a type of energy of its own, some means of accounting for the division and reproduction of the cell become at once apparent.

Nearly all, if not all, other known forms of energy are *phasic* or *cyclic* in character, and it is highly probable that biotic energy must be cyclic also, the conditions of the cycle being different in different types of cell, and under different conditions of environment, and hence at certain stages alterations must occur as the result of variation in the biotic energy of the cell for the time being.

In other forms of energy we recognise what has been termed the potential factor. Now, if we suppose that in the living cell there is produced a type of energy peculiar to living structures, from the non-living forms of energy supplied from without by means of the cell acting as transformer, then it may be supposed that if there is any tendency to accumulation of biotic energy, the capacity factor being fixed by the size of the cell, the potential factor must increase and lead to division and reproduction. Naturally any such process must be modified by other factors playing in upon the cell, such as food, temperature, and other conditions of environment, but it would be guided and controlled by the biotic energy of the cell.

The division and reproduction of the cell, therefore, furnish energy phenomena of a type not found outside living matter.

III. Heredity, and the reproduction of like species from like, shows that there is something present not dependent merely upon structure, but that the cell possesses a type of energy which causes a retention of properties, and a capacity for communication of these onward.

By variations in the factors of such a form of energy the character of the effects are capable of alteration, much in the same way as variation in vibration period can alter the effects produced by radiant energy, or alterations in constituent groups of the chemical molecule can alter the chemical energy.

The closest histological examination reveals no essential difference between the ovum of one species of mammal and that of another, yet the cells develop into widely different species. This cannot all be due to nothing but the operation of inorganic forms of energy upon structure so similar that the microscope can show no difference in design. Nor can the unicellular ovum contain already laid down in it structurally some representation of each cell or even each tissue of the animal which is to be formed from it. It is too minute and too simple in its organisation to render

such a view tenable. Nor is the chemical composition of the complete animal represented in that of the early embryo. It is evident that the course of cell-division and development by which a constant species is arrived at are not attained by means of structure in the embryo, the ingress of chemical energy from without, and the action of diffusion and osmosis. But if there be added to these the presence of a distinct type of energy peculiar to living matter which controls and regulates the energy phenomena of the growing embryo, and which is attuned initially to the species of living creature to which the embryo belongs, then a more feasible basis for the explanation of the course of development of the individual becomes apparent. At each step, this biotic energy will regulate the growth and division of the cells of the growing embryo. As each stage is reached, similar changes in the distribution of biotic energy will occur as in previous generations, for the embryo will arrive at them, with the same distribution as in past generations of its store of energy, and hence the same phases in the energy phenomena will repeat themselves, except in so far as these are modified by nutrition and environment in a secondary degree.

In the process of growth, the oxidation of the food yields the necessary energy, which is converted into biotic energy, and then this is utilised in building up fresh cellular material, in fabricating chemical substances for the use of the cells and in producing other forms of energy. Throughout the biotic energy retains certain intrinsic characteristics derived from the fertilised ovum, and by the impress given by these the process is directed.

IV. The fundamental properties possessed by living matter of *irritability*, *contractility*, and *conductivity*, are all energy phenomena characteristic of life, and nowhere manifested by the operation of other forms of energy in non-living matter. While it is undoubtedly true that the exhibition of these properties in living tissue are accompanied by manifestations of other forms of energy, such as chemical change, electricity and heat, and indeed necessarily must be, since the cell obtains the energy used in the production and propagation of these changes from such non-biotic forms of energy, yet the alterations in irritability are characterised by phenomena which are not chemical, electrical, or calorie, and cannot be placed under any of the known forms of non-biotic energy.

Take, as an example, the nerve impulse travelling along a living nerve-fibre. A similar phenomenon cannot be reproduced in any non-living structure, and while it is accompanied by an electric wave which travels at the same rate, it cannot be held that the electrical energy is the nerve impulse, any more than that the chemical change or electric wave accompanying a muscle contraction wave is the contraction wave itself; or, to choose an example from energy transformation in non-living matter, that the heat in the wire, and magnetic field around it, are the electric current travelling along the wire. In many other manifestations of irritability than the nerve impulse it is known that increased electrical negativity is associated with increased physiological activity, and hence the most logical view certainly is that the negative wave accompanying the nerve impulse betokens a wave of increased irritability propagated along the fibre. Here we have, then, a phenomenon of biotic energy in a typical form, and can even get at one property, namely, the rate at which the wave of biotic energy is carried along this particular type of conductor. In the muscle cell a similar wave is seen traversing a different form of conductor at a different rate.

V. The *metabolism* of the cell furnishes further proof that energy changes in the cell are produced by the action of a type of energy not found elsewhere than in living tissues. The production of the living protoplasm of the cell itself has already been alluded to as a proof of the existence of such a type of energy; but in addition to the substance of the energy-transformer itself, there are to be considered the products formed interstitially within the cell. Most of these are so complex that they have not yet been synthesised by the organic chemist; but even of those that have been synthesised, it may be remarked that all proof is wanting that the syntheses have been carried out in identically the same fashion and by the employment of the same forms of energy in the case of the cell as in the chemist's laboratory. The conditions in the cell are widely different, and at the temperature of the cell and with such chemical materials as are at hand in the cell no such organic syntheses have been artificially carried out by the forms of energy extraneous to living tissue.

Again, the regulation of the production and breaking up of

such substances, the variations in rate of action, and the regulation of the manifold intercurrent reactions running simultaneously within the minute compass of a single cell, even more powerfully than the mere synthesis of the substances point to a controlling and regulating type of energy different to anything outside living matter. The reversal of chemical action from period to period, and the sudden change in chemical activities produced by the activity of the nervous system, have no parallel outside the realm of living cells.

Much has been made of the fact that intracellular enzymes have been isolated from living cells which are capable of producing actions hitherto only observed in the presence of the cell, and it has been surmised that all or nearly all the chemical activity of the cell may be due to the action of a large number of such intracellular enzymes. It has, in fact, been supposed that if a solution could be prepared containing the proper number of enzymes, each in appropriate concentration, that the solution would act much like a cell.

Without disparaging the importance and value of such work of separation of intracellular enzymes, it may, however, be urged that there is in such a view no explanation of the phasic activity of the cell, no taking into account of the action of the living cell in co-ordinating, so to speak, the myriad activities going on within it whereby the whole process is regulated. Such a solution of enzymes compared with a living cell would be like a horde of savage warriors compared to a civilised and disciplined army of soldiers, or a mass of unicellular organisms compared to a highly differentiated mammal.

There must obviously in the cell be some type of energy controlling all this metabolic activity, and this is the rôle played by the biotic energy of the cell.

VI. The *osmotic* phenomena of the cell demand for their explanation the presence of a type of energy not found elsewhere than in living structures.

Even in the case of those cells of the body which in form look most like membraneous structures, viz. the air-cells of the lung and the thin endothelial cells of the wall of lymphatic and blood capillaries, it has been clearly shown that the laws of diffusion and osmosis as observed in the case of inert, non-living membranes are not obeyed. These structures are not inert membranes.

but living cells displaying biotic energy, taking up energy from the plasma in chemical form and using that energy by converting it into volume or osmotic energy, and effecting thereby separation of substances in solution in greater concentration—that is to say, such cells act as energy-transformers, the ultimate conversion being from chemical into volume energy.

Such a change is seen in the secretion of gases both in the swimming bladder of the fish, and, according to Bohr and other observers, in the mammalian lung, where the partial pressure of the carbon-dioxide separated in the alveolar air is higher than that in the blood of the pulmonary capillaries. Here the cells of the lung are acting against pressure, and, no matter what the intermediate steps may be, volume energy is increased in the process, and must be obtained from the chemical energy of the cell, the cell-protoplasm acting as the energy machine or transformer.

Similar instances of gaseous secretion are seen (with the difference in these instances that the gas is retained in solution, and the increased pressure is osmotic) in the case of the secretion of saliva and of bile, where in both cases the pressure of dissolved carbon-dioxide is greater in the secretion than in the venous blood flowing from the organ. Here just as truly as in the alveoli of the lung volume energy is increased in the process of secretion. In a similar manner hyper-tonic salt solutions are taken up from the serous cavities by the blood and lymph capillaries lining their walls against the gradient of osmotic pressure. The absorption of isotonic effusions, whether normal or pathological, is a process which also demonstrates that the cells lining the vessels are not inert membranes comparable in action to non-living membranes, whether permeable or semi-permeable; but are living cells, capable of acting as active absorbent channels, by behaving as machines possessing the important function of the conversion of chemical into volume energy. But it is in the case of the typical secretory and excretory cells of the body that this function of the living cell of acting as an energy-transformer between volume energy and chemical energy is seen developed to its highest degree. In these cases, it is observed not only that the amount of volume energy developed is larger, but that the action is eminently selective according to the nature of the dissolved substance.

This subject will be gone into in greater detail in the chapter on secretion and excretion; we need not therefore enter more fully

upon the matter here than to indicate that, in the kidney, for example, urea solution is concentrated from less than ·01 per cent. in the circulating blood up to 2 per cent. in the urine, and that in this process no matter what may be the intermediate stages, the kidney cells develop volume energy, against the usual laws applicable to inert semi-permeable membranes, just as much as a mechanical engine attached to a piston and cylinder would do in compressing a gas from a pressure of about 130 mm. of mercury and a volume of 75,000 c.c. to a pressure of about 6500 mm. of mercury and a volume of 1500 c.c., these being the volumes of the blood and urine and pressures of the ·01 and 2 per cent. solutions respectively.

In the face of this experimental evidence, surely it is time to cease regarding kidney cells as semi-permeable membranes to which the laws of osmosis apply. The case is exactly the reverse of the semi-permeable membrane, in which the solvent passes through, tending to equalise pressures and reduce volume energy; for in the kidney cell the dissolved substance passes through at a greater rate than the solvent, increasing the difference in pressure on the two sides and developing volume energy.

It is not intended at all to represent that the phenomena described are contrary to the laws of energetics, but to make clear that the cell does not play the part of an inert membrane, that the laws of osmosis deduced from observations on inert membranes do not apply, and that there is a form of energy and type of energy-transformer at work which are not to be observed elsewhere than in living cells.

The study of the properties of this particular energy-transformer, and the interactions between biotic energy and the inorganic forms of energy carried out by its action, is the province of the biologist, who must approach and has been approaching the subject in the same manner as the physicist and chemist approach the study of other types of energy—that is, by acting upon the cell with other types of energy, and studying its reaction to such treatment.

Experiments on any form of energy consist in observing the interactions between it and other forms, in studying the nature of the transformer, and of the changes, if any, which occur in it.

The structure of the cell must hence be taken up from the

point of view of its function; and we must study the chemical and physical composition, the effect of the several constituents upon one another, and upon the medium in which the cell lives, the nature and action of the input and output of the cell, including its secretions and how these are produced, the osmotic phenomena and the effects of changes in the surrounding medium, the characteristic accompaniments of stimulation of the cell and of conduction of stimulation from part to part, and the effects produced by cells possessing a life in common, as in the multicellular animal.

Physical chemistry affords us one of the most powerful experimental engines in conducting such inquiries for a reason which has already been touched upon, namely, that the living cell is in structure a complex solution containing both colloids and crystalloids, and that the chemical reactions occurring in the cell are reactions in solution. Accordingly, although the whole matter is profoundly affected by the fact that the cell is alive, it is evident that our knowledge of cellular activities must be based on knowledge of the properties of solutions, both of colloids and crystalloids; of reactions in solution, the velocity of such reactions and the conditions of equilibrium; of the mutual effect of crystalloids and colloids upon one another when in common solution, and of the effects of the living cell as a peculiar energy-transformer upon osmosis and diffusion.

CHAPTER II

CHEMICAL TRANSFORMATIONS IN LIVING MATTER AND ITS PRODUCTS—CHEMICAL EQUILIBRIUM AND REACTION—ENZYMES AND CELLS AS CATALYSTS OR ENERGY-TRANSFORMERS

The supply of energy necessary for all the changes observable in living structures arises from chemical reactions occurring in solution. The energy exchanges occurring in any such reaction are initiated and controlled by the cell and its secretions, or the substances present in the cell, acting as energy-transformers in the sense indicated in the preceding chapter. In the present chapter it is proposed to consider the conditions governing reactions in solution, and as far as possible how these are modified by the presence of the peculiar energy-transformers found in the living cell itself or its products.

Let us, first of all, take a general survey of the cycle of energy changes which occur in living structures as matter and energy are taken up from the inorganic world, sent through various changes in the living cell, in which many varied forms of matter are produced and different types of energy are exhibited, and finally both matter and energy are returned in other forms to the inorganic world. The matter involved in these changes is directly or indirectly taken from inorganic sources, and the energy from the light energy of the solar rays; for whether the food of an animal be of animal or vegetable nature, in the first instance it must have come from vegetable sources. In the green parts of plants, the chemical energy is first accumulated in matter which afterwards being carried to other parts of the plant, or being used as the food of animals, furnishes the store of energy used in carrying on the reactions of all other cells.

In the process of building up of organic bodies in the chlorophyll-bearing cell the chlorophyll acts as an energy-transformer, that is, it induces an interaction between forms of energy (in this case light energy and chemical energy), which would not occur

in its absence, or could only occur incomparably more slowly. In this process, the changes in the chlorophyll itself are so insignificant, in comparison with the amount of changes in light energy and chemical energy, that they may be left out of consideration, and we may regard the chlorophyll as a mechanism for carrying on the energy exchanges, just like a chemical catalyst or an enzyme, although the types of energy between which it works are different, as well as the limitations of its action. In the chemical modifications subsequent to the action of the chlorophyll, the various cells may be similarly regarded as catalysing agents differing somewhat in their mode of action upon the chemical materials supplied to them, and in the products formed, but agreeing in that they cause large amounts of interchange between chemical energy and other forms, without being themselves altered permanently, or proportionately to the reactions which they induce. The same is true of the enzymes formed by the action of the cells, which induce various reactions either within the cells, or after separation from them in the secretions.

Hence we may regard the processes occurring in plants and animals as energy reactions induced by the cells or their enzymes acting as energy-transformers.

Excepting in the green cell in the presence of sunlight, the net result of the energy interchanges induced by the cells and their secretions is that the chemical energy produced by the absorption and disappearance of the light energy is used up and converted into other forms, such as heat, mechanical energy, osmotic energy, electrical energy, &c. The process is accompanied by absorption of oxygen, and the matter passes back again into inorganic forms identical with or closely resembling those with which the process began, and containing little or no more energy than at the start.

In the intermediate reactions of metabolism the process is not, however, purely one of oxidation; the cell, on account of its peculiar properties as an energy-transformer, and probably by the production intermediately of its own peculiar type of energy, is capable of inducing synthetic processes in which chemical energy is taken up. The supply of energy for such a synthetic process is obtained from energy given out by other chemical processes running concurrently in which energy is set free.

It is in this respect that the more complex transformer which

is seen in the cell differs from the simpler inorganic catalysts and the enzymes formed by cells. As a rule, the simpler enzymes are only capable of inducing reactions in which no substances are formed with larger storage of chemical energy. But the cell is able from a supply of substance, such, say, as soluble carbohydrate, to oxidise part to yield energy, and utilise this energy to build up a smaller amount of a substance, such as a fat, possessing for an equal weight a higher amount of chemical energy.

The fluids with which the cells are bathed and which permeate them may be regarded as solutions containing, in a few common forms supplied universally to all the cells, energy in a chemical form which is utilised by the cells to carry on their reactions.

Depending upon the type of the cell and the enzymes which it contains, varying reactions are induced in this common medium, so that different products are formed, and different amounts and types of energy set free.

In following out the energy exchanges it is hence necessary to consider the properties of solutions, such as the velocity of reaction and the conditions of equilibrium between the reacting substances; the forms in which energy is present and the amount of change in energy as the substances react; and how the presence of catalysts alters the energy exchanges in the solution.

The law of conservation of energy teaches that the total amount of energy must remain the same, and hence the algebraic sum of the amounts of energy changing from one form to another must be equal to zero.

Since we possess no means of stating the absolute amount of energy contained by a system in any of the varied forms in which energy manifests itself, we can only estimate the *change* of energy in any given form as the system passes from one condition to another, and to obtain the total change in the system the amount of change in each particular form of energy undergoing change must be taken into account. The amount of change in each form of energy is obtained from the product of the capacity factor of the system for any particular form of energy, and the change in the potential factor of that form of energy.

In the case of certain forms of energy the capacity and potential factors are still unknown to us, and the amount of energy taken up or given out in these forms during a change

of energy from one given form to another can only be estimated by statement of corresponding changes in other forms of energy as a result of the reaction. The two forms of energy in which such changes of energy can best be expressed are heat energy and mechanical work or dynamical energy, and it is for this reason that energy exchanges are considered upon a thermo-dynamical basis.

Energy equilibrium or reaction is determined by the values at any given time of the potential factors of the various forms of energy, by the facilities presented in the system for potential equalisation, and by the manner in which energy is bound or related to particular forms of matter in the system. In so far as any particular form of energy is free to distribute itself throughout the system that particular form of energy tends to equalise its potential all through the system, and hence the energy always passes from a point of higher to a point of lower potential. Thus in any system where redistribution is possible heat always passes from a position of higher to one of lower temperature, electricity from a higher to a lower electrical potential, a fluid or gas from a higher to a lower level of pressure, and so on for every form of energy. In any such change the velocity of equalisation is directly proportional to the difference in potential, and inversely proportional to what is termed the *resistance*. The resistance, however, means only that the equalisation is opposed by the potential factor of some other form of energy, and that this opposition has to be overcome in effecting the equalisation. If the potential factor of this opposing energy is sufficiently high the equalisation may be entirely stopped, or its speed only may be lessened so that the equalisation takes longer and proceeds with diminished intensity.

It is impossible for equalisation of potential, or change towards equalisation of potential, of any form of energy to take place without conversion of a portion of the energy so changing potential into other forms, and all physical measurements of change in potential are based upon changes of the energy into other forms.

In any condition of equilibrium of a system there is a balance between the intensity factors of the various forms of energy capable of interacting with one another, and that balance is dependent upon the facilities provided in the system for interaction between the various forms of energy. Further, in the absence of equilibrium the velocity or intensity of reaction is dependent on

the same factors, namely, the values of the potential factors and the facilities for interaction.

Hence the state of equilibrium or reaction may be disturbed by addition or removal of energy so as to disturb the potential factors, or by alterations which change the ease of passage from one form to another.

It is the variation in the potential factor or intensity factor which appeals to our sense organs, and determines the results of all physiological stimulation, and it is for this reason that the potential factor and methods for measuring variations in it [1] were known to mankind long before the recognition of corresponding quantities of energy.

Chemical Energy and Chemical Equilibrium [2]

The energy changes which interest us most in connection with the biological chemistry of the cell are those in which chemical energy plays a part, and accordingly we pass on to the energy conditions in a solution. In order to simplify the matter we shall assume at first that there is no catalyst present, and investigate the equations governing energy changes in solution under different conditions in which energy interchanges are possible between the various forms of energy which can react in the solution. That is, we shall leave out of consideration at present the mechanisms by which the energy changes are brought about, which can alter the velocities with which the changes occur, or which, by bringing into play other forms of energy, can alter the conditions of reaction and the equilibrium point.

The three forms of energy which normally undergo alteration in value when a chemical reaction occurs in solution [3] are the chemical energy, the heat energy, and the osmotic energy. The law of conservation of energy teaches that the algebraic sum of the three alterations must be zero. Or, if C represent chemical

[1] Such, for example, as the thermometer in connection with heat energy, the monometer in connection with volume energy.

[2] The student who is unfamiliar with the mathematical method followed in the subsequent pages can omit the proof given therein, and confine himself to the experimental and more practical part of this subject.—(*Editor's Note.*)

[3] Or, in gaseous form, the investigation is the same for both conditions of matter.

energy,[1] H heat energy,[2] and V volume energy, for the change of any fixed quantity of matter from one chemical form to the other, we have :—

$$C + H + V = 0.$$

If attention be paid to change of sign any of these three quantities may be changed from one side of the equation to the other.

Hence if C represent chemical energy disappearing and given out in the other two forms we have :—

$$C = H + V.$$

Let us take it that a grm. molecule of the substance changes chemical form, and that the volume of the solution in which such a change occurs is so large that no appreciable change takes place in the osmotic pressures of the two substances in solution.

Then in the above equation C is the change in chemical energy accompanying a change in chemical constitution from the first form (A) on one side of the chemical equation to the other form (B) on the other side of the chemical equation. As C is dependent only on the change in chemical constitution it is a constant, the value of which is determined by the sum of the values of (1) the heat (H) for the change of a grm. molecule at given concentrations (P_1 and P_2) in solution of A into B, the two substances on the two sides of the chemical equation, (2) the change in volume energy due to the conversion of a grm. molecule of A at pressure P_1 into a grm. molecule of B at pressure P_2.

The heat energy produced by the change of a grm. molecule when the substance A has a pressure P_1 and the substance B a pressure P_2 is a variable quantity dependent upon the values of P_1 and P_2.

The value of V, the change in volume energy for the change of a grm. molecule of A at osmotic pressure P_1 into B at pressure P_2, is also a variable.

When as the result of a reaction a grm. molecule of a substance comes into solution at a definite pressure, a certain fixed

[1] This does not mean heat of reaction, but the total amount of chemical energy set free or absorbed when a given amount of substance changes form.

[2] The value of H is not the heat of reaction in the usual sense of the term, but the amount of heat energy due to the change of the fixed quantity at the definite osmotic pressures obtaining at a given instant or stage in the reaction.

amount of volume energy appears dependent upon the pressure, and as a result the heat produced will be less by this amount; and similarly when a grm. molecule disappears at another different pressure, a definite amount of volume energy disappears, and by this amount the heat of reaction will increase. Accordingly the difference in these two amounts will express V, the change in volume energy in the reaction.

The difference in volume energy before and after the reaction cannot be obtained by taking the differences in the total osmotic pressures before and after the reaction, because the quantity of substance appearing is not brought during the change from the one of these pressures to the other, but from the zero of pressure to the pressure at which it is finally found in the reaction, that is, to the partial pressure of the component substance to which it belongs; and similarly the grm. molecule of substance disappearing passes from the partial pressure at which that substance happens to be present in solution to zero pressure. Hence the amount of volume energy must be obtained separately for each substance taking part in the reaction.

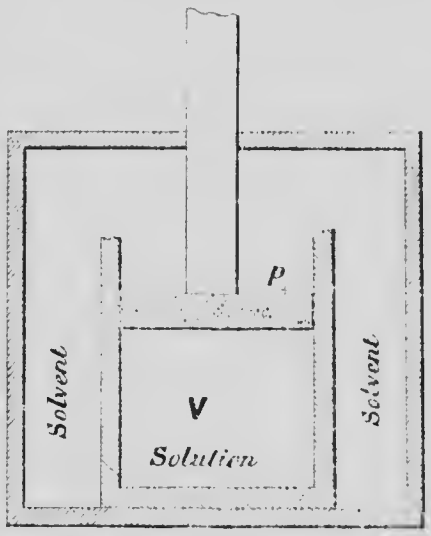

FIG. 1.—Work done in Compression of a Solution in a Semi-permeable Cylinder.

This amount of energy may be obtained as follows:—Suppose a definite amount of a substance to be in solution in a semi-permeable cylinder fitted with a semi-permeable piston, as sketched in the diagram, and surrounded by solvent. Then if the pressure on the piston be changed, just as in the case of a gas, the solution will correspondingly change in volume. The work done by the dissolved substance or *solute* is $P\delta V$, where P is the pressure and δV the small change in the volume. Or, using the gas law $PV = RT$, we have on differentiation $P\delta V = -V\delta P$, and again since $V = \frac{RT}{P}$, $P\delta V = RT\frac{\delta P}{P}$. On integrating this yields for the work done by the gas in expanding isother-

mally from pressure P_1 to pressure P_2 the well-known expression $W = RT \log \frac{P_1}{P_2}$.

This amount of work may be expressed as heat energy by giving the proper value to the constant RT, which for a grm. molecule of a substance which obeys the gas law works out to 5·8 K calories[1] at 0° C.

From this expression the amount of energy required to transform a solute from the minimum or zero pressure (P_0) to any given pressure (P), or *vice versâ*, may be expressed as heat energy, the expression being, $\pm RT \log \frac{P}{P_0}$.

When a chemical reaction takes place and molecules appear and disappear as a consequence, the change in volume energy is the algebraic sum of the changes in volume energy of each kind of molecule so appearing or disappearing. When a grm. molecule of a substance appears in solution at the pressure P the volume energy increases by RT log $\frac{P}{P_0}$, *and conversely when a grm. molecule of a substance disappears from solution the volume energy decreases by a like amount.*

This may now be applied to the energy changes taking place in different types of reaction.

1. The simplest case is that in which a grm. molecule of a substance A is formed and a grm. molecule of substance B disappears, as, for example, when a substance changes into an isomeric body, according to the equation,[2]

$$A \rightleftarrows B.$$

To simplify matters, we shall assume that the volume of solution is so large that a grm. molecule of A can change into B without causing any appreciable differences in P_A the pressure of A or P_B the pressure of B.

The disappearance of A causes a diminution of volume energy given by $RT \log \frac{P_A}{P_0}$, and the appearance of B an increase of volume energy given by $RT \log \frac{P_B}{P_0}$. Therefore the net increase in volume energy is $RT\left(\log \frac{P_B}{P_0} - \log \frac{P_A}{P_0}\right)$, which is $RT \log \frac{P_B}{P_A}$. Accordingly we have the equation

[1] The K (calory) is the amount of heat necessary to raise one grm. of water from 0° to 100° C., and is approximately equal to 100 grm. calories.

[2] The double arrow in a chemical equation is used, instead of the sign of equality, to signify that the reaction may proceed in either direction.

$$C = H + V$$

$$\therefore C = H + RT \log \frac{P_B}{P_A}$$

$$\therefore H = C - RT \log \frac{P_B}{P_A}.$$

In this equation P_B is the osmotic pressure at which B appears, and P_A the osmotic pressure at which A disappears. The value of C is constant, being the chemical energy set free by the passage of a grm. molecule from the form A into the form B, and the variable value H expresses the heat energy set free by the reaction when a grm. molecule changes form at those particular pressures.

If we begin at the point where nearly all the substance is present in the form A, then P_B has a very low value, and consequently $\log \frac{P_B}{P_A}$ has a large negative value, and this being prefixed by a negative sign, it follows that the heat of reaction[1] at this stage has a large positive value. As the reaction proceeds P_B continuously increases and $\log \frac{P_B}{P_A}$ decreases in negative value, accordingly the heat of reaction diminishes. As P_B still increases and P_A diminishes, $\log \frac{P_B}{P_A}$ becomes positive in sign and progressively increases in value, the heat of reaction accordingly still diminishes until when $RT \log \frac{P_B}{P_A} = C$, it becomes zero. Up to this point the reaction has been exothermic, that is to say, giving out heat; but from this point on it absorbs heat, the sign of H becoming negative, and the reaction is said to be endothermic.

If we take the reaction as running from B into A the same sequence of events in the reverse order occurs. Here C is negative, and $RT \log \frac{P_B}{P_A}$ becomes $RT \log \frac{P_A}{P_B}$, so that the equation runs

$$H = -C - RT \log \frac{P_A}{P_B}.$$

Starting with nearly all the substance in the form B, P_A is very small, $RT \log \frac{P_A}{P_B}$ has hence a large negative value. Accordingly $-C - RT \log \frac{P_A}{P_B}$ has a positive value, and the reaction runs exothermically until $RT \log \frac{P_A}{P_B} = -C$, or $RT \log \frac{P_B}{P_A} = C$, when the heat of

[1] That is, the energy set free in the reaction, which need not necessarily all be set free as heat energy; it is merely for simplicity that energy set free in the reaction is spoken of in the text as heat of reaction.

reaction is zero, and from this on the reaction is endothermic. It is hence evident that the reaction runs exothermically from either end down to the same point, where $C = RT \log \frac{P_B}{P_A}$, and at this point where $H = 0$, energy is neither given up to nor taken up from the surroundings. This point is accordingly the equilibrium point of the reaction. The equation for equilibrium is accordingly $RT \log \frac{P_B}{P_A} = C = \text{const.}$[1]

If the chemical energy involved in the passage from the form A to the form B, or *vice versâ*, is so small as to be negligible, as is usually the case in the passage of a substance from one isomeric form to another, we can write $RT \log \frac{P_B}{P_A} = C = 0$, and hence $\log \frac{P_B}{P_A} = 0$, $\frac{P_B}{P_A} = 1$, or $P_B = P_A$. That is, under such conditions equilibrium is attained when the osmotic pressures, and hence the molecular concentrations in solution, are equal.

If we write p_A and p_B for the osmotic pressures of the two substances at the equilibrium point, and P_A and P_B as before for the corresponding pressures at any given point in the reaction, another form can be given to the fundamental equation for the heat of reaction at any given point in the reaction.

For now $C = RT \log \frac{p_B}{p_A}$, and hence on substituting this value we obtain

$$H = RT \left(\log \frac{p_B}{p_A} - \log \frac{P_B}{P_A} \right), \text{ or } H = RT \log \frac{p_B}{p_A} \cdot \frac{P_A}{P_B}.$$

2. Let us take next the cases where two substances A and B interact to form reversibly two other substances C and D, and let P, with the appropriate suffix of the letter denoting the substance, represent the osmotic pressure of each substance.

Then since A and B disappear from solution and diminish the volume energy or osmotic pressure energy, and C and D appear and increase the osmotic pressure energy, the equation becomes

$$H = C - RT \left(\log \frac{P_C}{P_0} + \log \frac{P_D}{P_0} - \log \frac{P_A}{P_0} - \log \frac{P_B}{P_0} \right),$$

or

$$H = C - RT \log \frac{P_C \cdot P_D}{P_A \cdot P_B}.$$

[1] This equation may also be written $\log \frac{P_B}{P} = \frac{C}{RT}$ or $\frac{P_B}{P} = e^{\frac{C}{RT}}$.

The reaction, as before, can be shown to run exothermally from either end until $C = RT \log \frac{P_C \cdot P_D}{P_A \cdot P_B}$, at which point energy is neither given to nor taken up from the surroundings, hence at this point there is equilibrium, and the equation of equilibrium is $RT \log \frac{P_C \cdot P_D}{P_A \cdot P_B} = C = \text{const.}$[1]

At any other point in the reaction the heat of reaction for a grm. molecule changing form at the given pressures is given by the equation

$$H = C - RT \log \frac{P_C \cdot P_D}{P_A \cdot P_B},$$

or

$$H = RT \left(\log \frac{p_C \cdot p_D}{p_A \cdot p_B} - \log \frac{P_C \cdot P_D}{P_A \cdot P_B} \right),$$

or

$$H = RT \log \frac{p_C \cdot p_D \cdot P_A \cdot P_B}{p_A \cdot p_B \cdot P_C \cdot P_D}.$$

If at the beginning of the reaction A and B are present in equal molecular concentrations, then since an equal number of molecules of each always disappear in the reaction, and an equal number of C and D appear as the result, at every stage $P_A = P_B$ and $P_C = P_D$. Hence the equation of equilibrium simplifies to $C = RT \log \frac{P_A^2}{P_C^2} = 2RT \log \frac{P_A}{P_C}$, and that for the heat of reaction to $H = C - 2RT \log \frac{P_A}{P_C}$, or $H = 2RT \left(\log \frac{p_A}{p_C} \cdot \frac{P_C}{P_A} \right)$.

3. Take next a type of reaction which is one of the commonest, and that which is almost universally met in the problems of biological chemistry, namely, the type in which a single compound on one side of the equation breaks up into two or more on the other side, or a reaction in which, although two substances react on each side of the equation, one of them is

[1] As before, this equation may be put in the form $\frac{P_C \cdot P_D}{P_A \cdot P_B} = e^{\frac{C}{RT}}$, which if $P_A = P_B$, and $P_C = P_D$, may be further simplified to $\frac{P_C}{P_A} = e^{\frac{C}{2RT}}$.

identical with the solvent, so that it causes no change in volume energy when it appears or disappears in the reaction. Such a reaction occurring in aqueous solution, for example, as

$$\underset{(Maltose)}{C_{12}H_{22}O_{11}} + H_2O \rightleftarrows \underset{(Dextrose)}{C_6H_{12}O_6} + \underset{(Dextrose)}{C_6H_{12}O_6}$$

Using the same notation as before, let P_A be the osmotic pressure at any stage in the reaction of the single substance which undergoes change in osmotic pressure on the left-hand side of the equation, and P_B the osmotic pressure of either of the two substances produced on the right-hand side, for these being produced in equimolecular proportion, their osmotic pressures at any given stage in the reaction will be equal in value.[1]

Here, as a result of the reaction, a grm. molecule of A disappears, lowering the volume energy by $RT \log \frac{P_A}{P_0}$, and a grm. molecule of each of the two substances formed appears at a pressure P_B, raising the volume energy in each case by $RT \log \frac{P_B}{P_0}$, or in all by $2\,RT \log \frac{P_B}{P_0}$. Accordingly our equation becomes

$$H = C - RT\left(2 \log \frac{P_B}{P_0} - \log \frac{P_A}{P_0}\right),$$

or

$$H = C - RT \log \frac{P^2_B}{P_A \cdot P_0}.$$

As before, the reaction runs exothermically from either end until $RT \log \frac{P^2_B}{P_A \cdot P_0} = C$, at which point there is equilibrium.[2]

[1] If unequal quantities of the two substances formed on the right-hand side are present at the beginning along with the single substance, or a certain amount of one of them, then the osmotic pressures of these two (B and C) will not be equal; but if they are represented by the pressures P_B and P_C the only difference is that we will have $P_B \cdot P_C$ in the final equation instead of P_B^2, so that the equation becomes:—$H = C - RT \log \frac{P_B \cdot P_C}{P_A \cdot P_0}$, which for equilibrium leads to $\log \frac{P_B \cdot P_C}{P_A \cdot P_0} = \frac{C}{RT}$, or $\frac{P_B \cdot P_C}{P_A \cdot P_0} = e^{\frac{C}{RT}}$.

[2] As before, the equilibrium equation can accordingly be written

$$\frac{P^2_B}{P_A \cdot P_0} = e^{\frac{C}{RT}}, \text{ or } \frac{P^2_B}{P} = P_0 \cdot e^{\frac{C}{RT}}.$$

For the heat of reaction at any other point we have

$$H = C - RT \log \frac{P^2_B}{P_A \cdot P_0},$$

or using the same notation as in the other cases,

$$H = RT \left(\log \frac{p^2_B}{p_A \cdot p_0} - \log \frac{P^2_B}{P_A \cdot P_0} \right),$$

or

$$H = RT \log \frac{p^2_B}{p_A \cdot p_0} \cdot \frac{P_A \cdot P_0}{P^2_B}.$$

But the zero pressure P_0 and p_0 is always the same, hence

$$H = RT \log \frac{p^2_B}{p_A} \cdot \frac{P_A}{P^2_B}.$$

4. The most general form of the equation is where a number of substances, A, B, C, &c., react with one another to form a number of other substances, A′, B′, C′, &c., and where the number of molecules of each substances entering into reaction varies.

Let the chemical equation be represented by

$$aA + bB + cC + \&c. \rightleftarrows a'A' + b'B' + c'C' + \&c.,$$

in which the small letters represent the numbers of molecules of each substance respectively entering into reaction. Then the expression for the change in volume energy becomes

$$RT \left[a' \log \frac{P_{A'}}{P_0} + b' \log \frac{P_{B'}}{P_0} + c' \log \frac{P_{C'}}{P_0} + \&c. - (a \log \frac{P_A}{P_0} + b \log \frac{P_B}{P_0} + c \log \frac{P_C}{P_0} + \&c.) \right].$$

This expression can be written

$$RT \log \frac{P^{a'}_{A'} \cdot P^{b'}_{B'} \cdot P^{c'}_{C'} \cdot \&c.}{P^a_A \cdot P^b_B \cdot P^c_C} \cdot P_0^{[a+b+c+\&c.-a'+b'+c'+\&c.)}$$

For equilibrium, as before, this expression is equal to C, the energy evolved when a grm. molecule of each substance changes from the left-hand side of the equation to the right, and hence

$$RT \log \frac{P^{a'}_{A'} \cdot P^{b'}_{B'} \cdot P^{c'}_{C'} \cdot \&c.}{P^a_A \cdot P^b_B \cdot P^c_C \cdot \&c.} \cdot P_0^{[a+b+c+\&c.-(a'+b'+c'+\&c.)]} = C,$$

or

$$\frac{P^{a'}_{A'} \cdot P^{b'}_{B'} \cdot P^{c'}_{C'} \cdot \&c.}{P^a_A \cdot P^b_B \cdot P^c_C \cdot \&c.} = P_0^{[a'+b'+c'+\&c.-(a+b+c+\&c.)} \cdot e^{\frac{C}{RT}}.$$

Now the right-hand side is a constant, so the general equation of equilibrium becomes

$$\frac{P_A^{a'} \cdot P_B^{b'} \; P_C^{c'} \cdot \&c.}{P_A^{a} \cdot P_B^{b} \cdot P_C^{c} \cdot \&c.} = \text{constant.}$$

The results which can be derived from the types of reaction that we have considered may now be discussed. Writing K for the constant, we have the following results:—

I. Where a single substance A undergoes molecular rearrangement to form a single substance B:—

$$\frac{P_B}{P_A} = e^{\frac{C}{RT}} = K, \text{ or } P_B = K\,P_A.$$

If the chemical energy of transference from substance A to substance B is zero, the constant becomes unity and the equation is $P_B = P_A$. This condition is probably attained with stereo-isomers, and hence when the two isomers are formed in any reaction they are turned out in the condition of equilibrium, that is, in equal quantities, and we get, as in the case of the synthetically prepared sugars, the indifferent compound consisting of an equimolecular mixture of the two isomeric bodies.

II. Where two substances, A and B, interact to form two others, C and D:—

$$\frac{P_C \cdot P_D}{P_A \cdot P_B} = e^{\frac{C}{RT}} = K, \text{ or } P_C \cdot P_D = K\,P_A \cdot P_B,$$

that is, the product of the osmotic pressures of the one pair of substances is proportional to the product of the osmotic pressures of the other pair of substances.

If the substances A and B at the commencement are in equimolecular concentration, then $P_A = P_B$, and since the substances C and D are then also formed in equimolecular concentration also $P_C = P_D$, and hence the equation for equilibrium can obviously be simplified to $P_{(C \text{ or } D)} = K \cdot P_{(A \text{ or } B)}$. That is, the osmotic pressure of the substances formed always bear the same ratio to the osmotic pressure of the substances from which they are formed when the equilibrium point is reached.

It follows that for reactions of the type I., and for those of II. when the substances are present in the proper equimolecular

proportions for combining, that the equilibrium point is not affected by the concentration of the solution. That is, whether the reaction occurs in very dilute or in concentrated solution (within the limits at which the gas laws hold for osmotic pressures), the same proportion of A is turned into B in type I., and of A and B into C and D in type II. This is obvious, for if $P_A = K P_C$, then if P_A is doubled, so must P_C be in order that the equation may still hold, or, in other words, no matter what is the original concentration of the reacting substances, the same percentage is always turned into the substances formed before equilibrium is reached.

The law that the equilibrium point is fixed where the reaction is of the types I. or II., and is independent of the concentration, has been proved experimentally for the reaction between gaseous hydrogen and bromine vapour. Here a molecule of hydrogen and a molecule of bromine unite to form two molecules of hydrogen bromide, and there is no change in number of reacting molecules; thus $H_2 + Br_2 = HBr + HBr$, accordingly we have $P_{(H_2 \text{ or } Br_2)} = K \cdot P_{HBr}$, and no matter how the pressure of hydrogen, bromine, and gaseous hydrobromic acid are altered, the percentage of the three reacting constituents ought to have the same ratio; that is, there ought always to be the same percentage of bromine vapour converted into hydrobromic acid. This is found to hold experimentally; within fairly wide limits, no matter how the pressure is varied, the percentage of conversion remains the same.

III. Where a single compound splits up into two compounds, or *vice versâ* when the reaction is proceeding in the opposite direction. Under this class are also included those reactions in which, although there are two substances on each side of the reaction, one by passing out of solution or by blending with the solvent with which it happens to be identical, develops no change in osmotic pressure, and hence there is no work done on or by it, and it does not come into the reaction. Such a reaction would be included, for example, as a disaccharide splitting into its constituent hexoses or *vice versâ*, although the molecule of water added to or taken away from the disaccharides in the reaction makes the number of molecules *apparently* equal on the two sides of the equation. The molecule of water in the reaction, however, comes from or is returned to the great mass of water forming the

solvent for the disaccharide molecule, and hence has no osmotic pressure before, during, or after the reaction. Accordingly, it does not come into the equilibrium equation, for no change of osmotic energy takes place in connection with it, and hence such reaction is practically one in which one molecule forms two or *vice versâ*.

Nearly all the reactions of digestion and metabolism also belong to this type of reaction, with the slight modification that the number of simpler molecules formed is often three or more instead of two, and all the conclusions deduced for the simpler splitting into two molecules, or *vice versâ*, can be applied to such reactions with but slight modification. For example, the hydrolyses of neutral fats or triglycerides into fatty acids and of starches into sugars belong to this category.[1]

When a disaccharide is hydrolysed (for example, maltose into two molecules of dextrose), as in the equation

$$C_{12}H_{22}O_{11} + H_2O \rightleftarrows C_6H_{12}O_6 + C_6H_{12}O_6,$$

the equation of equilibrium is, as deduced above, $\frac{P_B^2}{P_A} = P_0 . e^{\frac{C}{RT}}$, where P_B is the osmotic pressure of the hexose at equilibrium, and P_A that of the disaccharide, this may be written $\frac{P_B^2}{P_A} = K$, or $P_B^2 = K\, P_A$. Expressed in words, there is a constant ratio between the osmotic pressure of the disaccharide and the *square* of the osmotic pressure of the hexose. As a result, the point of equilibrium is not fixed and independent of the concentration of the reacting substances as in the types of reaction previously discussed, but varies with the initial concentration of the solution, in such a way, that in dilute solutions, the equilibrium lies where the whole of the disaccharide is dissociated into its constituent hexoses, while as the solution becomes more concentrated, the equilibrium point lies more and more towards complete conversion into disaccharide. This is obvious, for if the concentration of disaccharide in the solution be doubled so that P_A becomes $2P_A$, then P_B only becomes $\sqrt{2} . P_B$, that is, with increas-

[1] *Vide* infra.

ing concentration P_B increases in a less ratio than does P_A; conversely on dilution, P_B decreases in a less ratio than P_A, and hence in dilute solutions P_B increases relatively to P_A.[1]

In the case of a neutral fat or triglyceride hydrolysing in solution,[2] three molecules of fatty acid and one of glycerine are formed from each molecule of the fat, and the equation of equilibrium becomes $P_B^4 = K \cdot P_A$. Accordingly, the effect of concentration becomes still more accentuated, and the tendency is still greater to form the fat in concentrated solution, and the fatty acid and glycerine in dilute solution. Similar results follow in the formation, or *vice versâ*, of the starches and proteids from their component simpler molecules.

It follows from the above considerations that in order th hydrolysis may proceed under the most favourable conditions, as in digestion, that the reaction should proceed in dilute solution, while in order that recombination may occur, as in storage in the cell, the process should take place in concentrated solution. Further, that any drop in concentration of a substance in solution in the cell will tend to produce again hydrolysis and re-solution of the stored-up substance. In fact, on the supposition that the enzymes are not capable of utilising any other forms of energy in the transformations which they induce, and merely act in hastening passage to the equilibrium point, hydrolysis during digestion, and building up again in the cell during metabolism can only proceed if the concentrations are low in digestion, and high in the cell during metabolism and accompanying storage.

The truth of the law deduced theoretically above with regard to the effect of concentration upon the point of equilibrium of reactions can also be shown by experiments. Thus Croft Hill

[1] It may be pointed out that an electrolyte, such as sodium chloride in aqueous solution, behaves similarly and for the same reason. In dilute solution, the electrolyte is practically dissociated into its ions, while in concentrated solution an amount proportional to the osmotic pressures of the different reacting substances is undissociated. The type of reaction is the same as that discussed above. If at equilibrium, the osmotic pressure of the undissociated molecules is represented by P_S and that of each ion by P_I, then as above for equilibrium we have $P_S = K \cdot P_I^2$ and the same reasoning as given above shows that P_I increases relatively to P_S with increasing dilution.

[2] The neutral fats are practically insoluble in water, but the reasoning holds for fats in solution in the cell protoplasm. (See p. 34.)

found that *in very concentrated solutions*, the ferment of malt, maltase, caused the formation of a disaccharide from glucose. In his earlier work Croft Hill thought the disaccharide formed was maltose, viz. the disaccharide on which the ferment naturally works in the grain, and that the process was hence a direct reversal of the action of the same ferment upon maltose in dilute solution. Emmerling, who repeated the experiments, thought the disaccharide formed in the concentrated solution of glucose was isomaltose. Then Croft Hill himself in later work found that the substance formed was a new disaccharide which he termed *revertose*. The important fact, however, remains that a ferment which in dilute solutions of a disaccharide causes a hydrolysis into hexoses or mono-saccharides; in concentrated solution, acts upon the hexose and causes a condensation to disaccharide. It was further shown by Croft Hill that in dilute solutions, containing less than 4 per cent. of glucose, the formation of the disaccharide does not occur.

It has since been shown by E. Fischer and E. F. Armstrong that lactase, a ferment obtained from kefir, causes under like conditions a formation of iso-lactose from a solution containing a mixture in equal concentration of its constituent hexoses, glucose, and galactose; and even from glucose alone a disaccharide was obtained.

Similar evidence has been obtained of reversibility in the case of certain esters of *somewhat* analogous constitution to the fats, by the action of lipase, the fat-splitting enzyme of the pancreas; from which by analogy the inference has been drawn that similar syntheses of neutral fats by reversed enzymic action may occur in the body.

Thus, Kastle and Loevenhart digested a mixture of butyric acid and ethyl alcohol with a fresh aqueous extract of pancreas, and were able to detect ethyl-butyrate by its odour, and, operating on a large scale, were able to obtain a few drops of a light oil with the odour and general properties of the ester. The changes did not occur when boiled pancreatic extract was used, and since in dilute solutions the same lipase can be used to convert ethyl-butyrate into butyric acid and ethyl alcohol, it becomes evident that the action is a reversible one. In a later paper, Loevenhart showed that a similar reaction was obtainable with a large number of different tissue extracts.

In a similar fashion, Hanriot showed that lipase is capable of forming mono-butyrin from butyric acid and glycerine.

In so far, however, as the syntheses of simple esters by lipase are considered to have a bearing upon the synthesis of neutral fats by the same enzyme in the body, it must be pointed out that the reactions are of somewhat different type, and that the equations of equilibrium, taken in conjunction with the solubilities of the neutral fats, show that the synthesis of neutral fats is a much more difficult process, and one which, granted that it may occur by reversed action of the enzyme under the conditions existing in the cell, is exceedingly unlikely to occur in aqueous solution in the test-tube.

The difference arises from the fact that the two syntheses achieved by the authors mentioned above of ethyl-butyrate, and mono-butyrin, are produced in reactions in which single molecules of each of the constituents unite to form a molecule of the ester; while in the case of the neutral fats three molecules of the fatty acid concerned unite with one molecule of the glycerine. If we represent the osmotic pressures (which are proportional to the molecular concentrations) by P_E, P_A, and P_B, for ester, acid, and base respectively, then the equation of equilibrium for the ethyl-butyrate and mono-butyrin becomes $P_E = K.P_A.P_B$ or $P_E = K.P^2_A$, and that for the neutral fat is $P_E = K.P^3_A.P_B$, or, assuming that the constituents are present in the proper concentrations for combination, $P_E = K.P^4_A$.

Contrasting the two formulæ $P_E = K.P_A^2$ and $P_E = K.P_A^4$, we see that in the case of such a reaction as that in which a neutral fat is formed, the tendency to remain dissociated in dilute solution, and to remain combined in concentrated solution are more exaggerated than in the simpler reaction. Accordingly, as we pass from a concentrated solution to a dilute solution the value of P_E falls very rapidly compared to the fall in P_A, or the relative amount of neutral fat becomes very small. In excessively concentrated solution practically all would be neutral fat, but very rapidly on reducing the concentration nearly all would be hydrolysed to free fatty acid and glycerine.

Hence a synthesis of neutral fat from fatty acid and glycerine is only possible in a highly concentrated solution. But the physical property of the fatty acids and neutral fats

of being insoluble in water renders the attainment of such concentrated solutions an impossibility in all attempts at synthesis hitherto made, and for this reason no satisfactory proof of syntheses of neutral fats by lipase have hitherto been furnished.

The theory of equilibrium in solution proves, however, that given the possibility of obtaining more concentrated solutions of the fatty acids the synthesis of neutral fats by enzymes is quite possible; and in the conditions obtaining in the cell, where the solvent is not water but the cell protoplasm, and where also other solvents, such as the bile salts, may be present in concentrated solution, the synthesis of fats may well occur by such means.

The synthesis of neutral fat from soap and glycerine solutions has been claimed by C. A. Ewald, and by Hamburger, by the action of the isolated cells of the intestinal mucous membranes; but similar experiments by the writer of this article, in which both the cells and cell-free extracts of the cells were used from the intestinal mucosa, lymphatic glands, and the pancreas, demonstrated that no trace of neutral fat was ever formed, the only action observable being a setting free of fatty acids from the soaps used. The observations of the authors quoted above, being obtained by difference between total ethereal extract and free fatty acid, were shown to be due to unaltered soaps dissolved out by the ether.

In regard to the synthesis of more complex carbohydrates from the sugars by reversed action of enzymes, it may be stated that Cremer has claimed to have observed a synthesis of glycogen from sugar by the action of Buchner's Zymase, but the result has not yet been confirmed.

IV. The most general case of equilibrium in solution is that where an indefinite number of substances react together, and the equation, as demonstrated on page 27, becomes

$$\frac{P_A^{a'} \cdot P_B^{b'} \cdot P_C^{c'} \cdot \&c.}{P_A^{a} \cdot P_B^{b} \cdot P_C^{c} \cdot \&c.} = K,$$

which may be written

$$P_A^{a'} \cdot P_B^{b'} \cdot P_C^{c'} \cdot \&c. = K \cdot P_A^{a} \cdot P_B^{b} \cdot P_C^{c} \cdot \&c.,$$

that is, at the equilibrium point the products of the osmotic pressures of the substances reacting upon the one side of the equation raised in each case to a power corresponding to the number of molecules entering into the reaction, bear a constant ratio to the similar products of the substances on the other side of the equation of reaction.

All the other special cases previously considered can obviously be deduced from this general equation of equilibrium. Otherwise it is of little practical interest, for reactions more complicated than those given under the special types are too difficult to deal with experimentally.

It may be pointed out that since the osmotic pressures are proportional to the molecular concentrations of the reacting substances, in all the above equations the P representing osmotic pressure may be replaced by a C representing the molecular concentration. This is the form in which such equations are usually given, but since the energy changes which are responsible for bringing about a reaction and establishing an equilibrium are dependent upon the osmotic energies of the dissolved substances it has been thought advisable to give the equations in the form shown above.

Before passing from the subject of equilibrium in solution to that of velocity of reaction, and the effect of enzymes and other energy-transformers upon reaction and velocity of reaction, it may be profitable to consider briefly the conditions which determine whether a reaction is *practically* reversible or not; that is to say, which determine whether the equilibrium point shall lie at an appreciable and practically measurable distance from either extreme end of the reaction.

In the first place, it is clear from the form of the general equation of equilibrium that the osmotic pressure, and therefore the molecular concentration, of none of the reacting substances on one side can be zero,[1] unless the osmotic pressure of one or more of the reacting substances on the other side also became zero. But a zero value on both sides could only mean that substances taking part in the reaction are absent, and this condition

[1] For if any one factor in the product $P_A \cdot P_B \cdot P_C$, &c., becomes zero, the value of the whole product is zero, and this cannot be equated to any finite value on the other side.

is therefore impossible. Hence there is theoretically an equilibrium point in all reactions, and every reaction is reversible. But the value of the constant may be, and in many reactions is, such that the point of equilibrium lies so near one of the end points that the position is indistinguishable experimentally from that of complete passage into the substances found on one side of the equation of reaction. Hence *practically* reactions may be divided into reversible or incomplete and irreversible or complete reactions, and we now proceed to consider the conditions which tend to cause a reaction to become practically reversible or incomplete.

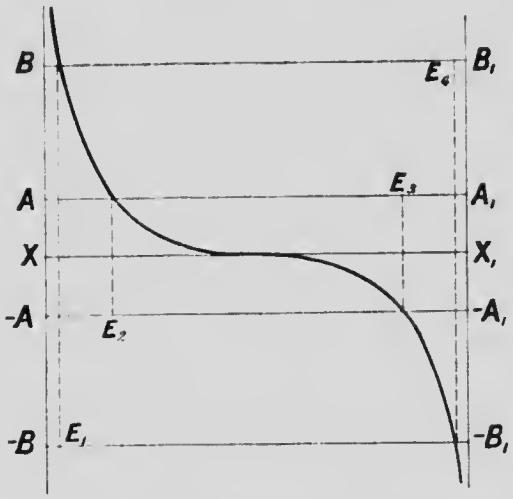

FIG. 2.—Graphic Representation of the Energy Changes in the Course of a Reaction.

We have already seen that in those reactions in which, as a result of the reaction, the number of molecules in solution changes, the concentration of solution changes the equilibrium point, and that such reactions tend to become reversible or incomplete in concentrated solutions and in dilute solution to become irreversible in that phase in which the number of molecules in the solution is largest, and the osmotic energy accordingly at a maximum. It follows for all reactions of this type, which involves by far the greatest number of reactions, that reversibility or the presence of the two phases is impossible in sufficiently dilute solution. This follows because in the equation $P_A = K \cdot P_B^n$, where n is greater than unity, that as P_A and P_B diminish together P_A must get relatively smaller compared to P_B, and with sufficient dilution tends to become infinitely small relatively to P_B.

The other factors which affect the position of the point of equilibrium and determine whether a reaction shall be practically reversible or not are the value of C, the chemical energy involved

in the change from the one phase to the other, and the absolute temperature of the reaction; for changes in either of these affect the value of the constant K.

The effects of changes in C and T upon the point of equilibrium can best be understood by following out the energy changes as the substances pass in the reaction from the one condition to the other. The matter can best be understood by graphic illustration as in the diagram on page 36, in which are represented the energy changes at each instant as the substance passes from the one form to the other according to the simplest type of equation, that in which $H = C - RT \log \frac{P_B}{P_A}$.

The base line XX′ represents the zero line of energy exchange, ordinates above the base line representing quantities of energy set free by the reaction at any stage, and ordinates below, energy required in order that the reaction may proceed. At X the substance is all in the form A and at X′ in the form B, at intermediate points fractions are in the two forms proportionate to the distances from X and X′. The horizontal line above or below XX′ represents by its height above or below XX′ the amount of chemical energy set free (positive when above XX′ and negative when below) when a grm. molecule passes from the form A to the form B. The curved line represents the osmotic energy set free at each point in the reaction when a grm. molecule changes form at the particular osmotic pressures present at that point. The height of this line above or below XX′ is given numerically by the expression $RT \log \frac{P_B}{P_A}$, and the sign is reversed in plotting it so that at each stage the difference in the heights above XX′ of the straight line and the curved line give the value of H, the energy set free at that stage in the reaction, for a grm. molecule changed, according to the equation $H = C - RT \log \frac{P_B}{P_A}$. Tracing now the value of the amount of energy set free in the reaction at each stage, we have at X where $P_B = 0$, and therefore $\log \frac{P_B}{P_A} = \log 0 = -\infty$. Therefore at X, and points close to it, $-RT \log \frac{P_B}{P_A}$ has a large positive value, which is to be added to the constant C in order to give the energy set free. Hence no value of C positive or negative can cause the equilibrium point to lie quite at X. As the reaction proceeds, however, and more and more of B is present, P_B rises in value, and the positive value $RT \log \frac{P_B}{P}$

rapidly drops. If now the value of C is negative, that is, if chemical energy is absorbed on change of substance A into substance B, then an equilibrium will be reached as soon as the value of $-RT \log \frac{P_B}{P_A}$ equals C. This will occur nearer X, as at E_1, if C has a large negative value than if C has a small negative value, as at E_2. Beyond the point of equilibrium so defined the positive value of $-RT \log \frac{P_B}{P_A}$ becomes still smaller, and hence H, which is $C - RT \log \frac{P_B}{P_A}$, becomes negative; that is, energy is absorbed, the reaction is endothermic, and cannot proceed without external energy being added, which is excluded under the conditions we are considering. But if C has a positive value the reaction will run farther towards X′ before the equilibrium point is reached. As it so runs P_B continually increases and P_A decreases. So long as P_A is greater than P_B, the fraction $\frac{P_B}{P_A}$ is less than unity, its logarithm is negative, and hence $-RT \log \frac{P_B}{P_A}$ has a positive value; but at the position where $P_B = P_A$, $\log \frac{P_B}{P_A} = \log 1 = 0$, and the curved line representing the change in the osmotic energy, crosses the base line for the osmotic energy set free at this point in the reaction is zero. From this point onward osmotic energy is absorbed instead of being given out in the reaction, for $\log \frac{P_B}{P_A}$ now becomes positive and goes on increasing in value, at first slowly, and later very rapidly as P_A becomes very small in the neighbourhood of X′ and the curved line becomes asymptotic to the ordinate. Hence at a certain point the distance of the curved line below the base line becomes equal to the distance above the base line of the horizontal line representing the positive value of C. Also the smaller the positive value of C the further from the end point will be the point of equilibrium.

The same reasoning applies if we start at X′ with the substance all in the form B, and proceed towards X. The diagram to suit progress in this direction is the mirror reflex in the base line of the one given for the opposite direction (X to X′). For if the value of C was positive in passing from A to B, it will be negative in passing from B to A, and value of $-RT \log \frac{P_A}{P_B}$ will be positive at the X′ end and negative at the X end, precisely as $-RT \log \frac{P_B}{P_A}$ was positive at the X end and negative at the X′ end. Hence the same equilibrium point is reached from whichever condition A or

B we choose to travel. The positions of the lines AA′ and – AA′ and of BB′ and – BB′ illustrate the effects of a small and a large value of C either positive or negative, and it is evident that a small value increases the distance of the equilibrium point from the end point, and hence increases the reversibility or incompleteness of the reaction.[1]

We learn accordingly that for a reaction of type $P_B = K \cdot P_A$ the smaller the chemical energy involved in the change from A to B, the more does the reaction become practically reversible. The same statement is also true, within certain limits, for other types of reaction.

Now the value of the chemical energy is not measurable experimentally, for heats of reaction as usually measured do not give either C or H of our equations, but instead the heat of reaction for a grm. molecule changed at varying values of P_A and P_B. This figure, which is the only experimental datum we possess, gives us an integration of a small fraction of H at each stage throughout the process.

However, the heat of reaction must vary somewhat in the same manner as C, and a small value of heat of reaction indicates a small value of chemical energy, and a large heat of reaction a high value of chemical energy.

Using this criterion as the best available, we find that experiment bears out the above conclusion. In all the typically reversible actions, such as the formation of esters, the polymerisation and hydrolysis of carbohydrates, and such reactions as we have seen above are reversible by enzymes, the heat of reaction is excessively low; so low indeed that it cannot be measured experimentally, since the effects obtained fall within the limits of experimental error.

Thus the heats of reaction for the formation of the following esters from their constituent alcohols and acids in rational calories are:—ethyl formiate, – 6 K; ethyl acetate, – 20 K; ethyl-hydrogen sulphate, – 49 K; amyl-hydrogen-sulphate, – 2 K; and ethyl oxalate, – 38 K. These amounts are scarcely measurable in the volumes of fluid in which they occur, and on account of the slowness with which the reactions take place, and in comparison with

[1] The shape of the curved line varies with the expression for the value of the osmotic energy, so that the effect of changes in low values of C is complicated to follow. But high values of C will always land the equilibrium point upon the asymptotic portion of the curve close to one or other of the two end points.

the total chemical energies of the substances reacting, as shown by combustion, are negligibly small. For example, the heat of combustion of ethyl alcohol is 3405 K.

The chemical energy required in the combination of hexoses to form disaccharides, a reaction which has been shown above to be reversible by enzymes, or in the formation of starches and fats from their proximate constituents, such as occur in the body cells, is also so small as to be incapable of measurement. But if we neglect small differences in heats of solution of the carbohydrates, we can obtain some idea of how small the heats of reaction are by taking the differences in heats of combustion of the substances on the two sides of the equation.

In the following equations for formation of the three best known examples of disaccharides from their constituent hexoses, the heat of combustion of each sugar is written below its formula, and the difference is given as the approximate heat of reaction in rational calories :—

1. $C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O - 31$ K.
   (*Glucose*) (*Fructose*) (*Cane-sugar*)
   6737 K + 6759 K = 13,527 K − 31 K.

2. $C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O - 33$ K.
   (*Glucose*) (*Glucose*) (*Maltose*)
   6737 K + 6737 K = 13,507 K − 33 K.

3. $C_6H_{12}O_6 + C_6H_{12}O_6 = C_{12}H_{22}O_{11} + H_2O - 81$ K.
   (*Glucose*) (*Galactose*) (*Lactose*)
   6737 K + 6696 K = 13,514 K − 81 K.

It is hence obvious how small the change in chemical energy, as shown approximately by heat of reaction, is in practically reversible reactions as compared with those reactions which run practically to completion.

The effect of change in temperature is *on the whole* opposed to that of change of chemical energy ; a rise in temperature having the same effect as a drop in chemical energy and tending to increase the reversibility of the reaction.

This is seen from the fact that the part of the constant K, which changes with alterations in chemical energy C and temperature T, is the expression $e^{\frac{C}{RT}}$, in which it is clear that similar changes

in C and T balance each other. In the simple form of reaction $P_B = K \cdot P_A$, illustrated in the diagram (p. 36), this is quite clear, for increase in T will cause similar increase in the expression $RT \log \frac{P_B}{P_A}$, and hence will place each point on the curved line farther from the base line, and so bring the equilibrium point, that is, the point where the curved and horizontal line lie at an equal distance from the base line XX', nearer to the mid point. Accordingly increase of temperature acts like low value of chemical energy and increases reversibility.

In the more complex forms of reaction, such as $P_B = K \cdot P^2_A$, &c., the form of the curved line representing change in osmotic energy varies; it is still asymptotic at both ends, but no longer crosses the zero line at the mid point between X and X'. Hence increases from zero in the value of C in one definite direction only, will up to a certain limit bring the point of crossing nearer the mid point, and so increase reversibility. Similarly, if the value of C is taken as fixed, increases in T up to a fixed limit will decrease the reversibility, but later for higher values will increase it. Hence in those cases where the number of molecules is altered in the reaction there is a certain given point in each case of change in C or change in T, reversal in the effect. For higher values of C or T, however, effects of increase of C to high positive or negative values is to throw the crossing farther along towards the asymptotic portion of the line at either end, and so diminish the reversibility; while effects of increase of T at higher values, C being fixed and moderately low, is to throw the crossings further from either end point and so to increase reversibility.

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CHAPTER III

VELOCITY OF REACTION, AND THE COMPARATIVE ACTION OF ENZYMES AND CELLS—EXPERIMENTAL OBSERVATIONS ON VELOCITY OF REACTION, AND THEIR DISCUSSION—ALTERATION OF CONCENTRATION OF ENZYME

The investigations of the conditions of equilibrium have shown that at a certain point in the reaction, at which the molecular concentrations or osmotic pressures of the different substances in solution and taking a part in the reaction bear a definite proportion to one another, there is no energy set free as a result of the reaction, and hence that the system is in equilibrium at this point. At all other points or stages in the reaction energy is set free as the result of the reaction, and hence the question arises why is a stationary position possible in the system at any other point in the reaction than the equilibrium point? If a system contains substances capable of reacting with one another and present at other concentrations than those of the equilibrium point, energy will be set free by any movement towards the equilibrium point; why then is the equilibrium point not instantly reached and the energy set free? Why, in some instances, is there a slow and measurable velocity of reaction towards equilibrium which may not be reached for days or weeks? Why, in other cases, is there no measurable movement at all towards the position of equilibrium, although the substances are left in solution for an indefinite time, until certain substances not permanently altered themselves in the slightest degree are added to the solution, and after the addition of such substances why does the reaction at once commence and continue until equilibrium is attained? That is, why do catalysts in general (and enzymes, which form a particular class of catalysts formed by the agency of living cells) induce reactions which cannot be shown to proceed in their absence, or cause reactions which proceed with infinite slowness to be hastened into a measurable velocity? Finally, in many reactions in living

cells, and notably in the anabolic processes of chlorophyll-containing cells of living plants, why does the reaction proceed *away* from the equilibrium point with storage of chemical energy, instead of energy being set free ?

There is no shadow of doubt as to the experimental facts which suggest these queries, all of which form a connected whole, and must receive their explanation on a common basis.

Sterilised solutions of cane-sugar, maltose, lactose, and starch can be preserved indefinitely without any measurable change into their simpler components. On the other hand, solutions in molecular proportions of the constituent hexoses which build up these more complex carbohydrates can similarly be kept in solution with no observable change for indefinite periods. There is hence no movement towards the equilibrium point from either side of that point. But let the appropriate catalyst be added under the proper conditions and at once the reaction towards equilibrium occurs.

Still more striking are the examples derived from the metabolism of living cells.

Carbon dioxide and water or water vapour may be left together indefinitely at such temperatures and physical conditions as obtain in the chlorophyll-containing cells, and no formation of organic compounds occurs. Similarly, the organic compounds produced can be left under like conditions, and no chemical changes are observed. But in the green plant cell, under the influence of the solar energy movement *directly away from the equilibrium point* of undirected chemical and osmotic energy takes place, and gives the origin to the whole system of vital processes on the planet.

The fundamental groundwork for an answer to the queries has been outlined in the introduction to this chapter. *For energy exchanges to occur it is necessary that there should be present the properly adapted machines or energy-transformers.* It is not sufficient that there should be differences in energy potentials in the system in order that a reaction may occur ; these give but the *possibility* for the reaction, which can never take place unless in addition conditions are present which allow the transference between the forms of energy possessing differences in potential.

The equations with which we have been dealing under the heading of chemical equilibrium teach us, that at other positions

than equilibrium, as a result of reaction energy will be set free, and the energy so set free is the driving force towards equilibrium, or a tendency towards chemical equilibrium. But whether there will be any movement towards equilibrium or the reverse, and the velocity of that movement, depend entirely upon how far there is opposition to such movement, by the resistance of other forms of energy present in, or brought to bear upon the system by any agency such as a catalyst, or upon how far such resisting forms of energy are diminished by the action of any such agent which may be present in the system.[1]

Chemical energy is not peculiar in this respect, and does not stand isolated from other forms of energy. Water standing in two reservoirs, unconnected by a channel through which the water can flow, will remain at a constant difference in hydrostatic pressure for ever, and if there be a channel of communication between them, the rate of flow, that is, the velocity with which the potential difference is equated out, will vary not only with the difference in potential but with the resistance to the flow of water in the connecting channel. Further, if there is a turbine, or properly constructed reversible pump, on the channel of communication between the two reservoirs, then the water as it flows from the higher to the lower level can be made to give out energy which by suitable transforming machines can be changed into any form of energy; or conversely, if external energy is supplied to the pump, at a sufficiently high potential, it can be made to work the pump in the opposite direction, and the absorbed energy can so be utilised to force the water in the opposite direction from the lower reservoir to the higher reservoir, so that the difference in hydrostatic potential increases instead of diminishing, as it would do if the system were not operated upon by other forms of energy from without.

Similarly in the case of electrical energy, if there is no path of conduction between two charged conductors at different potentials, there can be no equalisation of potential between the two conductors; if a path is provided the velocity of the energy

[1] Lest the reader should think that because these equations do not lead directly to expressions for the velocity of reaction that they are therefore useless, it may be pointed out, that they do give the conditions for equilibrium quite truly when no energy is imparted to the system from without. For although variations in resistance will alter the velocity with which equilibrium is reached, at the equilibrium point itself the velocity becomes zero, and the resistance has accordingly no effect upon the equilibrium point.

reaction varies with the resistance of the path; also by giving various forms to the conducting path, the electrical energy may be transformed into various forms; and lastly, if a sufficiently powerful dynamo be placed upon the conducting path and worked in the right direction, instead of the path being a means for equating potential it is converted into a means for heaping up difference in potential.

So also for any type of mechanical engine or motor, however driven; if the load is taken off, the engine races, as the load is increased the velocity lessens, and with a sufficiently heavy load the movement stops entirely. Always when the potential differences of the opposing forms of energy become greater than those of the form of energy driving the motor, the latter becomes ineffective and the engine must stop, or, if built so as to be capable of reversal, must run in the opposite direction.

Exactly similar reasoning applies to every known type of energy, and since the law of conservation of energy holds and a definite amount of chemical energy is equivalent to a definite amount of any other form, it is clear that the reasoning must hold for chemical energy also.

Hence we see that while the difference in chemical energy gives the driving force tending to cause chemical reaction, and a passage towards a definite point of equilibrium, there is present something in the nature of a resistance or load upon the engine, which determines by its amount whether a reaction shall occur at all, if it occurs the speed at which it shall occur, and according as the resistance is modified by other factors, the path of the reaction is determined and the very qualitative nature of the compounds formed by the reaction.

It is hence necessary for our purpose to inquire what is the nature of the resistance to chemical reaction, what are the forms of energy opposed to the reaction, and how is the action of these opposing forms of energy altered under different circumstances, so that the velocity of reaction becomes changed, the reaction stopped or its actual direction reversed, or finally the path of the reaction altered so that under different conditions, different products may be formed.

The obvious forms of energy opposed to chemical reaction are (1) molecular cohesion or chemical affinity, which must be overcome, before the molecule breaks up or is rendered capable

of reacting with another molecule, which also may require similar changes in its molecular constitution before it is fitted to react; and (2) the physical forms of energy which act between the dissolved molecule and its solvent, such as the velocity of movement of the molecule through the solvent, and, at any rate in the case of the colloidal molecule, probably surface tension at the surface between the dissolved molecule and its solvent.

Action between the dissolved molecule and its solvent, which must also come in as a factor in preparing for chemical reaction, or altering the resistance to chemical reaction, is seen in the different ionising powers of different solvents for salts, and in the different degrees of association of different dissolved substances in different solvents. For example, the ionisation of inorganic salts in water as compared to organic solvents, and the duplicate and higher molecular weights of organic substances in an associating solvent, such as benzol, compared with another solvent, such as alcohol. As proof of this connection, it may be pointed out that the more ionised a dissolved substance is the more quickly it reacts, showing that the molecular resistance to reaction has been broken down; and the very different velocities of reaction of the same substances in different organic solvents (see p. 132) must be due to corresponding differences in molecular resistance to reaction in these different media.

The first of these factors is that which will probably be most effective in entirely stopping a reaction, and the latter that which is effective in rendering reactions in solution as a general rule much slower than those which occur in gaseous mixtures.

It is by modifying the action of these opposing forms of energy, in some way,[1] that the catalyst or enzyme or living cell

[1] The mode of action is at present unknown to us, the hypotheses thereon will be given later (see p. 126); the important fact to realise here, apart from any hypothesis, is that the catalyst acts as an energy conductor or transformer for chemical energy, and varies the amount of energy necessary to be transformed in various other directions before the reaction can ensue, it makes the path easy between two forms of energy. Further, it may be pointed out that the *modus operandi* of energy-transformers lies always without the pale of our knowledge in the case of other forms of energy, just as much as in the case of chemical energy, where the matter has given rise to so much thought and discussion. We do not really know, for example, the mode in which an electric current heats a wire, or how an electric current magnetises iron, and no other substances; all we have is hypothesis, just as we have for how a catalyst produces its transformations of chemical energy.

produces its remarkable effect upon the duration and course of the reaction.

If the catalyst is not capable of acting upon external forms of energy, such as light energy in the case of the green plant cell, but can only utilise chemical energy and convert this into heat energy and osmotic energy, then the catalyst can only work towards the equilibrium point and not away from it. It can start a reaction held stationary by molecular attractions, or can modify a reaction already running by hastening or slowing it, but it must act in all cases towards the equilibrium point from either end. Also dependent upon the power of the enzyme, the reaction may be slowed and stopped at other points, usually corresponding to some definite stage in the reaction, which are called false equilibrium points (see p. 66). This follows, because as the reaction comes nearer the equilibrium point, the chemical potential aiding the catalyst in its work becomes less, and hence always the velocity becomes less, but also the resistance may become so great as to be insuperable for a particular enzyme, and the reaction may come to a dead stop. Take, for example, the relative action as catalysts upon starch solutions of dilute acid, and of diastase of malt. The diastase is powerless as a catalyst when all the starch has disappeared and been replaced by a mixture of dextrin and maltose, the resistances, for this catalyst, have been increased beyond the power it possesses and the reaction ceases. But the acid proceeds further, and converts the dextrin and also the maltose into dextrose; it possesses the power of breaking down the resistance which was insuperable for the diastase.

In both these cases, however, the movement is towards the point of chemical equilibrium, and it is by acting as a transformer of chemical energy into other forms that the catalyst does its work. The only difference is that at a certain stage the potential factor of the molecular energy of the substance being broken up, becomes too powerful for the diastase, and the reaction is stopped so far as that catalyst is concerned; but the more powerful hydrogen ion of the acid is still able to overcome the molecular cohesion, and to continue the reaction a stage farther.

But there are catalysts or transformers which can convert other forms of energy into chemical energy, and these form a distinct class from the others; for although they are similar to the former in not being themselves altered by the reaction they

induce, they differ in that they require external energy in order to do their work; instead of causing energy to be given out from the chemical system, they cause the system to take up energy, and instead of assisting the system towards the equilibrium point towards which it tends in their absence, they cause a movement away from the equilibrium point.

To this class of catalysts the living cells of plants and animals belong, and although the process is most clearly seen where chlorophyll is present, and is masked in other cells by preponderating action in the opposite direction, there is probably no cell in which anabolic processes do not occur, as shown by the building up, accompanied by storage of chemical energy, of complex organic substances, such as the organised proteid or protoplasm of the cell, and the granular deposits in the cell of amyloses, fats, and other reserve food-stuffs, from the soluble constituents of the plasma or of the circulating fluids by which the cell is nourished. In most types of cell the energy required for the anabolic processes is derived from chemical energy obtained by an oxidation process affecting a portion of the nutrient matter; the energy obtained from this reaction being used to run the anabolic reaction.

The linkage together in this way of a variety of complex chemical reactions is what distinguishes the cell as an energy-transformer from the simpler acting soluble enzyme, which is so often a product of its activity. Such linkage of reaction is never seen in the case of enzymes, which are exceedingly fixed and selective in their action (see p. 114). The enzyme acts usually upon one type of molecular arrangement only, often failing in attacking even the sterco-isomer, but the cell carries on a wide commerce of reaction with many types of matter, and modifies the reactions in many varied ways; and also differently at different periods according to its condition, and the manner in which it is affected by concurrent reactions taking place in other cells in the body, or by the influence of the nervous system upon it.

Actions similar to those of the cell in storing up chemical energy are also seen in physical transformers; an example of such is the synthesis of compounds by the electric current and the electrolysis of conducting solutions. Here the electrodes, two conductors at different potentials, act as energy-transformers for converting electrical into chemical energy.

The analogy between chemical energy transformations and those of other forms of energy is so clear and the action is so obviously of the same nature, that we may summarise the action of a catalyst or energy-transformer as follows: —

1. *The action of the soluble or unorganised catalyst or enzyme may consist* (*a*) *in commencing a reaction which does not proceed at all in its absence;* (*b*) *in altering the velocity of a reaction which does proceed in its absence, and such action may be positive, increasing the speed of the reaction, or negative, diminishing the speed of the reaction: but* (*c*) *the direction of reaction must always be towards the point of equilibrium, as defined in the previous section, because the enzyme does not yield energy itself, and is unable to act as a transformer to external energy, or to link two chemical reactions so as to obtain energy from one for the performance of the other.*

2. *The living cell as an energy-transformer, in addition to the actions* (*a*) *and* (*b*) *of the enzyme, can store up chemical energy, either by using energy in other forms and converting it into chemical energy, or by linking several reactions together and transforming the chemical energy obtained from one back to chemical energy which is stored up in others. Finally, the cell can modify its activities, and alter in its action as a transformer changing entirely the course of the reactions it induces and the products obtained, while the type of action of the enzyme is simple, selective, and entirely fixed.*

There is no doubt whatever that the cell makes use of the action of many intracellular enzymes for the chemical transformations it induces, but in all cases the action of such enzymes is adapted, controlled, and co-ordinated by the cell.

It is necessary to point out that the above view as to the action of enzymes is different in many essential points from the one which is usually accepted at the present time.

The currently accepted view is that a reaction which is influenced by catalysts is already proceeding in the absence of the catalyst, and that all the catalyst can do is to alter the speed of reaction, and bring the reaction more quickly or slowly to that equilibrium point which it would inevitably have come to in its own time in the absence of the catalyst.

The statement is based on the fact that the catalyst itself altered in the reaction, and hence neither takes nor gives out energy to the system, accordingly it cannot alter the amount of energy in the system, and must lead to the same equilibrium

point. For if the equilibrium were different in the presence and absence of the enzyme, then by working a cycle with the catalyst in the solution when removing the catalyst and excess product of activity, and replacing the [illegible] energy could continually be manufactured from nothing. Working in the opposite direction energy would be destroyed. Both which results are obviously contrary to the law of conservation of energy.

To this reasoning the following objections may be taken:—

1. The assumption is wrong that because the catalyst is not permanently altered in the process it cannot therefore take up or give out energy to the system, because it excludes (*a*) the possibility of the catalyst operating upon external energy, as is done, for example, by the chlorophyll-bearing cell; (*b*) the possibility of the catalyst using part of the chemical energy of the system, to run another reaction in which energy is absorbed, as is done by all living cells.

2. Even for catalysts in the restricted sense, there is nothing in the reasoning to show that the catalyst cannot take up and give out energy in a vibratile fashion, so that as a net result its own condition and amount of energy is unaltered, and its condition at the end is the same as at the beginning, and yet by this means can induce a reaction which would not occur at all in its absence. For example, an electro-motor is not altered at the end of a period of running from what it was at the beginning, but by intermediately taking up an amount of energy it is capable of converting a large amount of electrical energy into mechanical energy which would never have occurred if it had not been in the electric circuit. So when a chemical reaction is *absolutely* stationary on account of opposed molecular attractions present in the molecule having a tendency to react, the enzyme by imparting momentarily a small amount of energy to the molecule may overcome the molecular attractions, and in the break up of the molecule may receive back all the energy previously given out, so as to remain unchanged in the process.

Finally, as has been pointed out above, the presence of the catalyst may not cause the reaction to proceed completely to the equilibrium point, because as the reaction proceeds, and the concentration of the substance reacting changes, the potential tendency backing the action of the catalyst may fall to such a level that the energy in the first stage which the catalyst

is capable of yielding is insufficient to cause the cleavage to occur. Hence the reaction may cease at a different point with different catalysts (see p. 66).

The only point essential with the simple type of catalyst is that it shall work towards the equilibrium point; but it can cause movement towards that point not occurring before, alter velocity of reaction, and may fall short of reaching the equilibrium point.

The experimental evidence with regard to the action of enzymes is entirely in accord with the view expressed in this article.

If, as has already been pointed out, the appropriate solutions on which the digestive enzymes act be kept in sterilised condition, not the slightest change is observable in any one case, no matter how long the solution is preserved, but if the enzyme is added its effect is apparent in a few minutes.

Further, the nature of the reaction and of the products formed, as well as the relative amounts of the latter, are often determined by the nature of the catalyst added to the same medium. As, for example, in the catalysis of proteid by pepsin, trypsin, acid, and alkali respectively. Here, in all four cases, the products and their amounts are different. Are we, then, to suppose that all these different reactions to as many different equilibrium points are running concurrently in any given proteid solution, but at so slow a rate as not to be observable? It is an interesting theoretical speculation; but it would appear more probable that these different catalysts possess a specific affinity for attacking some definite molecular groupings in the complex proteid molecule, and in each case *started* a reaction which was not possible until that particular catalyst was present in the solution.

Equations for Velocity of Reaction

The various expressions deduced for the value of the energy set free in the reaction give us the driving agent in the reaction, but the unknown values of the resistances opposed to this, and the amount of the effect of catalysts upon them, render the velocity theoretically indeterminate from a knowledge of the energy set free in the reaction only. We have hence, in order to obtain formulæ for the expression of the velocity of reaction under different conditions, to introduce empirical constants to denote

the resistance to reaction of the substances concerned, or rather the reciprocals of these resistances (that is, the chemical conductivities). The velocity of change of each substance is then proportional to the product of the osmotic pressure or molecular concentration of that substance and the constant[1] which represents the reciprocal of the resistance to change. For (as is shown in deducing the conditions of equilibrium) the energy set free by the reaction will vary with the osmotic pressure, and the resistance is inversely proportional to the constant, and accordingly the product of these two is proportional to the velocity of reaction.

In all cases the tendency to react is proportional to the osmotic pressure or molecular concentration of each substance in solution, and this, the fundamental law of chemical kinetics, is called *the law of mass action*. When two or more substances tend to unite to form a single substance, the tendency on the part of each substance is by the law of mass action proportional to its pressure or concentration in the solution, and hence the velocity of formation of the combined substance will be proportioned to the *product* of the concentrations of the uniting constituents. Accordingly, in any equation of velocity, the velocity of formation of a substance may be set down as equal to the product of the concentrations of its constituents in the solution, multiplied by a constant (k) which represents, and is the reciprocal of, the resistance to the reaction. The value of k will hence vary with the amount of catalyst present, with the temperature and other factors which alter the resistance, but these factors being supposed kept constant, the value of (k) will remain constant throughout the reaction.

We are now in a position to state equations for the velocity of reactions.

Let two substances, A and B, in solution react to form two others, C and D, according to the equation

$$A + B \rightleftarrows C + D.$$

Further, let the molecular concentrations of the four substances (or their osmotic pressures which are proportional to these concentra-

[1] It will be shown later (p. 78 *et seq.*) that the assumption that the resistance is constant throughout the reaction is only an approximation, and that the resistance really varies somewhat with the ratio between enzyme and substratum.

tions) be represented by c_A, c_B, c_C, c_D, and the constant of reaction for conversion of A and B into C and D (as explained above) be k_1, and the constant for reaction in the opposito direction of C and D into A and B be k_2. Then the rate of formation of C and D is given by $k_1 \cdot c_A \cdot c_B$, and the rate of formation of A and B (that is, of disappearance of C and D) is given by $k_2 \cdot c_C \cdot c_D$. Hence the net velocity of formation of C and D is the difference of these two expressions, or, $k_1 c_A \cdot c_B - k_2 c_C \cdot c_D$. But the velocity of reaction is the limit of the change in concentration divided by the change in time when both change and time are infinitely small, that is, velocity $= -\frac{dc_A}{dt}$, the negative sign being used because c_A is decreasing.

Accordingly the equation for the velocity of reaction is

$$[1]. \qquad -\frac{dc_A}{dt} = k_1 c_A \cdot c_B - k_2 c_C \cdot c_D.$$

This equation holds, under the conditions as to constancy of resistance laid down above, throughout the course of the reaction, and hence if a, b, c, and d be the initial molecular concentrations of the four substances, and at the end of a time t, the molecular concentrations of A and B have changed by an amount $-x$, and become $a-x$, and $b-x$ respectively, while C and D have changed also by an equal amount $+x$, and become $c+x$ and $d+x$ respectively, then the equation becomes

$$[2] \qquad \frac{dx}{dt} = k_1 (a-x)(b-x) - k_2 (c+x)(d+x).$$

If the initial concentrations and the values of k_1 and k_2 are known, the course of the reaction can accordingly be determined, and the amount of x after any given time be determined by integration of the above equation, remembering that when $t=0$, $x=0$. The values of k_1 and k_2 can be determined by making measurements at sufficiently close intervals of the value of x at different times during the reaction, the initial concentrations being known and substituting in the equation.

The ratio of the two constants k_1 and k_2 in the equation for velocity of reaction is equal to the value of the constant K of the equation of equilibrium, for at equilibrium $-\frac{dc_A}{dt}$ is zero, because no change is occurring in the substance, therefore from the equation $-\frac{dc_A}{dt} = k_1 c_A \cdot c_B - k_2 c_C \cdot c_D$, we have at equilibrium $k_1 c_A \cdot c_B - k_2 c_C \cdot c_D = 0$,

or $$\frac{c_C \cdot c_D}{c_A \cdot c_B} = \frac{k_1}{k_2},$$

but in the equation of equilibrium

$$\frac{c_C \cdot c_D}{c_A \cdot c_B} = K,$$

therefore $$K = \frac{k_1}{k_2}.$$

It follows from this that the resistances k_1 and k_2 are the same respectively in whichever way the reaction is going, and that anything which slows or hastens respectively the reaction in the direction $A + B \rightarrow C + D$ must also equally slow or hasten it in the direction $A + B \leftarrow C + D$.

Hence a catalyst or enzyme which at one set of concentrations increases the velocity of a reaction in one direction must equally hasten it at another set of concentrations in the opposite direction. In other words, all catalytic action must be reversible, although in most instances the equilibrium point lies so near one end that the action of the enzyme on the velocity of reaction in one of the directions cannot be demonstrated experimentally.

The equation for the velocity of reaction between two pairs of substances A, B and C, D, given above, can be much simplified, if A and B be present in the proper molecular concentration for reacting upon each other at the commencement, and C and D be initially absent. For then the initial molecular concentration of A and B will be the same; let it be represented by a and let x be the change in molecular concentration at time t after the commencement of the reaction. Then the equation for the velocity of change in x at time t, by using equation [2] and remembering that $a = b$, and $c = d = 0$ becomes

$$\frac{dx}{dt} = k_1 (a - x)^2 - k_2 \cdot x^2 \qquad [3].$$

Similar equations for the velocity of reaction can be deduced by using the same processes of reasoning, for the other types of reaction which have been discussed under the heading of equilibrium.

For example—

1. For a single substance undergoing conversion by molecular rearrangement into another single substance, such, for example, as an isomeric change :—

$$\frac{dx}{dt} = k_1 (a - x) - k_2 (b + x),$$

where a and b are the original molecular concentrations of the substances A and B, and x the change in molecular concentration at time t. This equation obviously takes the form

$$\frac{dx}{dt} = K' - K'' x.$$

2. For a single substance (A) undergoing conversion into two substances (B and C), the common type of action of enzymes and many other catalysts :—

$$\frac{dx}{dt} = k_1 (a - x) - k_2 (b + x)(c + x),$$

where a is molecular concentration of single substance, b and c those of substances into which it is converted, and x the change in concentration in time t. If, as is usually the case, B and C are absent in the beginning, and a still stands for the initial concentration of substance A, then the equation obviously simplifies to

$$\frac{dx}{dt} = k_1 (a - x) - k_2 x^2 \qquad [4].$$

3. For completeness we may add the formula deduced at length above, for when two substances (A and B) react to form two others (C and D)—

$$\frac{dx}{dt} = k_1 (a - x)(b - x) - k_2 (c + x)(d + x).$$

When initially $a = b$ for A and B, and $c = d = 0$ for C and D, then the equation becomes

$$\frac{dx}{dt} = k_1 (a - x)^2 - k_2 x^2 \qquad [5].$$

The integration of the above equations of velocity is necessary in order to obtain x, the quantity of the substance (or substratum) changed in a given time t by the action of any catalyst, which is the quantity usually observed in experiments on reaction velocity, and is used to determine the constant or constants of reaction. Such integration is, however, difficult, and leads to complicated expressions for the value of x, on account of the presence of the second member on the right-hand side prefixed by the negative sign. Now this expression, which introduces the difficulty, arises from the supposition that the reaction is reversible, it is the expression in the equation which represents the tendency of the substances to react in the reverse direction from right to left instead of from left to right.

Accordingly, if the degree of reversibility is small, that is, if the equilibrium point lies close up to the end point corresponding to the substances being practically all converted into the forms represented on the right-hand side of the equation of reaction, the value of the second expression on the right-hand side of the equation of velocity becomes very small compared to the value of the first expression, and may be neglected. That is, the reaction may be taken as running irreversibly, and this is what is usually done in obtaining the equations for the velocity of most reactions which concern us.

This means that in those equations which run almost to completion, the value of the constant k_2 is small compared to k_1. Now the constants are the reciprocals of the resistances to the reaction running in the two opposed directions, and hence this means that the resistance to such a reaction running from left to right is small compared to the resistance for it running from right to left.

It must be pointed out, however, that in the later stages of the reaction running from left to right, when $a-x$ has become very small compared to x, although k_1 is large compared to k_2, the second expression may cease to be negligible, and hence although the equation obtained by neglect of the second expression, may truly represent the course of the reaction throughout the greater part of its length, there may be a difference between observed and calculated results at the later stages of the experiment.

The discrepancy will be less, the lower is the initial concentration of the substratum, and, as we have seen in speaking of equilibrium in dilute solutions of type 2, where one substance is resolved into two others, the equilibrium point lies close up to complete resolution into the two substances. Accordingly for this type of reaction in dilute solution, which includes all the digestive processes, the second expression can be allowed to drop without sensible error.

In concentrated solutions, for this type of reaction, as the reaction approaches completion, and the *tendency* to reversion begins to become potent, the velocity of reaction must, however, fall off, and the velocity constant calculated by neglecting the reversibility expression ($k_2 x^2$) must fall off in value, as it is actually found to do by experiment.[1]

This important fact has been neglected by most experimenters, and the drop in velocity has been attributed to combination between the catalyst and the products of reaction. This explanation of the effect of products of reaction in slowing the reaction is no doubt

[1] See p. 72.

experimentally correct, for the enzyme does combine with the products or one of them. But such combination is also the preliminary stage in the process of reversal, and the ferment must equally combine with the substratum when the reaction is running from left to right. The subject will be returned to when the results of experiment upon strong solutions of carbohydrates are considered; for the present, with this word of warning as to the danger of neglecting the reversal factor in such experiments, we may proceed to the derivation of the equations, connecting x the quantity converted in time t, and the velocity constant, when the second expression is neglected as small in value. Since the second constant falls away in this process, we can replace k_1 by k, when the typical equations become:—

Nos. 1 and 2. Where a single substance undergoes change into either one or two others:[1]—

$$\frac{dx}{dt} = k\,(a - x).$$

In words, this means that the velocity of reaction is proportional to the molecular concentration, at the moment, of the substance undergoing change.

The above equation can be written

$$\frac{dx}{a - x} = k\,dt,$$

this when integrated gives $-\log \text{nat.}\,(a - x) = kt + \text{const.}$, and since when x is 0, t is 0, the constant is $-\log \text{nat.}\,a = \text{const.}$ Subtracting, we get $\log \text{nat.}\,a - \log \text{nat.}\,(a - x) = kt$,

or
$$\log \frac{a}{a - x} = kt,$$

and, for the value of the constant of reaction,

$$k = \frac{1}{t} \log \frac{a}{a - x}.$$

The curve showing the quantity of substance changed in different times, for such reactions in which only one substance undergoes change, is accordingly a logarithmic curve.

[1] Or indeed any number of others; the same equation would hold, for example, for a triglyceride, breaking up into three molecules of fatty acid and one of glycerine. This arises because there is only one substance changing on the left-hand side, and the back action of those on the right side is negligible.

Such reactions are termed mono-molecular, and include all those reactions induced by enzymes, in which a single substance breaks up into two or more, and the quantity of ferment active throughout the reaction remains constant.

No. 3. Where two substances on the left-hand side of the equation of reaction react to form two (or more)[1] substances on the right-hand side.

Here the velocity equation becomes

$$\frac{dx}{dt} = k\,(a-x)\,(b-x) \qquad [1],$$

or if the substances concerned (A and B) are in equimolecular concentration a at the outset—

$$\frac{dx}{dt} = k\,(a-x)^2 \qquad [2].$$

The first equation [1] may be written

$$\frac{dx}{(a-x)(b-x)} = k\,dt,$$

and this

$$\frac{1}{a-b}\left(\frac{dx}{b-x} - \frac{dx}{a-x}\right) = k\,dt,$$

on integrating this equation yields

$$-\frac{1}{a-b}\Big[\log(b-x) - \log(a-x)\Big] = kt + \text{const.}$$

To obtain the value of the integration constant, we have when $t=0$, that is, at the start of the experiment, $x=0$, for x is the amount changed at time $=t$, and hence when $t=0$, $x=0$. Therefore, substituting these values in above equation, we get

$$-\frac{1}{a-b}\Big[\log b - \log a\Big] = \text{const.},$$

and subtracting the two equations we obtain

$$\frac{1}{a-b}\Big[\log(a-x) - \log(b-x) + \log b - \log a\Big] = kt,$$

which is

$$\frac{1}{a-b}\log\frac{b\,(a-x)}{a\,(b-x)} = kt,$$

giving for the velocity constant (k)

$$k = \frac{1}{(a-b)\,t}\cdot\log\frac{b\,(a-x)}{a\,(b-x)}.$$

[1] As before, the number of substances on the right-hand side has no effect if these do not react back on the progress of the reaction from left to right.

The second equation [2] of p. 59 may be written

$$\frac{dx}{(a-x)^2} = k\,dt,$$

or since $dx = -d(a-x)$

$$-\frac{d(a-x)}{(a-x)^2} = k\,dt,$$

which on integrating yields

$$\frac{1}{a-x} = kt + \text{const.},$$

as before when $t=0$, $x=0$, hence $\frac{1}{a} = \text{const.}$, and on subtracting the two equations

$$\frac{1}{a-x} - \frac{1}{a} = kt,$$

or

$$\frac{x}{a(a-x)} = kt,$$

and for the reaction constant (k)

$$k = \frac{x}{t \,.\, a \,.\, (a-x)}.$$

These reactions, where two substances undergo change in concentration on the left-hand side of the equation of reaction when the reaction is proceeding from left to right, are spoken of as *bi-molecular reactions*. The best known examples are where an ester, containing two monad radicles, such as ethyl-acetate, is saponified by an alkali. The reaction is different from that with an acid, for the alkali as well as the ester is changing its concentration during the reaction, while in the hydrolysis by the acid, the hydrogen ion concentration which affects the change in the ester remains constant, and the water produced being merely added to the water of the solvent has no effect on the progress of the reaction.

Tri-molecular reactions and higher are rare, from the tendency of the reaction to break down into stages. For a tri-molecular reaction, in which three molecules react together on the left-hand side of the equation, the equation of velocity, supposing the three substances present in equi-molecular proportions at the outset, would be

$$\frac{dx}{dt} = k(a-x)^3,$$

which on integration yields

$$k = \frac{1}{2t} \cdot \frac{x(2a-x)}{a^2(a-x)^2};$$

and very similar equations can be evolved for equations of a higher order.[1]

Experimental Observations on Velocity of Reaction Induced by Enzymes

We may now pass on to the examination of the experimental results on chemical kinetics, and to the investigation of the extent to which such observations are in accord with the equations theoretically deduced above.

At the outset we are met by great apparent divergence of experimental results, and different statements by different observers. More recent work has, however, tended to show that the apparent discrepancies are due to different observers having worked at different portions of the reaction, that there are several different factors involved which disturb the velocity of the reaction when there is not due attention given to the relative amounts of catalyst and the substratum on which it acts, or to the conditions obtaining at the extreme ends of the reaction.

In the case of the enzymes it has recently been shown, particularly by the researches of Horace Brown and Glendinning and of E. F. Armstrong, that the simple logarithmic law deduced above holds only at some distance from the beginning or end of the reaction, and when there is a due proportionality between ferment and substratum.

The experiments on velocity of reaction may be divided into :— (1) those in which, with a constant amount of catalyst throughout the experiment, the course of the reaction is followed from the initial point at which only the substratum or substance upon

[1] For an n-molecular equation the equation of velocity is

$$\frac{dx}{dt} = k(a-x)^n,$$

which yields on integration

$$k = \frac{1}{(n-1)t} \cdot \frac{a^{n-1} - (a-x)^{n-1}}{a^{n-1} \cdot (a-x)^{n-1}}.$$

which the catalyst acts is present, to the end-point at which the substratum has been as completely converted into the products of reaction as is possible under the conditions of experiment; (2) those in which the effects upon the velocity of varying the concentration (a) of ferment and (b) of substratum are observed in the earlier stages of the experiment; and (3) those in which the effects of addition of one or other of the products of reaction at the initial stage are studied, or the effect of addition or removal of such products upon the end-point or apparent equilibrium point of the reaction. The experimental measurement in all cases is that of the quantity or percentage of the substratum converted in a given time, and this has been measured in many different ways. The enzymes which have been most employed have been those which act upon starches or sugars, that is, the amyloclastic and sucroclastic enzymes in the terminology of H. E. Armstrong,[1] but recently an important paper has been communicated by Bayliss on the velocity of reaction caused by trypsin in different proteid solutions. Determinations of the rate of change in the sugar solutions have been made chiefly by the polarimeter, but estimations by various modifications of Fehling's method have also been employed. In the case of trypsin, Bayliss has used the method of determining change in electrical conductivity.

The results of experiment may be stated either in the form of a curve in which the co-ordinates represent time from the commencement of the experiment and percentage of substance converted, or by calculating the values of the constant of reaction (k) at each period at which a determination is made.

The velocity of reaction induced by inorganic catalysts, and particularly by acids and alkalies, has been the subject of many researches which cannot here be reviewed. It must suffice to state that the results follow fairly closely the formulæ deduced above, as shown by the close values obtained throughout the experiment for the value of the constant of reaction deduced on the basis of the formula.

[1] It is pointed out that the older terminology, amylolytic, &c., is open to confusion with electrolytic, hydrolytic, &c., because amylolytic meant that the starch is split up, while electrolytic does not mean a spliting up of electricity but by means of electricity, hence the author suggests the more rational terms, amyloclastic, &c.

In the case of dilute acids, acting upon esters or upon disaccharides, the reaction is mono-molecular, and the curve showing the ratio of percentage conversion to time of reaction is as the formula indicates, a logarithmic curve.[1]

The velocity is proportional in each case to the dissociation of the acid, that is to say, to the concentration of the hydrogen ions, but the proportionality is by no means exact. Thus a 0·5 normal hydrochloric acid inverts cane-sugar at 6·07 times the rate of a 0·1 normal solution, although its concentration in hydrogen ions is only 4·64 times as great. Hence the negative ions of the acid must also possess a certain secondary action in increasing the power of the hydrogen ions as catalysts, as suggested by Arrhenius. That this is the cause of the discrepancy is further shown by the action of the ions of the neutral salt when present along with the acid, for although the neutral salt alone does not catalyse, the presence of an equivalent amount of the neutral potassium salt of the acid used increases the rate of catalysis by the acid alone by about 10 per cent.

An application of this inverting power of the acid or hydrogen ion, as a test for free hydrogen ions or acidity, is of great importance to the biological chemist. The method is of highest value when dealing with a secretion of acid reaction,[2] where it is important to determine whether and in how far the reaction may be due to the presence of a feebly dissociated acid, such as carbonic, acetic, lactic, &c., or to a strongly dissociated acid such as hydrochloric.

Here the usual methods of titration of the acid entirely fail; for example, a deci-normal solution of acetic acid will give on titration in presence of an indicator the same acidity as a deci-normal solution of hydrochloric acid. But the real effective acidity of the two solutions is entirely different, the acetic acid only possessing about 3 per cent. of the hydrogen ion concentration of the hydrochloric acid, and being in consequence for most physiological purposes correspondingly weak in its action.

[1] See, however, Armstrong and Caldwell. These authors find with very dilute acid that there is an initial period, in which the amount converted increases more than proportionally to the logarithmic law, indicating an approach to a linear law.

[2] A similar application can be made in the case of alkaline solutions, in determining by inversion the concentration of the alkali, or hydroxyl ion.

Could the two acids be obtained in pure solution the determination of their relative effective acidity could easily be made by measuring their relative conductivities; but as the physiological chemist has to deal with them, as, for example, in the gastric juice, they are present in solution with inorganic salts of high conductivity, such as sodium chloride, and hence the electrical conductivity method fails.

It is just here that the method of determining concentration of hydrogen ions and corresponding effective acidity, first suggested by Ostwald and carried out experimentally by F. A. Hoffmann, becomes of such immense value in enabling a determination of this important point to be made in gastric juice.

The best substratum to employ is methyl-acetate, and in using the method recently in a large number of pathological cases for the determination of the amount, and, by means of control with ordinary titration methods, the nature of the acid in the gastric contents, the writer has found it a most reliable method.

The recognition of the fact that it is not the total amount of acid or alkali in a secretion or body fluid, as shown by titration with an indicator, which confers upon the fluid its activity or modifies its activity as a catalyst, or as an active agent upon living cells, but rather its effective concentration in hydrogen or hydroxyl ions, is of the highest importance, and the supplying of methods for determining such factors, of which an example has been shown above, is one of the most important services that physical chemistry has rendered to biology.

On passing from the action of the simpler catalysts, such as acids, alkalies, and inorganic salts, to the enzymes, we find that the disturbing elements, of which we have had some evidences above in the action of the negative ion, and of neutral salts, in effecting the catalytic power of the hydrogen ion, become more predominating, and often, especially at certain stages in the reaction, the velocity does not experimentally obey the logarithmic law at all, although the reaction is quite clearly a mono-molecular one.

As a result of measurements with different strengths of solutions and of enzyme, and of the experiment only being carried through the earlier stages by some observers, while others carried on observations until the reaction came to a stand-

still, very different expressions were obtained for the law of velocity of reaction of the same enzyme upon the same substratum, and it is only recently by the observations of Henri, Horace Brown and Glendinning, E. Frankland Armstrong, and Bayliss that we are beginning to be able to understand the results, and to bring the different observations into accord with one another.

O'Sullivan and Tompson were the first observers who studied the velocity of action of an enzyme quantitatively throughout the course of the reaction.[1] They employed the action of invertase on cane-sugar, and found that the reaction was mono-molecular, obeying the mass action law, and giving a logarithmic curve. Henri, however, who later worked at the same subject, found that the value of the constant K, deduced from their figures, by using the formula deduced above (p. 60), $\frac{1}{t} \log \frac{a}{a-x}$, did not remain quite constant throughout the reaction, but slowly increased in value in the ratio, for example, of 298 near the beginning to 332 near the close of the reaction, thus showing that the velocity of reaction only approximated to the logarithmic law.

Tammann, in a series of researches, investigated not only the action of invertase on cane-sugar, but of emulsin on different glucosides (salicin, amygdalin, arbutin, æsculin), and found that the reaction never proceeded to completion. He observed that the velocity of reaction was retarded in increasing amount by the presence of the products of reaction as these accumulated in solution. The percentage which remained unconverted varied with the temperature, the concentration of ferment, and the concentration of substratum. Increasing the temperature caused the reaction after it had come to rest at the lower temperature to recommence and proceed further towards completion. With a constant quantity of enzyme (emulsin) increased concentration of substratum (amygdalin) increased the total quantity converted, although not proportionately, the percentage conversion being diminished; also, addition of sub-

[1] That is to say, the progress of the reaction when a definite amount of enzyme had been added initially; the effects of variation in amount of enzyme acting for equal times had previously been studied by Brücke, Schütz, and others.

stratum after the reaction had ceased caused a fresh quantity to be converted.

Removal of the products of conversion also had the effect of causing conversion of further quantities. This last result is confirmed by other observers.

These results of Tammann with regard to the alteration of the position of the incomplete end-point by precisely such factors as influence a true equilibrium point, are interesting in view of the usual statement that the point of rest in such incomplete reactions are so-called *false* equilibrium points. That the equilibrium point is false in the sense that it cannot be reached by the same catalyst working in the reversed direction must be admitted, but it is a false equilibrium point in this sense only; and as far as the entire system, *including the catalyst*, is concerned, for the given concentrations and temperature it is in true equilibrium at this point, else why should the system come permanently into rest and the reaction cease? As has been pointed out above, the action of the catalyst is to diminish the resistance opposed to the driving force due to energy set free in the reaction. Now, as the system approaches the true or absolute equilibrium point which it would reach in the absence of resistance, the driving force diminishes, and the movement against resistance will cease at a point, dependent upon the power of the catalyst in diminishing the resistance, short of the equilibrium point for no resistance in no matter which direction the reaction is proceeding.

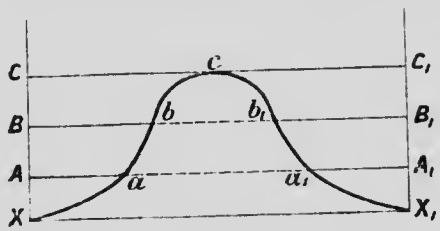

FIG. 3

The matter may be illustrated graphically, as in the annexed diagram. Let the horizontal line XX′ represent the path of the reaction, the substance being supposed to be all in one form at X and all in the other form at X_1, at intermediate points varying percentages are in the two forms. Let the curved line, by its height above XX′, represent the opposition to reaction[1] at each stage. Then the opposition

[1] That is $\frac{R}{P}$, where R is resistance and P the potential tending to reaction of which R remains approximately constant while P continually diminishes as the equilibrium point is approached, hence, as shown in the diagram, the opposition to reaction $\frac{R}{P}$ increases as the equilibrium point is approached.

where it is low will be overcome up to a certain distance from either side, dependent upon the power of the catalyst. A catalyst of sufficient power will carry the reaction up to the same point, in no matter which direction the change is going, *this* point is what is ordinarily called the equilibrium point or true equilibrium point. But for a less powerful catalyst the diagram shows that there will be two points of equilibrium, one on each side, according to the direction in which the reaction is proceeding, each falling short of the true or absolute equilibrium point. For example, a catalyst which by reason of its properties and concentration has a power represented as at AA′, will when the reaction is proceeding in the direction from left to right (X to X′) carry the reaction up to the point a, and the system will be in equilibrium there, and when the reaction is proceeding in the opposite direction (X′ to X) will carry the reaction up to a^1, and leave the system in equilibrium there. Hence it is quite erroneous to speak of these equilibrium points in incomplete reactions as *false* equilibrium points, for under the given conditions the system is as truly in equilibrium as it would be if all resistance to reaction were removed and it had reached the absolute equilibrium point in the absence of a catalyst.

Barth, and later Duclaux, found that in the earlier part of the reaction induced by invertase upon cane-sugar, when moderately concentrated solutions were used, that the amount of the sugar hydrolysed was not proportional to its concentration. Thus Barth observed that using the same amount of invertase, and varying the concentration of sugar, that between 5 per cent. of sugar and 15 per cent., practically the same amount was hydrolysed in equal time. Below 5 per cent. the amount hydrolysed increased with the concentration, but not proportionately, so that the percentage hydrolysed was less, and with percentages higher than 15, the absolute amount hydrolysed actually fell off.[1] Duclaux further showed that with the same concentration of enzyme and sugar, the amount hydrolysed up to the point at which 20 per cent. had been inverted was simply proportional to the time, so that the curve representing the progress of the reaction up to this point was a straight line and not a logarithmic curve. At a

[1] This is just the opposite result to that obtained in the case of hydrolysis by acids; here the amount hydrolysed increases more than proportionately to the increase in concentration of the sugar, so that the value of the constant K is increased. See E. F. Armstrong, "Proc. Roy. Soc.," 1904, vol. 73, p. 530.

later stage, as a result according to Duclaux of the retarding action of the products of reaction, the curve began to obey the logarithmic law. Henri later showed, however, that even in this portion Duclaux's results do not give the logarithmic law, the constant K all the time increasing with the progress of the reaction.

In an extensive series of experiments on the inversion of cane-sugar by invertase, Henri showed that the value of K calculated on the basis $K = \frac{1}{t} \log \frac{a}{a-x}$ continually increased throughout the reaction.

He based, on the fact that the value of the constant increased with the percentage of invert sugar, an empirical formula in which the constant K was replaced by $K_1\left(1 + \epsilon \frac{x}{a}\right)$, so that the equation for velocity of reaction became $\frac{dx}{dt} = \epsilon\left(1 + \epsilon \frac{x}{a}\right)(a-x)$, leading on integration to $K_1(1+\epsilon) = \frac{1}{t}\left[\log \frac{a}{a-x} + \log\left(1 + \epsilon \frac{x}{a}\right)\right]$. Calculating the value of the empirical constant ϵ from his experimental results, Henri found that it varied within narrow limits around the value unity, and hence the above formula simplifies to $K_1 = \frac{1}{2t} \log \frac{a+x}{a-x}$.

The formula utilised by Henri was that deduced by Ostwald, to satisfy the condition that the products of reaction have an *accelerating* influence. But the accumulation of products of reaction, or their addition at the beginning of the reaction, in the case of the reaction of invertase upon cane-sugar, as shown experimentally by Henri himself, retard the reaction instead of accelerating it. The formula can hence only be taken as an empirical one, and not as showing that the increase in velocity above the logarithmic value is due directly to the products of reaction. The formula, however, as demonstrated by Henri, gives a close approximation to a constant both for his own results and those of earlier experimenters, and hence is to be preferred to the simple logarithmic formula.

Henri showed experimentally that the invertin is not attacked or altered during the reaction. He also demonstrated that the

retarding action of the products of reaction is due almost entirely to the fructose and not to the glucose, a result of great interest in view of E. Fischer's discovery that it is only those disaccharides which yield fructose on hydrolysis which are acted upon by invertase, and showing that it is the fructose to which the enzyme is adapted, probably by possessing such a chemical constitution that it forms an unstable, incomplete, equilibrium compound with that particular hexose.

Henri further examined the action of emulsin upon salicin, and here found, contrary to the preceding case, that the velocity of reaction was less than that indicated by the logarithmic law; while in the case of the action of amylase upon starch, which he also tested, he found that the law was closely obeyed.

In addition to the empirical formula given above, Henri has also deduced a somewhat complicated equation on a theoretical basis, which yields for all the three enzymes worked with a fairly close concordance with the experimental results obtained.

Henri supposes that the enzyme is in part free, in part combined with the hydrolyte or substratum, and in part combined with one of the products of reaction. Further, that the latter portions in combination, are unstable and determined by the usual equations of equilibrium.

Utilising these equations, and on the supposition that either the free ferment or the portion combined with the cane-sugar is responsible for the reaction, the following expression can be deduced for the velocity of reaction—

$$\frac{dx}{dt} = \frac{K\,(a-x)}{1 + m\,(a-x) + n\,x},$$

in which m and n are the two equilibrium constants of the two compounds of the ferment with the cane-sugar and fructose respectively.

This equation on integration yields

$$K = \frac{a}{t}\left[(m-n)\frac{x}{a} + n \log \frac{a}{a-x}\right] + \frac{1}{t} \log \frac{a}{a-x}.$$

From his experimental data Henri deduced the values $m = 30$, $n = 20$. In the case of emulsin acting upon salicin, where the velocity of reaction is less than that demanded by the simple logarithmic formula and continuously decreases as the reaction advances,

according to Henri, the equilibrium constants for the compounds between the enzyme and the two bodies to which it is united are the other way round in relative magnitude ($m = 40$, and $n = 120$); and on taking these the value of the constant does not decrease but varies slightly around a mean value. For amylose acting upon starch, where the logarithmic law is followed,[1] according to Henri, the value of m and n are equal. It must be admitted that this ingenious hypothesis and formula of Henri's are capable of explaining the deviations on both sides from the logarithmic law; but they do not furnish an explanation of the experimental observations of E. R. Armstrong,[2] to be described later, that there is first a linear period, then a period when the velocity constant calculated by the simple logarithmic formula increases, and finally a period when the constant decreases.

An interesting difference in action between diastase of malt and the pancreatic juice obtained by the action of secretin is noted by Henri. It has usually been stated that the same total amount of starch is hydrolysed by diastatic enzymes in the same time independently of the concentration of starch in the solution; but Henri found that this only holds for more concentrated solutions of starch, and the limit at which the velocity of hydrolysis becomes constant varies in the case of the different diastases. Thus, in the case of diastase of malt, the quantity hydrolysed increases with the concentration of the starch solution until a strength of 0·75 per cent. has been reached, after which the amount hydrolysed in a given time becomes constant; while in the case of the amylose of the pancreatic juice the maximum conversion is not reached until the concentration passes 2 per cent. The result may be due either to a greater concentration of enzyme in the pancreatic juice, or more probably to a more powerful enzyme.

Variation in the concentration of cane-sugar in the case of invertase gave the result that in dilute solutions the velocity increased with the dilution; in solutions of intermediate strength (3·6 to 18 per cent.)[3] the velocity is independent of the concentration: while in more concentrated solutions the velocity actually

[1] See, however, Horace Brown and Glendinning.

[2] Armstrong points out that a similar effect is seen in the measurements by Horace Brown and Glendinning.

[3] That is, 0·1 to 0·5 normal in cane-sugar.

falls off. These results are similar to those of Barth mentioned above. Similar results with regard to the variation of the hydrolyte in concentration were also obtained for the action of emulsin on salicin.

In accord with O'Sullivan and Tompson, Henri found that within limits in which the enzyme was not too concentrated the amount of hydrolysis in the case of invertase and cane sugar was directly proportional to the concentration of the enzyme. The laws governing the velocity of reaction have also been studied by Adrian Brown in the case of zymase for conversion of glucose into alcohol, and for the action of invertase upon cane-sugar. In the case of the alcoholic fermentation it was found by this author that the velocity of reaction was not represented by a logarithmic curve, but by a straight line, that is to say, the velocity of reaction was constant. On the other hand, it was found, as in Henri's experiments, that the velocity of reaction in the case of invertase and cane-sugar increased more rapidly than it ought on the basis of the logarithmic law, the value of $K = \frac{1}{t} \log \frac{a}{a-x}$ increasing steadily throughout the series. Adrian Brown also obtained similar results to those of Barth, Duclaux, and Henri for variations in concentration of the cane-sugar in not too dilute solutions, the amount converted showing a constant weight and not a constant proportion for equal times. To explain this result, he supposed that a compound is formed between enzyme and sugar which persists for an appreciable time,[1] and that as a result a molecule of enzyme can effect only a limited number of complete molecular changes in unit time. Accordingly, whatever the available concentration of sugar may be at any given instant, no increase of conversion above a fixed maximum can occur. It is hence only when the concentration of the sugar falls below a certain definite level relatively to the amount of enzyme present that the amount of conversion can fall below this maximum, and the velocity of reaction can begin to obey the logarithmic law. It is hence only in dilute solutions (com-

[1] It is difficult to see why an appreciable time is insisted upon; all the reasoning follows whatever be the time-interval of the combination, from an hour to a millionth of a second; the assumption required really is that the time-interval of the combination shall be constant in all cases, no matter how the concentration varies.

pared to the amount of enzyme) that the amount of sugar converted per unit time can be proportional to the amount present, and this condition Adrian Brown found to be experimentally realised.

It is clear that this conception of A. Brown's is really coincident with that of Arrhenius of the "active mass," applied later by E. R. Armstrong to this reaction.[1]

The catalysis of starch by the action of diastase was next studied by Horace Brown and Glendinning, who showed that the velocity curve in this case also is at first represented closely by a straight line, but later approximates to the logarithmic curve. These authors also assume a combination between the enzyme and its substratum, and that at first the concentration of enzyme is small compared to that of the substratum. As before, as long as the amount of hydrolyte is large, the amount of combined hydrolyte and enzyme will remain constant, the amount therefore converted in unit time will remain constant, and the velocity curve will be a straight line. Later, when the concentration of hydrolyte falls off, the amount in combination will begin to vary directly as the concentration at any moment of the hydrolyte and the logarithmic law will begin to hold.

It remained for E. R. Armstrong to demonstrate experimentally that there is a third phase in the course of the same reaction, succeeding the period at which the constant calculated on the simple logarithmic basis had been increasing, in which the constant begins to diminish. This later stage is, according to Armstrong, due to a removal of enzyme by combination with one of the products of reaction.

The experiments of E. R. Armstrong were made upon the action of the enzymes, lactase, emulsin, and maltase upon lactose and maltose, and show clearly that there are stages in the reaction varying in extent with the particular enzyme and hydrolyte, and with their relative and absolute concentration, in which the curve of velocity is (1) rectilinear, (2) logarithmic, and (3) falling off from the logarithmic curve. To explain the results, the author takes as his basis the "active mass" hypothesis and the formation of combinations of the enzyme both with the substratum and with one of the products of reaction. The

[1] *Vide* infra.

conception of an "active system" is introduced on the hypothesis that the enzyme acts upon a portion of the sugar only at any given time, if S be the total amount of sugar, and s that portion which is in combination with an amount of enzyme e; "it will be convenient to speak of the combination $s+e$ as the *active system*."

The author divides the consideration of the question into four sets of conditions, which may be given in his own words, as follows:—

"*Case I.*, in which, whatever the amount of sugar present, the quantity of enzyme is relatively small.

"*Case II.*, in which there is a difference from Case I., inasmuch as the quantity of enzyme is relatively considerable.

"*Case III.*, in which the amount of enzyme diminishes as the action proceeds.

"*Case IV.*, in which the amount of sugar present is varied.

"*Case I.*—As hydrolysis proceeds, assuming that the enzyme itself is not affected by the work it does, since the magnitude of the active system depends on the amount of enzyme present, it is obvious that in the initial stages if the total amount of the sugar present S be large compared with s, the enzyme will be in presence of enough sugar molecules to establish the maximum possible number of effective combinations; or, in other words, the magnitude of the active system will remain constant and the change will be expressible, as Brown and Glendinning have pointed out, as a linear function of the time. As hydrolysis proceeds, the amount S of sugar present decreases until it is no longer negligible compared with that of the active part s, and hence the enzyme will no longer effect the maximum possible number of combinations: the proportion of sugar s undergoing change will then be a function of the total mass, and the formation of active systems will be governed by the law of mass action. The rate of change will be a logarithmic function of the time.

"This explanation is fairly in accordance with the observed facts in the case of invertase and diastase, the only enzymes hitherto experimented with, which have always been used in very small quantities.

"*Case II.*—If, on the other hand, the quantity of enzyme used be relatively large, the active mass will be a function of the total mass from the very beginning of the experiment, so that the linear part of the curve will escape notice. O'Sullivan and Tompson seem to have used a relatively large proportion of enzyme, and therefore

it is easy to understand why they found the action of invertase to follow the logarithmic law, whilst subsequent observers using relatively small quantities of enzyme have noted departures from this law.

"*Case III.*—When the amount of enzyme does not remain constant but for some reason decreases, the magnitude of the active system will not only be a function of the amount of sugar but also of that of the enzyme; it will therefore be represented by an equation of the second order, in which both of two interacting substances decrease—as, for example, is the case in the interaction of an alkali and methylic acetate. Such an expression corresponds to a curve falling off from a logarithmic curve and therefore giving a series of decreasing values for K when this is calculated for the simple logarithmic law. In such a case, the change in its early stages will still be a linear function of the time, as the diminution in the amount of enzyme will not at first materially influence the magnitude of the active system.

"Stated shortly, the ordinary equation of mass action $\frac{dx}{dt} = K(S - x)$, where S is the total sugar and x the amount changed in time t, is applicable only to the period during which a constant relatively large proportion of enzyme is present together with a continually decreasing amount of sugar but uninfluenced by the products of change.

"During the final period, when the products of change exercise an influence by withdrawing enzyme from the sphere of action,

$$\frac{dx}{dt} = K(S - x)(E - y),$$

where E is the total enzyme, y the amount withdrawn in combination with the products in time t.

"During the period when the proportion of sugar present is very large, x becomes negligible compared with S, so that $\frac{dx}{dt} = KS = k$, where k is a constant.

"The apparent duration of the linear period must be affected not only by x becoming no longer negligible compared with S, but also by the extent to which the products of change make their influence felt.

"It may here be pointed out that Henri's formula combines in a single expression the linear and logarithmic periods, but does not take into account the last period during which the products of change exercise a retarding influence.

"The action of invertase appears to be much less affected by invert sugar and that of diastase by maltose than is that of lactase,

emulsin, or maltase by the products to which they respectively give rise. Consequently, for these latter enzymes, the linear period is of short duration, and the logarithmic period is barely perceptible owing to the rapid reduction in the rate.

"*Case IV.*—When the amount of enzyme and water is kept constant whilst that of sugar is increased, it may be supposed that the magnitude of the active system will increase until $s+e$ reaches a maximum, a definite equilibrium being established between enzyme, sugar, and water, the whole of the enzyme, perhaps, becoming combined with the sugar. It may be assumed that if the amount of sugar be further increased, the equilibrium will remain unaffected, notwithstanding that an addition of sugar is practically equivalent to a withdrawal of water.

"But if $s+e$ remain unaltered, whatever the proportion of sugar present beyond a certain minimum, a constant amount of hydrolyte will undergo change in a given time, although the proportion changed as also the value of K will decrease as the concentration is increased. This conclusion is entirely in agreement with the facts elucidated especially by Adrian Brown, and with my own observations."

With regard to the effects of varying the amount of enzyme, E. F. Armstrong states that the weight hydrolysed in a given time by varying amounts of enzyme was approximately proportional to the amount of enzyme, provided that the amount was not too large, and also that the comparison was made during the earlier stages of hydrolysis before the secondary products began to exert a marked influence.

In a recent paper Bayliss has published an extensive series of observations on the hydrolysis of various proteids, chiefly caseinogen and gelatin, by the action of trypsin. The method used by Bayliss was that of measuring the increase in electrical conductivity, and it appears from his statement to be both convenient in carrying out experimentally and to give reliable results.

With regard to the course of the reaction, Bayliss found that the curve representing quantity converted and time, fell off continuously and rapidly throughout the experiment from the logarithmic curve, the velocity constant decreasing in value to the end. The form of the curve (which tends to become asymptotic to the base line) shows that the velocity of reaction tends to become zero, that is to say, that there is an equilibrium point with

the reaction incomplete. It was found that the position of this point of rest altered with the same factors as have already been described as causing an alteration in Tammann's experiments,[1] viz. alteration of concentration of substratum; alteration of concentration of enzyme; alteration of concentration of the system as a whole; removal of products of reaction; and alteration of temperature.

A certain amount of evidence in favour of the reversibility of the reaction was obtained by subjecting a 40 per cent. solution of the products of reaction of caseinogen and trypsin to the action of fresh trypsin, when in a period of four days a considerable diminution of conductivity was observed, which is the reverse of the increase in conductivity observed when the caseinogen is acted upon, and is presumptive evidence that the reaction was proceeding in the reverse or synthetic direction.

Bayliss, in discussing the interpretation of his results, expresses the opinion that the mode of treatment by E. F. Armstrong given at length above, meets the case of trypsin better than any other, and quotes *verbatim* the four cases given by Armstrong.

In experiments upon the effects of alterations in the concentration of the trypsin, Bayliss found (1) that in the initial stage of the reaction the velocity is in linear proportion to the amount of enzyme, but (2) that this relationship is more and more departed from as the reaction proceeds, so that a stage is eventually reached at which the velocity is practically identical for different amounts of trypsin. The explanation of the equality of rate of change given is, that as the concentration of the substratum diminishes there will come a time when there is only sufficient for a small amount of enzyme to combine with and hydrolyse. If the amount of enzyme present is not very small there will be sufficient of it, in all the cases with the varying amounts, to act upon all the available substratum.

The following is a summary of other important results recorded by Bayliss in this valuable paper:—

The velocity of reaction is proportional to the concentration of the substratum up to about 4 per cent. in caseinogen: above this and up to about 8 per cent. it is independent of the concentration, and above 8 per cent. it is inversely proportional to it. In no case,

[1] See p. 65.

however, is the proportionality a linear one, the effect being proportionately less as the concentration rises.

If the effect of diminishing the concentration of the substratum as well as that of increasing products of reaction be eliminated, the curve becomes a straight line.

There is no evidence of actual destruction of trypsin in digesting solutions up to the seventh or eighth hour at 38° C., but trypsin solutions alone rapidly lose activity when kept even at 0° C.

When kept for some time at a warm temperature, trypsin in solution becomes converted into a body resembling a "toxoid," which may be termed a "zymoid." This body appears to have retained its power of combination with the substratum while becoming comparatively inactive as regards its proteoclastic powers.[1]

The chief cause of the increase of the electrical conductivity in trypsin digestion is probably the splitting off of the inorganic constituents of the substratum molecules; and also, in the case of caseinogen, to the conversion of organic phosphorus into inorganic phosphates. The change of internal friction has, apparently, very little part in the effect. This production of electrolytes is insufficient to account for the retarding action of the products of reaction, they being present in too small a concentration in the total products.

There is some evidence that amino-acids are more active as retarding agents than the constituents, such as albumoses and peptones, present as chief constituents of the products of the earlier stage of the reaction.

The retarding action of the products is on the enzyme rather than on the substratum; and their mode of action is, most probably, by combining with the enzyme and withdrawing it from the sphere of action. This is supported by the fact that they are at least as active as the substratum in protecting trypsin from destruction by heat.

Trypsin is capable of acting on caseinogen at a temperature as low as 0° C.

An anti-trypsin is present in egg-white and serum, which slowly disappears during the reaction with trypsin.

The biuret reaction commences to disappear as early as $2\frac{1}{2}$ hours after the commencement of the action of trypsin on caseinogen. The viscosity of caseinogen solutions undergoes a rapid diminution and then becomes constant, while the conductivity curve continues to rise fairly rapidly.

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[1] This statement is based on the fact that warmed trypsin solution, when added to gelatine, causes a marked and immediate fall in conductivity.

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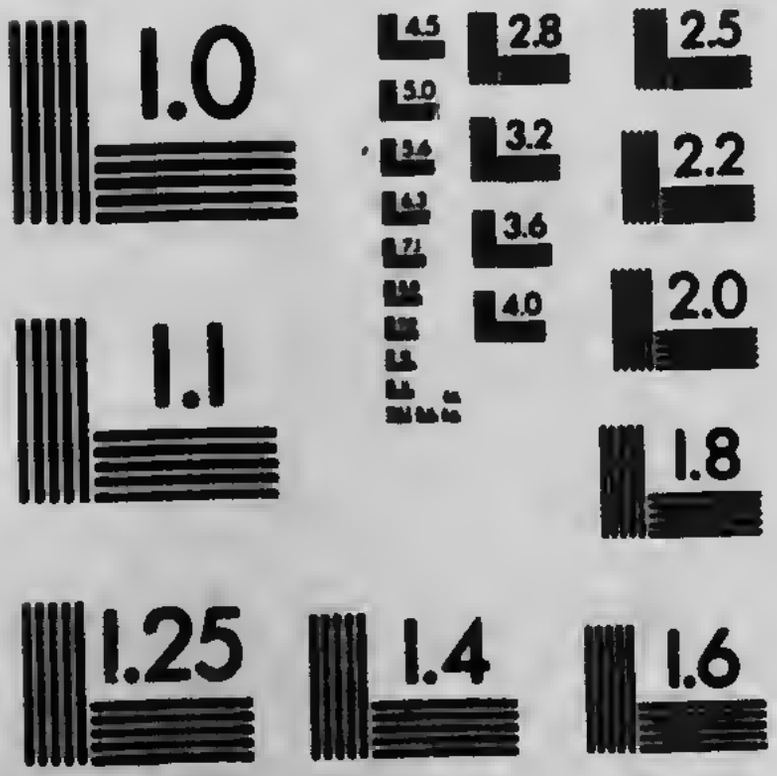

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Discussion of Experimental Results on Velocity of Reactions induced by Enzymes

It is clear from the foregoing account of the experimental results obtained by different authors, that in the case of the enzymes the simple logarithmic law fails widely to suit the general course of the reaction. The formula of Henri, although it fits the earlier portion where the velocity is expressed as a linear function, or the portion where the curve increases above the logarithmic law, fails entirely to cover the portion, occupying in some cases the greater part of the reaction, where the products of digestion retard the reaction. Its form shows that it is based in fact on an accelerating action of these products. Finally, the mode of treatment adopted by Armstrong, while it gives a verbal explanation of the cause of the variations at different stages in the reaction, fails to unite these into one formula, or to give any expression which will show the velocity of the reaction at any stage.

It may be inquired, therefore, whether any assumption has been made in the deduction of the velocity equation for such reactions as we have been considering which has been the means of introducing the differences between theory and experiment.

The equation from which the simple logarithmic expression, $k = \frac{1}{t} \log \frac{a}{a-x}$, is derived is $\frac{dx}{dt} = k\ (a-x)$, which simply expresses that the rate of change at any moment is proportional to the concentration of the unchanged substratum at that moment. The entire action of the enzyme, as far as the formula is concerned, is contained in the constant k; the more powerful the enzyme the greater is the value of k, and the less powerful the less is the value of k. Regarding the ferment as acting by reducing the resistance to reaction in the system, as described above, we may regard k as the conductivity factor in the reaction. Hence by writing $\frac{dx}{dt} = k\ (a-x)$, we have assumed that the effect of the enzyme upon the conductivity is constant throughout the reaction. Expressed in another way, the assumption has been made that throughout the whole of the reaction, no matter what

the concentration of the substratum, the effect of the enzyme is the same *upon each molecule of the substratum*. Now this need not necessarily be the case experimentally, and probably is not so; for as the number of substratum molecules decreases, there will be continually a larger number of enzyme molecules relatively, and there may be in consequence a greater amount of action upon each substratum molecule, that is, the value of k on this account will increase throughout the reaction. Thus indirectly k becomes a function of x, the quantity inverted, but not (as Henri's formula would indicate by its derivation) on account of a directly favouring action upon the enzyme of the products of the reaction. There is no experimental basis for the assumption that the effect of the enzyme upon each molecule of substratum (if the expression may be allowed) is the same, no matter what the concentration in substratum; and when the concentration of the substratum falls in the course of the reaction, then the available substratum upon which the enzyme acts being lessened, the effect upon each molecule must be increased. Therefore although the velocity of change diminishes as the mass action law indicates, on account of the diminution in unchanged material, there is a factor of increase on account of greater activity being exercised by the constant number of enzyme molecules upon each of the now smaller number of substratum molecules.[1] A second assumption which is made in applying the law $\frac{dx}{dt} = k(a-x)$ to enzymic action has already been alluded to in the derivation of the equation (see p. 52), viz. that the action is taken to be irreversible, or that the tendency to reversion may be neglected.

This assumption is in all probability not experimentally justifiable, especially at the later stages of the reaction. For it does not follow that because a reaction runs practically to completion, as, for example, that induced by invertase upon cane-sugar, that

[1] A kinetic analogy may make the contention clearer. Suppose the enzyme molecules are a fleet of battleships, firing at a number of targets which are gradually sunk as a result. Then as the targets sink, the rate of disappearance will decrease, and provided the number of targets is large enough the rate will be proportional to the number at any instant, that is, the law $\frac{dx}{dt} = k(a-x)$ will hold. As the firing goes on, however, there will come in a factor of increase in the rate of sinking, because each target will be attacked by an increased number of ships.

therefore the effect of the *tendency* to reversion in decreasing the velocity of reaction in the late stages can be neglected.

The same causes which produce the actual reversibility seen in the case of strong solutions must be present in dilute solutions, and emphasis must be laid upon the fact that, on either side of the equilibrium point for some distance, the tendency for the reaction to run in the opposed direction must be present and ever-increasing in amount as the equilibrium point is neared, so as to stop the reaction at the equilibrium point. Hence before the equilibrium point is reached there must be a decrease in velocity due to the tendency to reversion.

Accordingly it is not safe to assume that because a reaction runs to 99 per cent. and over before equilibrium is reached, and is therefore regarded as a complete reaction, that it will run up to 99 per cent. with the same velocity as if there were no equilibrium point and no tendency to reverse near the end-point.[1]

As pointed out above, Bayliss has shown that there is a tendency, at least, to reversion in the case of caseinogen and trypsin; actual reversion has been shown with other enzymes: and even in the case of the action of *acids* upon disaccharides. E. F. Armstrong and R. J. Caldwell have demonstrated that there is a tendency to reversal indicated by the rotation of the plane of polarised light beyond the maximum value corresponding to complete hydrolysis.

In fact, the retardation due to products of reaction which causes the velocity in the later stages to fall off from the logarithmic expression may in all cases probably be ascribed to the tendency to reversion. The usual view that the drop is due to removal of enzyme from the sphere of action by its combination with one or more of the products of reaction is not incompatible with this supposition. For just as it is supposed that, in order that the action may proceed from left to right, it is necessary for the enzyme to enter into some relationship or combination with

[1] Visser (quoted by Hamburger, *Osm. Druck und Ionenlehre*, vol. iii. p. 97, 1904) found that the action of invertase upon cane-sugar was not quite complete, as always 1 per cent. of the cane-sugar was left. Visser deduced a formula which gave a constant with his own results and those of Henri. In this he first, as recommended above in the text, retained the reversibility expression; and secondly, introduced a variable for the alteration in intensity of action of the enzyme throughout the reaction. The method suggested in the text for making the second of these two corrections is different from that of Visser.

the substratum, so it must be supposed that some such relationship is necessary with the reaction products, or one of them, in order that the reaction may proceed in the opposite direction from right to left. Nor is it any objection to the view that slowing by the products of reaction is due to the tendency to the establishment of the reverse reaction, that such slowing is caused by one only of these products in each case; but rather the contrary, for the enzyme in whichever direction the reaction is going will probably act upon one of the cleavage products only, and dependently upon the relative concentrations, either attach it to the other cleavage product or detach it from it. But while there is nothing in the formation of chemical compounds between the ferment and either the substratum or one of its cleavage products to negative the view that the retardation caused by the products is anything else than the expression of a tendency to reversion; it must be pointed out that the formation of such chemical compounds is a hypothesis invented *ad hoc* to explain the retardation, and that there is no experiment as proof of the existence of such compounds.

That the enzyme enters into some relationship with the substratum, as a result of which the velocity of reaction is established or increased, is certain; and it is equally certain that the enzyme also enters into some relationship at a later stage in the reaction with one of the products of the reaction, as a result of which the reaction is slowed. Or, when a position is considered beyond the equilibrium point, as a result of which the action is made to proceed in the opposite direction. But it is by no means certain that this relationship is that of a chemical compound in the ordinary sense of the word; there have no such compounds been isolated, there is no exact relationship pointing to any chemical combination between enzyme and substratum, and the amount of enzyme compared to that of the substratum which it can act upon at the same instant or in an exceedingly short time interval is such as to preclude in all probability the existence of a chemical compound in the ordinary sense of the term.

It is hence most probable that the influence of the enzyme as an energy-transformer is one of a physical character, and at any rate the formation of chemical compounds must at present be taken as unproven. Accordingly it is much safer to make use of a point of view which leaves the question open, and to regard

the retardation due to products of reaction as the sign of the tendency to reversal or influence of the products tending to react in the opposite direction, rather than being due to removal of enzyme by combination with such products in a reaction which is regarded as irreversible.

The matter becomes clearer when we consider the reaction as proceeding in the opposite direction, as, for example, in any of the syntheses by enzymes mentioned on p. 31 *et seq.* Here the reaction slows down also as it nears the equilibrium point, and we might consider the slowing as due to the product of reaction, and state that this combined with the enzyme and removed it from the sphere of action. But for the reaction proceeding from left to right, it is just this combination of enzyme and product of reaction (now substratum) which is regarded as giving rise to the action of the enzyme. It is evident, then, that our explanation must be symmetrical on both sides of the equilibrium point, if the expression may be allowed, and that it is better to regard the relationship between enzyme and substratum on the one hand, or cleavage product on the other, as favouring the reaction in a determinate direction in each case, rather than as doing this in one case, and simply inertly removing enzyme in the other.

Returning to the consideration of a formula to suit the entire course of the reaction, after the above discussion it becomes clear that in the first place we must not remove the expression $k_2 x^2$ or there will be experimental variation in the later stages increasing as the equilibrium point is neared; and, secondly, that into the portion of each expression which represents the action of the enzyme we must introduce a factor expressing that this action is not constant throughout, but intensifies as the concentration of the substratum diminishes, and here it must be remembered that for the expression $k_1 (a - x)$ the concentration of the substratum is $a - x$, and for the second expression in the equation of velocity of reaction $k_2 x^2$, the concentration of the substratum is x. In other words, the influence of the enzyme in either direction is not a constant but is some function of the concentration of the substratum. As a simple approximation the factor introduced by Henri $\left(1 + \frac{x}{a}\right)$ may be used, so that k_1 becomes $k_1 \left(1 + \frac{x}{a}\right)$; only it must be remembered that such a correction equally applies to the reversed reaction, and hence

instead of k_2 we must put $k_2\left(1-e_1^{\frac{a-x}{a}}\right)$, when the equation for velocity of reaction becomes

$$\frac{dx}{dt}=k_1\left(1+e^{\frac{x}{a}}\right)(a-x)-k_2\left(1-e_1^{\frac{a-x}{a}}\right)x^2.$$

This formula is too complicated for application to experimental results on integration, but it includes all the observed experimental cases, that is, it shows a stage when x is small where the reaction is linear, a stage where the reaction is more rapid than the simple logarithmic law demands, as in Henri's experiments, a stage showing a falling off from the logarithmic values, as in the later stages of the experiments of Armstrong and of Bayliss, a zero stage at the equilibrium point, a reversed velocity, which also at the very end tends to become linear.

To make the investigation of the equation easier,[1] we may suppose, since Henri found experimentally that the value of e was approximately unity, that $e=e_1=1$, when the equation becomes

$$\frac{dx}{dt}=k_1\left(1+\frac{x}{a}\right)(a-x)-k_2\left(1-\frac{a-x}{a}\right)x^2.$$

This may be written

$$\frac{dx}{dt}=k_1(a-x)+\frac{x}{a}\left[k_1(a-x)-k_2x^2\right],$$

and in this form we may now investigate how the velocity, that is, the value of $\frac{dx}{dt}$, will vary at different stages of the reaction.

First, let the value of x be small compared with a as in the earlier stages of the reaction, then x^2 and higher powers of x may be neglected as small magnitudes of the second or higher orders and the equation reduces to

$$\frac{dx}{dt}=k_1(a-x)+x\,.\,k_1=ak_1.$$

That is, the velocity of reaction is constant, and the curve expressing it is linear.

Secondly, for higher values of x (that is, later in the reaction), but where x is not yet large compared to $(a-x)$, since k_2 is small com-

[1] The same results follow with the formula as it stands, only the expressions are more complicated.

pared to k_1, k_2x^2 is small compared to $k_1(a-x)$ and may be neglected, when the formula becomes

$$\frac{dx}{dt} = k_1(a-x) + \frac{x}{a} \cdot k_1(a-x).$$

This may be written

$$\frac{dx}{dt} = k_1\left(1+\frac{x}{a}\right)(a-x),$$

which is Henri's formula—that is to say, during this period when k_2x^2 is small compared to $k_1(a-x)$, or in other words, when reversion may still be neglected, Henri's formula holds. The curve of velocity shows a greater value than is given by the simple logarithmic law, and the constant calculated on the simple logarithmic basis will increase in this stage of the reaction, while a constant calculated on the above formula will remain approximately constant.

As x goes on increasing, however, the value of k_2x^2 will cease to be negligible as compared with $k_1(a-x)$, and in the end $k_1(a-x)$ will become less than k_2x^2, when the expression within the square brackets will become negative in value. Just around the point where $k_2x^2 = k_1(a-x)$, the simple logarithmic law will approximately hold, for then the expression in square brackets will be almost zero and negligible in comparison to $k_1(a-x)$, so that the equation becomes $\frac{dx}{dt} = k_1(a-x)$, which yields the simple logarithmic formula. But as x goes on increasing, and $a-x$ diminishing, it is obvious that the negative value of the expression in square brackets will rapidly increase, and that the reaction will proceed much more slowly with ever-increasing deviation from the logarithmic law.

Finally, the reaction will come into equilibrium, and beyond this point will be reversed.

For the reversed reaction *near the end-point*, since $a-x$ is small compared to x and hence $\frac{a}{x} = 1$, the equation becomes $\frac{dx}{dt} = k_2a^2$, or the velocity of reaction is constant and the curve expressing it a straight line as at the beginning of the reaction. Since, however, the whole of the reversed stage is short, and an appreciable amount of $a-x$ relatively to x is soon formed since the reaction runs $\frac{dx}{dt} = k_2x^2$, and x is here large, the straight line portion on this side is infinitely short and cannot be demonstrated experimentally.

The equation given above hence serves to demonstrate that the law governing the reaction is the same throughout, and that the

deviations from the logarithmic law arise from the assumptions having been made in the derivation of that law (1) that reversibility can be neglected, and (2) that the intensity of action of the enzyme, per molecule of substratum, can be taken as constant throughout the reaction.

Effects on Velocity of Reaction of Alterations in the Concentration of Enzyme

The effects of variation in the amount of enzyme initially added upon the velocity of reaction, in the case of the sucroclastic enzymes and of trypsin, have already been mentioned in describing the experiments upon the course of the reaction. Experiments upon this question, to be comparable with one another, should be made under such conditions that the concentration of the substratum remains constant throughout the experiment. Otherwise the ratio of enzyme and substratum is continually varying during the experiment, and the effect of variation in concentration of enzyme is not obtained pure, but a combination of this with variation in concentration of substratum. Also the effects of accumulation of products of reaction must be avoided. Hence the ideal condition is that in which there is excess of solid substratum, the products of action are continuously removed by dialysis, and the enzyme is present in constant strength throughout each experiment. Such an arrangement as is suggested, for example, by Bayliss,[1] of a bell-jar filled with solid gelatine containing the enzyme and dialysing into a larger vessel (the bell-jar being attached to one end of a lever which records the rapidity of action by the loss in weight, and writes a record on a smoked paper surface) would be an ideal arrangement for such a purpose, on the supposition that the enzyme did not dialyse out, as would probably be found to be realised within the experimental limits, as the rate of dialysis of enzymes is so slow.

Such experiments have, however, not been yet carried out and the next best are those in which the observations have been recorded at the initial stages of the reaction where the amount

[1] *Loc. cit.*

of substratum has been large and not very widely varied before the measurement has been taken, and especially those in which, where possible, a solid substratum has been employed.

As Bredig has pointed out, the result would be more certain, and more definite conclusions could be drawn if, in such experiments, instead of measuring the different amounts of substratum converted in *equal* times by varying amounts of enzyme, determinations were made of the varying intervals of time necessary to convert the same percentage of substratum as the concentration of enzyme is changed. For in the latter case whatever the law may be governing the course of the reaction, and as we have seen above this may be somewhat complicated, since the reaction in such case runs to the same stage, in deducing the ratio of the increases in velocity, due to the two different concentrations of enzyme, this complicated factor eliminates out, being the same in each case, and the ratio in the activation by the two quantities of enzyme is simply inversely proportional to the two time-intervals for production of the same percentage change.

While it must be admitted that this method of varying time-interval and constant percentage of conversion is the more scientific, it must, however, be stated that it is in most cases of zymolytic action most difficult or impossible to carry out experimentally. For in most such cases we have no indicator to show when a certain definite percentage of the total change has occurred, and a somewhat elaborate measurement or determination must be made[1] in order to discover the state of affairs in the solution, so that the experimenter is reduced to making measurements at definite time-intervals instead of at definite amounts of conversion. Nor will it do to take the end-point of the reaction in most cases on account of the slowness with which that point is reached, although in certain cases where the end-point has special physical or chemical characteristics this has been used, as, for example, the coagulation point for an enzyme such as rennin, or the disappearance of the colour test with iodine in the case of starch and diastase.

It may be further added that in many cases the objections of

[1] This does not apply to the electrical conductivity method of Bayliss, who has utilised the method suggested by Bredig, and determined the times at which equal changes in conductivity occur.

Bredig have little practical weight, as, for example, where solid substratum is used in determining activity in varying concentration of proteoclastic enzymes, or where the amount of substratum is initially large compared to the amount of enzyme, and the measurements are taken early in the course of the reaction before there is any large alteration in percentage of the substratum. In such cases none of the variations in the reaction detailed above occur in the short stage of reaction utilised for the determinations, and the degree of activation by the enzyme may be safely taken as directly proportional to the amounts converted in equal times.

The law connecting velocity of action and concentration of the enzyme varies with the nature of the enzyme. In many cases where the question has been accurately examined in recent times, the ordinary law which applies to inorganic catalysts applies also to enzymes, viz. that the effect is in simple direct proportion to the concentration in enzyme. There is this difference, however, in the case of enzymes, that a maximum is soon reached beyond which further addition of enzyme produces no noticeable effect whatever, and it is hardly necessary to add that for concentrations somewhat short of the maximum, the linear law does not hold, as the linear portion of the curve gradually rounds off to the asymptotic line which marks the maximum velocity with increasing concentration. Having regard to the high molecular weight which enzymes possess and correspondingly low molecular concentration, and also the low percentage amount present when the maximum amount of increased effect with concentration is obtained, we have here incontrovertible evidence of a difference in mode of action of enzymes and inorganic catalysts; such, for example, as sucroclastic enzymes and the hydrogen ion of acids. Here the sucroclastic enzyme is already in possession of its maximum effect at a molecular concentration, at which the action of the hydrogen ion is practically imperceptible, and the hydrogen ion goes on increasing in effect, as the concentration is increased, at a rate considerably greater than corresponds to the increased ionic concentration, while the action of the enzyme remains at a constant level.

The enzymes which within the limits indicated above obey the law of direct proportionality between concentration and activity are: Invertase (O'Sullivan and Tompson, Henri, E. R.

Armstrong), Rennin (Segeleke u. Storch, Soxhlet, Löreher, Duclaux, Fuld), Lipase (Kastle u. Loewenhart), Trypsin (Bayliss).

In the case of other ferments, however, although the same falling off to a maximum value at an upper limit, at which the percentage of ferment is still very low, is observed in all cases, it is found that even well below this limit of maximum effect the linear law is not obeyed of direct proportionality between concentration of enzyme and intensity of action.

In most such cases, the majority of experimenters have arrived at the conclusion that the law, first empirically deduced from experiments upon pepsin by E. Schütz and known as "Schütz's law," is that which best expresses the effects of concentration upon intensity of action within a certain range. The law is that the intensity is directly proportional to the square root of the concentration, or put conversely, that the relative concentrations of enzyme are directly proportional to the squares of the intensities (that is, the amounts changed in equal times). Expressed in an equation, if k_1 and k_2 are the velocity constants (or quantities converted in equal times) at two different concentrations of enzyme c_1 and c_2, then "Schütz's law" is that

$$\frac{k_1}{k_2} = \left(\frac{c_1}{c_2}\right)^{\frac{1}{2}}, \text{ or } \frac{c_1}{c_2} = \left(\frac{k_1}{k_2}\right)^2.$$

The law has been most worked out in the case of pepsin by E. Schütz, J. Schütz, Huppert and J. Schütz, and Borissow; but according to Pawlow and his co-workers, using Mett's method, it also holds for the tryptic and diastatic enzymes of the pancreas, in addition to pepsin.

The whole subject, however, deserves to be thoroughly worked out anew, for there is a want of concordance in methods and results amongst the different workers, who have not only employed different methods and different stages in the reaction for different enzymes, but also for the same enzyme. Thus in the case of trypsin Bayliss finds, as stated above, that the law at any rate for dilute solutions is approximately a linear one, while Pawlow, using a different method (Mett's), finds the "Schütz law" followed. Again, while Huppert and J. Schütz found the Schütz law followed for not too concentrated solutions on using dissolved proteid (egg albumin), they found with the Mett's tube method on coagulated egg albumin, that this law was not obeyed,

but rather that the length of albumin dissolved was nearly directly proportional to the concentration in enzyme. On the other hand, Borissow, using the Mett's method, found that pepsin in its action in dissolving coagulated egg-white obeyed Schutz's law.

In the writer's own experience with the Mett's method, and active preparations of commercial pepsin of various origin, the Schutz's law is by no means followed. With stronger solutions, the length of egg-white dissolved off is approximately equal: as the concentration in enzyme is diminished, the intensity of action falls off very slowly, much less than in direct linear proportion, but there is no period at which the Schütz law is closely obeyed, and with very dilute solutions the length of egg-white is so little as not to be accurately measurable, so that the method is useless for testing very dilute solutions. Even in stronger solutions the slowness of fluid diffusion in the narrow tubes tending to accumulation of products of digestion at the active interface, and the irregularity with which the column of egg-white is eaten away, form grave objections to the employment of this oft-described method.

In the case of the experiments, such as those of J. Schütz and Huppert and Schütz, in which the activity is determined from the amount of secondary albumose formed in equal times with varying concentration of enzyme, the objections of Bredig described above must be taken, for here the concentration of the substratum is altering all the time of the experiment, and the percentage of conversion and concentration of products of reaction will be greater in the solutions containing more concentrated enzyme, and hence there will be a greater factor of retardation in the more concentrated solutions.

That this is the case is seen from the extension of the Schutz law which is advocated by Huppert and J. Schutz. These authors give as a result of their experiments the formula, $S = kA\sqrt{t}.p.s$, in which S = the amount of secondary albumose formed, k = the reaction constant, t = the time of experiment, p = the concentration in pepsin, and s = the concentration in acid, provided this does not exceed 0·2 per cent. Now such a formula cannot express more than an empirical coincidence throughout a certain short range of experiment, for apart from the improbability of exactly the same law being followed in the case of three such different factors as time, enzyme concentration, and acid

concentration, it is evident that as the optimum amount of acid lies very little if any above 0·2 per cent., that there must, as in the case of all other optimum points, be a considerable range in acid concentration below this point throughout which change in acid concentration has a very slight effect compared to what it has at the lower and minimal concentrations of acid. Again, if we take all the other factors in the formula except the time as constant, the formula for amount of conversion and time becomes $S = K\sqrt{t}$, now this is quite different from all the other formulæ deduced experimentally or theoretically for velocity of enzyme reaction (see pp. 58–61). At the same time this casts a light upon how under certain conditions such a formula can be obtained empirically from experimental results, and can for a certain distance give an apparently close coincidence to the results of experiment, and appear to give a law for expressing them, although if under the conditions it were possible to carry the experiments farther to either side, the law would be shown not really to exist. For the above equation may be written, on squaring both sides, $S^2 = K^2 . t$. This is the equation of a parabola, with its axis horizontal, if the quantities converted are plotted as ordinates, and the times as abscissæ. Now, if the action of pepsin is similar to that of trypsin as experimentally investigated by Bayliss, instead of a parabola we should have first a straight line portion, then a more or less logarithmic portion, and finally a portion where the velocity of conversion fell off and finally became very small, the line running almost asymptotic to the axis. But with the exception of the initial straight line portion, which was probably missed, and the later portion of the curve where the velocity is falling off most rapidly, the intermediate portions of the two curves are roughly parallel, and hence observations confined to this region might easily give the impression that the law $S = k\sqrt{t}$ gave the course of the reaction.

The explanation of the "Schütz law" is probably of a similar nature, that is, it holds for a certain range only, and in this range is an empirical law which gives an approximation to the truth.

The writer considers this a more probable explanation than the one given by Höber and F. Hofmeister, although this is very ingenious. It will be remembered that when a substance dissociates into two others in equal molecular concentration, the equation for equilibrium runs $c_1 = k\, c_2^2$, where c_1 is the concentra-

tion of the undissociated substance and c_2 that of either of the two dissociated cleavage products. Now if we suppose that pepsin is a substance which is very feebly dissociated, and that it is only the dissociated portion which is active as an enzyme, then there follows an easy explanation of the Schutz law. For, the substance being but feebly dissociated, c_2 is very small compared to c_1, and if C be the total concentration in enzyme we can write instead of the above equation $C = k c_2^2$. But the activity is proportional to the dissociated portion c_2 and hence to $\sqrt{C}$, which is Schutz's law.

However, it is a quite unproven hypothesis that an enzyme dissociates into an active and an inactive part, and considering the nature of enzymes as colloids, an exceedingly improbable one.

Medwedew found for the oxydase of liver tissue the law that the intensity of action of the enzyme is directly proportional to the *square* of the concentration and not the square root as in Schütz's law. This might receive an "explanation" by making the hypothesis that the ferment is practically all dissociated, and that it is the smaller undissociated portion only which retains activity: but the writer prefers to believe that it is an approximate empirical expression for a small portion only of the curve expressing the relationship between concentration and activity.

The conclusion may therefore be drawn that in the case of each enzyme, there is in dilute solutions a range of concentration throughout which the activity increases approximately directly as the concentration, and as the concentration increases a further period in which there is also an increase but at a less rapid rate than the concentration, and that finally a maximum effect is obtained beyond which increasing the concentration has no action in increasing the activity.

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CHAPTER IV

INFLUENCE OF OTHER FACTORS UPON ENZYMES AND CELLS —TEMPERATURE—PHYSICAL AND CHEMICAL AGENTS —NEGATIVE CATALYSTS, ANTI-CATALYSTS, ANTI-FERMENTS, AND ANTI-ENZYMES — ZYMO-EXCITATORS OR KINASES —AUTO-CATALYSIS AND INFECTION — SPECIFIC NATURE OF CATALYTIC ACTION—THE CHEMICAL AND PHYSICAL NATURE OF ENZYMES — THE SO-CALLED INORGANIC ENZYMES OR METAL-SOLS

It is characteristic of all enzymes that they are very sensitive to changes of temperature both as regards their stability and as regards their rapidity of action.

The stability of the different enzymes varies very widely, but as a general rule it may be stated that the rate of destruction rapidly increases with the temperature, although this may lie much below that at which they are most rapidly destroyed. The amount of enzyme destroyed also varies with the substances present in solution: thus the products of reaction in all cases exercise a protective action against rise in temperature which has been ascribed to the formation of a compound between the enzyme and the products of its activity.

All enzymes in aqueous solution are rapidly destroyed at about the coagulation temperature of proteids (65–75° C.), and a coagulum usually appears in the solution.

Even at body temperature and below it many are, however, rapidly destroyed, especially in the absence of a protective substratum. Thus Vernon has shown that trypsin in nearly pure solution is rapidly destroyed at 38° C., but is protected from such rapid destruction by the presence of proteid. The subject has been further investigated by Bayliss, who finds that trypsin in solution loses activity even at 0° C.

The action of heat upon enzymes in organic solvents is much less marked than in aqueous solutions; thus Pavy finds that

the diastatic ferments of the liver and pancreas are not destroyed by boiling in alcoholic solution, and Fermi and Pernossi find that enzymes are only slowly acted upon in amyl alcohol solutions.

In the dried condition it has been shown that enzymes can be heated to as high as 160° C. without losing their activity.

Exposure to low temperatures does not appear to have any marked effect upon enzymes.

With regard to the effect of alterations of temperature upon the rapidity of action of ferments, it is found that each ferment is most active at a temperature called the *optimum* temperature, which varies in the case of each ferment, and also in the same ferment with the conditions of solution, presence of neutral salts, reaction, and temperature to which the solution has previously been exposed. As a general rule the optimum temperature lies between 35° and 45° C., but according to Roberts the action of trypsin increases even up to 60° C., at which temperature it is however rapidly destroyed.

It is stated by Bredig that the existence of the optimum temperature and the decrease in rapidity of reaction at higher temperatures than the optimum is due to two opposing factors. The first factor is the increase in reaction velocity which rise in temperature always occasions, and the second is the destruction of a portion of the enzyme which gradually occurs, more rapidly as the temperature is increased, and finally outbalances the positive effect due to increased temperature *per se*. Ernst working in Bredig's laboratory tested the rate of action of Bredig's platinsol upon water-gas, and found conformably to this view that it also possessed an optimum temperature of action, the exact position of which varied with the previous history of the platinsol, the period during which it had been kept at the higher temperature previously to starting the reaction, &c.

Accordingly it would appear that the optimum temperature is not a peculiar characteristic of enzymes, but depends upon their instability at the optimum temperature and above it. It must be added however, that in presence of their appropriate substrata, some ferments are very stable at their temperature of optimum activity, *e.g.* pepsin, the rate of destruction being practically inappreciable, and hence it appears to the writer that although Bredig's view may hold in some cases it is not a universal ex-

planation of the existence of an optimum temperature of action for all ferments.

The temperature coefficient, that is, the variation in the velocity constant with the temperature, which is usually expressed for each 10° C., has been measured by Bayliss in the case of trypsin. It was found that it required 5·3 times as long to effect an equal change at 20·7° C. as at 30·7° C.; between 30·7° C. and 38·7° C. the ratio of velocities was 2·6 : 1, giving 3·3 as the coefficient for an interval of 10° C.; also a determination of the velocity at 0° C. gave a coefficient for each 10° interval between 0° C. and 30° C. of 12. In the case of emulsin, between 60° and 70°, Tammann found for the temperature coefficient the value of 7·14; Senter for the peroxidase of blood the value of 1·5, between 0° C. and 10° C. (Quoted from Bayliss *loc. cit.*)

The living cell in its reaction to temperature changes, in so far as it is not controlled in the higher animals by the temperature regulating mechanism, obeys exactly the same laws as the enzyme. Its activity is only possible, as in the case of the enzyme, between certain well-defined limits, which vary from cell to cell, as from enzyme to enzyme, and somewhere in the range there is an optimum point of maximum activity which is variable under like conditions as in the case of the enzyme. Also at the point of maximum activity, the living cell is working above its safety point, and prolonged action at this point leads to a break-down in the cell's activity, and to death.

There is an apparent exception in the case of warm-blooded animals in the fact that a slight fall in temperature leads to increased activity, but this is merely due to the action of one cell upon another, to stimulation by the nervous system, and when on account of continued fall in temperature the regulatory mechanism is overcome, the cells of the warm-blooded animal obey the general law just as do those of cold-blooded animals.

On account of the regulatory mechanism, as a result of the action of which the cells of the warm-blooded animals are rarely exposed to any appreciable variations in temperature, the cells have lost their power to respond to temperature variations throughout so wide a range, the minimum and maximum points are close together, and so arises the great danger of temperature variations after the regulating mechanism has been overpowered by greater than normal variations in temperature of external surroundings.

It is, therefore, in unicellular organisms, and in the earlier stages of development of multicellular organisms, that the variation in activity with alteration in temperature is most clearly seen. Here it is found that at a certain minimal temperature the activity just begins to be perceptible, as the temperature rises the activity increases with it until an optimum point is reached, lying usually, as in the case of the enzyme, a few degrees above the usual temperature of the action of the organism, and beyond the optimum temperature the life of the organism becomes again more sluggish, its activities lessened, and if the high temperature is maintained it dies. The optimum point of activity for the living cell probably arises in the same manner as in the case of the enzyme, by the simultaneous action of two opposing causes; viz. (1) the hastening of all chemical reactions by rise in the temperature, (2) a similar hastening from the same cause of the bye-reactions which lead to a using up and diminishing of the cell substances which act as catalysts to these reactions. As a result of this it follows that in the earlier stages of the range of temperature, as the temperature rises, the chemical reactions in the cell will increase in velocity, while as yet there will be no appreciable destruction of the more stable cell substance, or of the catalysts. But in the later stages, at the optimum point and beyond it, destruction of catalysts, cellular enzymes, and cell substance will also proceed at an ever-increasing rate, and although the velocity of reaction of the catalysed reactions (under No. 1) is still increasing, the diminution in catalyst more than outbalances this, and the cell activities are lessened.

The same differences are to be noted at the two extreme ends of the range in the case of enzymes and living cells; as the temperature falls, the enzyme and living cell merely become dormant and temporarily pass out of activity, but neither is killed unless the fall in temperature is enormous compared to the rise in temperature which would cause total permanent loss of activity or death upon the other side of the active range. Nor is the reason far to seek; the lower limit is reached by gradual fall in activity until the zero point is reached, while the upper limit is reached by gradual increase in activity, accompanied by gradual destruction finally surpassing increase in activity, until the cell stops from destruction in hyper-activity.

The level at which living cells are *rapidly* destroyed by increased

temperature closely resembles that at which enzymes are similarly destroyed, and lies around the coagulation temperature of proteids. As in the case of enzymes, the point varies with the nature of the cell, some cells being more resistant than others. The resistance appears to vary inversely as the state of activity of the cell, inactive spores being more resistant than the active cells.[1] As in the case of enzymes, the amount of water present has a powerful effect upon the rate of destruction. Dried bacteria, and their spores in the dried condition, can be raised to temperatures above the boiling point for an appreciable time without destruction, although in suspension in aqueous fluids they are readily destroyed by such a temperature; and dried seeds can be exposed to low temperatures without injury, while in the moist condition they lose their vitality. The latter effect is probably a physical one due to disruption of the cell by the expansion of the water in freezing.

Influence of Various Physical and Chemical Agencies on Enzymes and Living Cells

Many enzymes are rapidly destroyed in aqueous solution by sunlight. The subject has been investigated in the case of the diastases by Green, who found that all parts of the spectrum are not equally active in this respect, the most active part is the ultra violet, but the green rays are also destructive. Certain portions of the red, orange, and blue appear at first to increase the amount of diastase, but this positive effect soon disappears and is followed by a destructive action. Green ascribes the first stage to a conversion of zymogen into active enzyme. The different diastases are not equally affected; thus the destruction in the case of malt diastase amounted to 68 per cent., in salivary diastase to 45 per cent., while diastase from green leaves was only affected to the extent of 8 per cent., but in this latter case it is probable that the chlorophyll acted as a protective.

A similar action is seen in the case of the living cell in the marked germicidal action of sunlight upon bacteria of many kinds, and in the higher animals in the subtle influence of sunlight in

[1] The more active an enzyme preparation is the more rapidly it is destroyed by variations in external conditions.

the preservation of a normal physiological condition of the body; in the formation and action of pigment cells; in the powerful effect upon epidermal cells of sunlight apart from the heating effect; in the probable effects of insufficient sunlight, in producing the cretinism found in the inhabitants of certain valleys; in the effects of light under certain conditions in producing ophthalmia. Here may be mentioned also the action of various other forms of radiant energy upon living cells, such as the X rays and Finsen rays, and the radiations of radium, which act as such powerful stimulants upon living cells.

Action of Acids and Alkalies and of Neutral Salts upon Enzymes and Living Cells.—In considering the value of food-stuffs such as proteids, carbohydrates, and fats as sources of energy to the body, we are too apt to forget that energy is not the only thing required, and that in order to use this energy, the integrity of the mechanism for its conversion or transformation, viz. the living cell, is no less important. For the preservation of this integrity, the simple inorganic salts, and a due proportionality between acid and alkali, are no less important, and such simple substances are no less indispensable than the organic food-stuffs. It has been shown by Ringer and others that normal physiological activity is not possible in the presence of the organic or energy-yielding constituents alone, that these may be present in abundance, and yet the tissue be entirely incapable of functionating unless it is also fed with certain inorganic constituents. Ringer further showed in the case of the frog's heart that there must be a certain balance maintained between the various inorganic constituents, that sodium chloride alone could not maintain the activity, but that it was necessary to have present both potassium and calcium in certain balanced proportions. Working on the basis of his experiments, he devised "Ringer's solution," containing these substances in the proper concentrations for the preservation of physiological activity, which has since, in various modifications, been employed by most subsequent workers on the subject.

The work begun by Ringer has been continued by many workers, and extended into observation of the effects of variation of the inorganic salts, and of acids and alkalies, not only upon a maintenance of physiological activity, which was the problem chiefly studied by Ringer, but also of the effects upon rapidity of cell growth and division and of reproduction.

The physiological balance of salt solutions first discovered and investigated by Ringer, in the case of the frog's heart, has been extended by Loeb to skeletal muscle and to marine organisms, and shown to be a general law.

In addition, Loeb made the most important discoveries that cell division can be initiated and carried to an advanced stage of development, in the unfertilised eggs of several organisms, by variations in the saline conditions only; that the conditions requisite for fertilisation and cross-fertilisation vary with the composition in inorganic salts of the medium, and with its reaction, and that the rate of growth varies with the degree of alkalinity.

These valuable results obtained by many independent observers show the immense importance to the growth and activity of living cells of their inorganic constituents, and this division of biochemistry is rapidly acquiring an immense literature of its own.

In such an action due to variation in inorganic salts, the writer believes that the key will ultimately be found to the secret of the cause of irregular cell division in the body, giving rise to malignant growths. For the production of what must be described as a pathological division in unfertilised eggs, and the production of pathological cell divisions such as have been noted by Galleoti by the action of inorganic salts such as the iodides, must be problems of the same order as the causation of the ungoverned and pathological divisions, often of very similar type, found in malignant growths.

As a general rule it may be stated that for the same enzyme the intensity of action of a given concentration of an alkali or acid varies approximately directly as the concentration in HO^- or H^+ ions, the effect of the other ion being only of secondary importance. Thus in all cases free alkalies such as sodium or potassium hydrate are many times more powerful than the corresponding carbonates in consequence of their almost complete ionisation as contrasted with the low ionisation of the carbonates.[1] Again, ammonia, which is but feebly ionised (about $\frac{1}{8}\frac{1}{8}$ of that of sodium hydrate) has a correspondingly feeble destructive action. The same holds in the case of acids, the effect here being mainly

[1] In deci-normal solutions sodium carbonate has only about 3 per cent. of the concentration in hydroxyl ions found in sodium hydrate (Shields, *Zeitsch. f. physik. Chem.*, vol. 12, p. 167).

due to the hydrogen ion; thus organic acids solutions, such as acetic, which are only ionised to the extent of two or three per cent., have a correspondingly weak destructive effect, while inorganic acids, such as hydrochloric, which in dilute solution are almost completely ionised, break up the enzymes with great rapidity.

The degree of resistance as compared in different ferments is subject to wide variation, dependent doubtless upon the chemical constitution of the different enzymes, and arising usually from the environment in which the enzyme has been developed. The most exceptional enzyme in this respect is pepsin, which is most active in a concentration of acid (*i.e.* of hydrogen ion; which would be almost instantly destructive to nearly all other enzymes.

The great majority of enzymes are produced and act in media of alkaline reaction, and although in certain instances it has been shown that even in the case of these ferments the degree of activity is increased by a slight decrease in hydroxyl ion and increase in hydrogen ion beyond their usual normal reaction, there is no doubt that they act well in fluids of alkaline reaction, and according to some observers are at their maximum activity in such media.

While there is no doubt that all the enzymes occurring in alkaline media in the body are quickly paralysed in their action and rapidly destroyed by more than the merest trace of free acid, and also, that a slight trace of *free* alkali above the amount necessary to form bicarbonate with the carbonic acid present has a similar effect; there is much difference of opinion in the literature of the subject as regards the point of optimum action of each ferment. The subject is made very hazy by the fact that earlier workers, not realising that the all-important point was the concentration in the solutions tested of the hydroxyl and hydrogen ion, have worked indiscriminately with free alkalies in some cases and carbonates in others. Kanitz,[1] who is one of the more recent workers on the subject, has examined the effect upon the activity of trypsin of different alkaline hydrates and carbonates, and states that the action is dependent upon the concentration of the hydroxyl ion, and that the range of greatest activity lies between $\frac{1}{70}-\frac{1}{200}$ normal.

It is interesting to observe that the same effect of acids and

[1] *Zeitsch. f. physiol. Chem.*, 1902, vol. 37, p. 75.

alkalies, and the same dependence upon hydroxyl and hydrogen ions, is observable in the case of living cells as has been described above in the case of the enzymes.

The rate of growth and cell division, and the regularity of the latter process, are dependent in large measure upon the reaction of the medium which bathes and permeates the cell. Solutions of various neutral salts in addition have a marked influence upon cell growth and upon the maintenance of a physiological condition of normal metabolism in the cell, but it is the alkalies and acids, and these proportionately to their concentration in hydroxyl and hydrogen ions, which exercise the profoundest influence.

A normal balance in the ratio of hydroxyl and hydrogen ions must be maintained, or the whole of the metabolism and life of the cell becomes abnormal and morbid.

The position of pioneer in this subject must be ascribed, as mentioned above, to Sidney Ringer, who first showed the enormous importance of even minute doses of certain inorganic salts in maintaining a normal condition, and proper performance of physiological functions in living cells.

Ringer, working before the advent and about the time of the introduction of the modern ionic theory of solutions, did not express his results in the language of that theory, and spoke of "the action of calcium or of lime of potassium or of potash salts," instead of, as in modern terminology, of "the effects of molecular concentration of the calcium or potassium ion," and of the effects of acids and alkalies instead of those of hydrogen and hydroxyl ions. But there is no doubt that Ringer thoroughly appreciated that the effects he obtained were due to the one ion of the combination he used although he did not speak of it by that name, and that he recognised without naming them as such the antagonistic action of different ions.

It is necessary to point out the importance of this early and classical work of Ringer's, because it appears to be in danger of becoming forgotten by modern workers, who often do not refer to him in their account of previous work, and appear to use "Ringer's solution," or modifications of it, with little knowledge of its history, or of the fact that Ringer by its use had shown the all-importance of ions for the maintenance of physiological activity, and had demonstrated the action of sodium, potassium,

and calcium ions, and recognised it though not by name as ionic activity, when yet the ionisation theory had obtained but little credence.

Ringer's experiments upon the effects of acids and alkalies were, however, confined to the action of these in maintaining physiological activity, and it remained for Loeb to demonstrate upon the fertilised eggs of the sea-urchin that the rate of growth is appreciably increased by very minute amounts of alkali added to the sea-water, larger amounts stopping the growth entirely.

The writer, led to the subject from its relationship to malignant growth, by the fact that the secretion of the acid in gastric juice was suppressed or diminished, and the alkalinity of the blood increased in cancer, has recently carried out, in conjunction with H. E. Roaf and E. Whitley, a series of observations on the effects of acids and alkalies, and of acids and alkaline salts upon the rate of growth and character of the cell division in the fertilised eggs of *Echinus esculentus*.

It was found that a mere trace of added sodium hydrate, viz. $\frac{1}{10000}$ normal,[1] increased markedly the growth even in the earlier stages, and not merely after one or two days, as Loeb had previously found, while the addition of half as much more sodium hydrate practically stopped the growth altogether, the cells not proceeding beyond the four-cell stage. Addition of double the quantity, $\frac{1}{5000}$ normal, stopped the growth entirely. Addition of hydrochloric acid slowed the growth from the beginning, and at $\frac{1}{5000}$ normal the growth was stopped entirely, all the cells remaining in the single-cell stage.

The action of alkaline and acid salts, such as the phosphates and carbonates, corresponding to their lessened concentration in hydoxyl or hydrogen ions, were less effective, and these salts had to be added in greater concentration.

In addition to the alteration in rate of growth, it was found that the addition of alkali, in more than the minimal concentration of $\frac{1}{10000}$ normal, led to marked irregularity in the size and shape of the cell, and to irregular nuclear division. In the large undivided cells, multiple nuclei were found, and many division

[1] That is, one c.c. of $\frac{N}{10}$ alkali per 100 c.c. of sea-water: this amounts to only one part by weight of sodium hydrate in 25,000 parts by volume of sea-water.

figures were seen with the chromatin reduced in quantity, and in number of chromosomes. In many cases division with three and four centrosomes was observed.

In the cells to which acid had been added, no such increase in nuclei was observed, nor nuclear division figures, and in many cases the chromatin appeared to have been acted upon chemically and to have disappeared.

The experiments illustrate the extreme sensitiveness of the living cell to variations in concentration of the hydroxyl and hydrogen ion, and the importance of a normal reaction of the medium for cell growth and division.

Action of Antiseptics and Protoplasmic Poisons

There is here a great *quantitative* difference in action upon enzyme and living cell respectively, which probably has for its cause the more complex and highly organised chemical structure of the cell, causing it to enter more readily into combination with the antiseptic. That the difference is a quantitative and not a qualitative one, however, is shown clearly by many experiments which go to prove that many of those substances which affect cells, and either render them inert or permanently destroy them, have in greater concentration a similar action upon enzymes. Thus alcohol, chloroform, salicylic acid, carbolic acid, thymol and sodium fluoride, which were at one time regarded as affecting living cells only and without action upon enzymes, have now been shown by various observers to more or less retard the action of enzymes also, and to destroy them in greater concentration, although the action varies in degree in different instances and is always less than that upon the cell. The greater degree of action of such substances upon cells has been often taken advantage of as an experimental aid in observing the nature and products of reaction of enzymes, especially of those proteoclastic enzymes which act in an alkaline medium. For such substances stop the growth of the putrefactive bacteria at a concentration in which they have little action upon enzymes. This experimental use of antiseptic agents was first made by Kühne in studying the products of action of trypsin. In choosing such an antiseptic, one ought to be selected which possesses a strong action upon cells, but as little as possible upon

enzymes, and one which is now often used for the purpose on this account is toluol. Another substance said to be almost without action on enzymes, but most toxic for cells, is hydrocyanic acid; it stops, however, the property of acting upon hydrogen peroxide which is common to nearly all enzymes. Such substances as antiseptics and anæsthetics produce their effects by combination with proteid or protoplasm; and since all enzymes and cells must be allied in consisting of colloids related in character to proteid, it is evident that an ideal substance which will affect the cell and not the enzyme is an impossibility. As a result, we have no hard-and-fast criterion as to whether a given effect is produced by an enzyme or a living cell, except where living cells can be ruled out by microscopic examination. For, while active cells are thrown out of activity by protoplasmic poisons much more readily than enzymes, there exists, bridging over the interval, the sporing form of the cell, which shows the same resistance to the chemical reagents that we have already seen in the case of changes in temperature.

The only true test is that of being able to grow and produce the cell in pure culture, and then obtain with it the previously observed chemical or biological effect. Unfortunately, this in many instances fails, notably in the case of many of the commonest infectious diseases, and we are left unable to trace with certainty the causation of the disease to any particular micro-organism.

A good example of this is to be met with in the case of ordinary vaccine. As is now well known, thanks to the labours of Copeman and of Green, this can be kept in contact with glycerine, or better, with chloroform water, until all extraneous organisms have perished, and nothing can be grown from the preparation in culture media; yet the virus is still present in almost unabated power, as is shown by obtaining the typical effect on vaccination.

The virus may here either be a very resistant spore, which remains alive after all the other organisms have been destroyed by the chloroform, which cannot be cultivated upon ordinary nutrient media, and only commences to develop in the serum of the living body after vaccination; or it may be that the virus of vaccine, as suggested by the writer,[1] is an enzyme with the

[1] "A Chemical Theory as to the Propagation and Development of Certain Infectious Diseases," *The Journal of State Medicine*, April 1901.

property of reproducing itself in the manner indicated under the heading of auto-catalysis (see p. 112).

The theory of infection held at the present day includes as an axiom that all infection must be carried by micro-organisms or parasites. Now, although this has been incontrovertibly demonstrated in many cases, in just as many others, and these including the most common infectious diseases, in spite of innumerable attempts no causal connection between any definite parasite or micro-organism has been shown to exist.

If the above-mentioned axiom is granted, then it follows that vaccine prepared with chloroform must contain an undemonstrable living germ, but otherwise the experimental evidence is far more strongly in favour of the virus being an enzyme, reproducing itself in the manner described under auto-catalysis.

At any rate for the present, the case may serve as an example of how difficult it often is to decide whether a given action is the result of an enzyme or a living cell, because the same agencies which affect one similarly affect the other.

Negative Catalysts

The catalysts which we have hitherto been considering are those which, by their action in diminishing resistance, increase the velocity of a reaction, but a number of substances are known which increase the resistance and so diminish the velocity of the reaction without being changed themselves in the process. Such bodies have been termed by Ostwald negative catalysts. In the language of our formulæ these bodies diminish the value of k, the constant of reaction velocity. That these substances are truly catalytic in their action is demonstrated by the extremely minute quantities necessary to slow the reaction in comparison with the quantities of substratum acted upon. Thus Bigelow has shown that the presence of such a minimal trace of mannite as 0·000,001,4 grm. per cubic centimetre reduces the velocity of oxidation of 800 times as great an amount of sodium sulphite in solution by one-half, and S. W. Young has shown that the oxidation of stannous chloride or sodium sulphite is similarly reduced by mere traces of many organic substances, such as nicotine, brucine, morphine, quinine, aniline, mannite, and potassium cyanide. As

first shown by Graham, the oxidation or ignition of phosphorus is also prevented by traces of organic substances such as turpentine, alcohol, ether, and ethyl iodide.

The number of such negative catalysts known to us is not so large as that of those which increase the velocity, but this is perhaps due to the fact that the interest and attention of the chemist have been chiefly directed towards those substances which cause or hasten reaction rather than to such as stop or retard it.

We have already seen that the reactions caused by enzymes do not proceed with a measurable velocity in the absence of the enzymes, in fact, do not appear to occur at all, and hence there is no necessity for the existence of negative enzymes in the body, and none such have hitherto been described.

It will be pointed out later that the existence of negative catalysts, as has been urged by Ostwald, is a point of evidence against the view that the mode of action of catalysts is *in all cases* by the formation of intermediate compounds.

Anti-Catalysts, Anti-Ferments, and Anti-Enzymes

Under the name of "Antikatalysatoren" or "Paralysatoren" Bredig has designated those substances which by their presence in small quantity retard or stop the action of a catalyst. Such bodies differ from the "negative catalysts" mentioned above in that they do not retard the reaction by directly acting upon the substratum, but by acting upon a positive catalyst which is present and preventing or retarding its action. Bredig places these substances in analogy with the antitoxins. Strictly speaking, all those substances ought to be placed in this group, which have already been described above as affecting enzyme action, such as acids and alkalies, neutral salts, anæsthetics and antiseptics; but it is better to reserve the term, if it is to be used at all, for substances which act after the fashion of catalysts of a second order, so to speak, in quantities small compared to the amount of the primary catalyst.

An example of such an action is the "paralysis" of the action of solutions of colloidal platinum upon hydrogen peroxide, by the addition of traces of hydrocyanic acid. Thus, Bredig, Müller von Berneck, and Ikeda found that the addition of 0·000,000,001

grm. hydrocyanic acid per cubic centimetre to a colloidal platinum solution containing 0·000,006 grm. platinum per cubic centimetre reduced the intense action of the platinsol upon hydrogen peroxide to half its original value. Here it is to be noted that although the quantities of both platinum and hydrocyanic acid are small, that of the platinum is 6000 times as large as the hydrocyanic acid; hence there is no stochiometric relationship, and the action cannot be ascribed to any chemical combination in definite molecular relationship between the colloidal platinum and the hydrocyanic acid. The paralysing effect of the hydrocyanic acid can be removed by passing a stream of air through the solution and so removing the hydrocyanic acid. The "recovery" shows that the catalyst is not destroyed by the "poison" of the anti-catalyst but only inhibited during its presence.

The catalytic action of platinum upon hydrogen peroxide or water-gas is also anti-catalysed by traces of many substances of which the following list is given by Bredig: Iodine, mercuric chloride, hydrogen sulphide, sodium thio-sulphate, carbon-monoxide, phosphorus, hydrogen phosphide, hydrogen arsenide, mercuric cyanide, carbon-bisulphide.

In this group must also be placed the anti-ferments or anti-enzymes which have been shown to exist in the case of the majority of the enzymes. These have usually been obtained by the process of injection into an animal of solutions containing the enzyme in question for a period, and then separating the animal's serum and demonstrating that it contains a substance capable of stopping the action of the enzyme.

The first anti-enzyme was shown to exist by Morgenroth in the case of anti-rennin ("Antilab."); he obtained it in a similar fashion to an antitoxin by injection of increasing doses of rennet solution, and found that both the serum and the milk of the injected animal possessed in a high degree the power of preventing the coagulation of caseinogen by the action of rennin. Anti-bodies have since been obtained to pepsin, trypsin, fibrin ferment, laccase, urease, and tyrosinase.

It has been urged that this reaction of the tissue cells to ferments shows that the toxins of disease and various poisons of animal and vegetable source act similarly to enzymes, and produce their effect in a similar catalytic fashion, until their action is paralysed by the production by the tissue cells of the appropriate

antitoxin. Although there is no doubt whatever as to the production of the antitoxins and of the anti-enzymes, we do not yet know enough regarding the processes to generalise as to the action of toxins and enzymes being the same, and the wide generality in the reaction of the cells in producing an anti-body to almost anything which is presented to them, as has now been demonstrated for hundreds of bodies, indicates that the formation of the anti-body is a general act of protection of the tissue cells and not one specially directed against catalysts or enzymes only.

Weinland states that anti-trypsin exists ready formed under normal conditions in the cells of intestinal worms and in the cells of the intestinal mucosa, as shown by the fact that cell-free extracts of these protect fibrin against tryptic digestion. The proteids of the serum in unaltered form show a great resistance against trypsin, which is lost when they have been chemically altered by coagulation, or the action of chemical reagents, and this resistance has been ascribed to the presence of an anti-trypsin.

Since the discovery of enterokinase (see under) the view has been advanced by Dastre and by Delezenne that the effect of preventing the action of the trypsin is not due to an anti-trypsin but to an anti-kinase, which prevents or opposes the activation of the trypsin by the enterokinase (see p. 111).

Zymo-Excitators or Kinases

The various enzymes are not at first produced in an active form in the cells of the glands which secrete them, but as inactive substances called zymogens or pro-ferments. This was first shown by Langley in the case of pepsin, and the precursor of the enzyme was termed pepsinogen. The method of separating pepsinogen and pepsin, by their varying resistance to alkali, which attacks pepsin with much greater rapidity than it does pepsinogen, was later given by Langley and Edkins. Trypsinogen, the zymogen of trypsin, was later shown to exist by Heidenhain, and since then the existence of a pro-ferment has been shown for most of the enzymes. These zymogens, as has been stated, are inactive while in the cell and exist in granular form visible under the microscope; they are converted into the active form, either at the time of

secretion or later, on coming in contact with certain substances which have been termed zymo-excitators, or, in certain cases, kinases.

This action is possessed by all dilute acids, and it is probably in this way that pepsinogen and prochymosin, the zymogens of pepsin, and of rennin or chymosin, are activated in the stomach.

In the case of the trypsinogen of the pancreatic juice it has been shown by Pawlow and Chepowalnikoff that the activation takes place by means of a substance secreted by the intestinal mucosa. It was found that while the secretion from a pancreatic fistula had scarcely any action upon proteid, the addition of a small quantity of succus entericus caused it rapidly to become very active. Such action took place only upon the trypsin and not upon the other pro-ferments of the pancreatic juice, but an increase in the activity of the lipo-clastic enzyme occurred on the addition of bile.

The substance which so behaved as a zymo-excitator or kinase to trypsinogen was called *enterokinase* by Pawlow, and has since been the subject of much investigation and discussion as to whether it is itself a true ferment, a "ferment of ferment," as it was styled by its discoverer, who regards it as a ferment on account of the small quantity necessary to activate a much larger amount of trypsinogen, and the fact that it is destroyed, although slowly, at the usual temperature of destruction of enzymes (65° C.).

Delezenne and Dastre and other French observers deny that enterokinase is an enzyme, but consider that it forms a compound with trypsinogen, which is the actual proteoclastic ferment trypsin. This view is based upon the observations that a definite amount of enterokinase was required to develop the maximum amount of activity in a given amount of trypsinogen. They hence regard the enterokinase as an "amboceptor" in the language of Ehrlich, which serves to link together the attacked proteid and the trypsinogen, and so invokes the proteid cleavage. A further support for this view was the supposed observation that enterokinase combines with fibrin and can be so removed from solution.

Other French observers have pointed out as evidence against enterokinase being an enzyme, that it is much more slowly destroyed by heat than are most other enzymes. Thus Largnier des Barcels claims to have obtained activation although in lessened

degree on extraction of the mucous membrane with boiling saline, and Biery and Henri state that they have heated enterokinase for twenty minutes to 120° C. without entirely destroying its action.

Bayliss and Starling, however, have brought forward strong evidence in favour of enterokinase being a ferment. Thus, they have shown that there is no stochiometric relationship between the amount of trypsinogen and the amount of enterokinase necessary to activate it, as little as 0·0001 c.c. of an active enterokinase being capable of activating 5 c.c. of pancreatic juice provided it was allowed two or three days to act. The rate of activation was also found to be proportioned to the amount of enterokinase added. Bayliss and Starling accordingly consider that the observation of Delezenne, that a definite amount of enterokinase is required to produce full activation, was due to a sufficient attention not having been given to the time relationships of the reaction, so that the full effects of the smaller quantities of added enterokinase were not allowed to develop, and secondly to slow autodestruction of the trypsin first formed in the longer period necessary to effect the conversion with the smaller quantities of enterokinase.

It is an observation dating back to Kühne's earlier experiments that in preparing active extracts of pancreas, the trypsinogen of the fresh gland cells can be activated by extraction with very dilute acids, such as acetic acid, and that such treatment always yields more powerful extracts.

Since the discovery of enterokinase, Vernon has also shown that an inactive pancreatic extract can be rendered active by addition of an active preparation, or of an active commercial trypsin preparation. This was attributed by Vernon to the presence of an enterokinase different in some of its reactions, such as greater sensitiveness to alkalies, from intestinal enterokinase.

Bayliss and Starling found, however, that this did not apply to pancreatic juice, which they found remained inactive not only when treated with active glycerine pancreatic extract supplied by Vernon, but also when acidified with weak or strong acid and then neutralised or made alkaline after varying periods, or when left in contact with fibrin, yeast, taka-diastase, platinum black, gastric juice in acid or alkaline media, saliva, hydrogen

peroxide, and sodium amalgam. The juice stood most of these treatments extremely well, that is, it could be activated afterwards by enterokinase. The authors accordingly regard the action of enterokinase as absolutely specific.

They were unable to confirm the statement of Delezenne mentioned above, that fibrin took up enterokinase from solution, as also that enterokinase could be obtained from lymphatic glands or Payer's patches as stated by Pelezenne, but found it specifically confined to the mucous membrane of the small intestine in its upper portion, extracts of the mucous membrane of the ileum being inactive.

Further evidence brought forward in their second paper by Bayliss and Starling against trypsin being considered as any compound of trypsinogen and enterokinase was derived from the formation of the anti-body (anti-kinase) in the animal's serum, as a result of subcutaneous injection of enterokinase. On the view of Delezenne, the anti-tryptic action of normal serum must be due to anti-kinase, trypsin consisting of kinase (enterokinase) and trypsinogen, and hence subcutaneous injection of kinase (enterokinase) should increase the anti-tryptic action of serum. This, however, it was not found to do, but to produce a directly anti-kinasic body instead, neutralising enterokinase if it were allowed to act upon this before the mixture was added to a trypsinogen solution.

The authors, therefore, conclude that the anti-trypsin of normal serum is really anti-trypsin or the anti-body of a specific substance trypsin, and not anti-kinase.

Activation in the case of the superoxydases is produced by minute traces of alkalies, which cause a marked increase in the action upon peroxide of hydrogen possessed by the solutions of most enzymes and tissue extracts.

The action of the manganese salts in increasing the power of laccase, and of calcium salts in increasing the rapidity of action of the coagulating enzymes, may also be mentioned in this connection.

In inorganic reactions examples of similar effects are seen in the action of minute traces of copper salts in aiding the catalysis of hydrogen iodide and hydrogen peroxide by iron salts, and in the action of traces of alkali in increasing the power of colloidal platinum solutions upon hydrogen peroxide.

Auto-Catalysis and Infection

In the course of any reaction the products formed may themselves in certain cases act as catalysts and alter the velocity of the reaction. For example, in the hydrolysis of esters by water, the process is at first so slow as to be inappreciable, but as the process goes on, the hydrogen ions of the acid set free in the reaction itself act as a catalytic agent upon the portion of ester still undecomposed and hasten the reaction. To such a process Ostwald has assigned the term of "auto-catalysis," and has pointed out that such a process may play an important rôle in biological processes, and that the course of such auto-catalytic reactions bear a close analogy to the phenomena of fever.

If the substance produced acts as a negative catalyst it will have only the effect of making the reaction run more slowly, and as its effect will increase with rising concentration, the result will be that the course of the reaction will resemble that of an ordinary reaction, save that the tendency to run more slowly as the equilibrium point is approached will be increased.

When, however, the substance formed in the reaction acts as a positive catalyst, the course of the reaction becomes markedly changed in a most interesting fashion.

For while the ordinary non-catalysed reaction, or a reaction in which the concentration of catalyst remains constant, as in all those which we have previously considered, the velocity of reaction diminishes steadily onward from the beginning, in an auto-catalytic reaction, as the quantity of auto-catalyst increases as the reaction proceeds, the reaction is correspondingly hastened. Hence a reaction of this type may begin by being barely perceptible, but gathering way as it proceeds like a descending avalanche, may in the end become stormy or explosive.

Examples of such auto-catalytic reactions are seen in the tendency of many explosive substances to spontaneous explosion. Thus if gun-cotton be not most carefully washed from oxides of nitrogen, these products, present in too minute quantity at first to cause any change, may set up a slow and at first inappreciable reaction, which, slumbering at first, gradually increases in velocity and finally fires off the gun-cotton.

A good example of auto-catalysis is quoted by Ostwald in the case of the action of nitric acid in dissolving metals. As has been noticed by several observers, pure nitric acid free from nitrous acid scarcely at first attacks many metals, such as copper, mercury, and zinc, although the impure acid readily oxidises them with production of nitrous acid. If a trace of a nitrite be added, however, the reaction at once commences and momentarily becomes more energetic, as it is continually auto-catalysed with increasing energy by the nitrous acid formed in the reaction.

The writer has pointed out that such a process of auto-catalysis, induced by a trace of enzyme, may be the means of infection, and of the reproduction of the virus in many of those infections diseases, such as the acute exanthemata, in which notwithstanding much bacteriological research no causal connection of any living organism has yet been demonstrated.

The incubation period would be that required for the production of the auto-catalyst in sufficient quantity to cause a general reaction with the tissue cells. The auto-catalyst, which would act as the toxin of the disease, would still go on increasing in quantity until it had attained the concentration for maximum effect; but at the same time the tissue cells would react to it as to an ordinary toxin and produce the antitoxin, by which the toxin would be neutralised and rendered inert, and so the course of the disease would be limited.

The length of time and the exactness of duration of the incubation period form no objection to such a view, for the time of the initial period of auto-catalytic reactions is often prolonged, and the duration of the incubation period would be determined by the reaction of the tissue cells affected, and would but little depend upon the amount of the trace of auto-catalyst which originally carried the infection, unless this were very large.

Further, it may be pointed out that in many cases in which the toxin arises from the products of bacterial growth, the length of the incubation period depends upon the period of reaction of the tissue cells, and not upon any cycle of development of the parasite, as, for example, in enteric fever, where the Bacillus typhosus possesses no period of growth corresponding to the period of incubation.

Finally, variations in immunity in different individuals here,

equally as in parasitic infection, will depend upon the individual variations in the blood as a suitable medium for such a reaction, upon the presence or absence of an anti-body at the outset capable of neutralising the trace of auto-catalyst bearing the infection, and upon the reactive power of the tissue cells to the auto-catalyst or toxin.

The Specific Nature of Catalytic Action

It has already been mentioned that enzymes are very specific in their action, and in the character of the products which they produce from the substratum.

It has been well pointed out by Bredig that this cannot be converted into a criterion for distinguishing the action of an enzyme from that of an inorganic catalyst. For although such specific action is seen at its maximum in the enzymes, it is also observable in the action of many inorganic catalysts. Thus while certain catalysts, such as the hydrogen ion, are very general in their action, catalysing most hydrolytic reactions, such as cleavage of all disaccharides, amyloses, and esters, other catalysts are quite elective in action. Examples quoted by Bredig are potassium bichromate, which energetically catalyses the oxidation of hydriodic acid by bromic acid, but does not act upon that of the same acid by iodic acid or potassium persulphate; iron and copper salts, which intensely catalyse the oxidation of potassium iodide by potassium persulphate, but not the oxidation of sulphurous acid by the same oxidising agent.

On the other hand, as also pointed out by Bredig, emulsin in addition to hydrolysing the glucoside amygdalin with which it is naturally associated, similarly acts upon many other substances, such as Arbutin, Helicin, Salicin, Phloridzin, Daphnin, Coniferin, Aesculin, and Lactose.

There is no doubt, however, although no fundamental difference can be deduced therefrom, that the vast majority of the enzymes are more highly selective in their action than the inorganic enzymes.

Thus, as has been beautifully shown by the researches of E. Fischer, an enzyme may act upon one stereo-isomer and not upon the other, the action being thrown out by the change in position of a single group.

To use Fischer's striking analogy, the ferment and its sub-

stratum must fit like key and lock, or the reaction does not occur.

A similar selective action is seen in the case of the organisms which induce fermentation, as was known before the date of Fischer's researches upon the enzymes, as a result of the investigations of Pasteur, who showed that only dextro-rotatory racemic acid was attacked, and was able to separate the lævo-rotatory stereo-isomer by such means. It is only those sugars with six or nine carbon atoms that are fermentable, and of these only certain of the stereo-isomers belonging to the "*d*" series. This fact has been used by Fischer for the purpose of separating the "*l*" stereo-isomers from the inactive mixture of "*d*" and "*l*" sugars obtained by synthetic procedures.

That the hydrolytic action of enzymes upon the sugars depends upon the stereo-isomeric form has been clearly demonstrated by E. Fischer, E. Fischer and E. Frankland Armstrong, and other observers, by the action upon derivatives of the fermentable or hydrolysable sugars. By combination of the hexoses with methylic alcohol, the methyl-hexoses are obtained. In each such case two stereo-isomeric compounds are obtained, which Fischer termed α and β compounds. Thus from glucose and methyl-alcohol one obtains α-methyl-glucoside, and β-methyl-glucoside. If now the effect of the yeast enzymes is tested upon these two artificial compounds, which only differ in their stereo-chemical relationships, it is found that only the α-modification is hydrolysed, the β one being quite resistant. If instead of the yeast enzymes, however, the emulsin enzymes be employed, the β compound is now the one which is attacked, similarly to a large number of naturally occurring glucosides.

In collaboration with E. F. Armstrong, Fischer extended his observations to show that the same laws held in the case of other sugar derivatives, such as the osones and artificial disaccharides.

It can accordingly be predicted, when the constitution and stereo-chemical relations of a body are known from its derivation, whether it will be attacked by a given enzyme or not.

It is worthy of note that it is not whether a sugar is artificial or natural which determines the attack, but whether it possesses a certain molecular configuration the identity of which must extend even into stereo-chemical exactness. Hence it follows that the specific action does not mean that an enzyme can attack one sub-

stance only; it may attack many hundreds if only they all possess a given molecular and stereo-identical grouping, and it is this relationship upon which the specific action is based. Also the extent of action depends on the group attacked. Thus both invertase from yeast, and emulsin from bitter almonds, attack the glucoside, amygdalin; but invertase only detaches a molecule of glucose, leaving the remainder untouched, while emulsin, attacking a different grouping, breaks the amygdalin up into benzaldehyd, hydrocyanic acid, and glucose. Other natural glucosides, not possessing the particular grouping attackable by invertase, are resistant to that enzyme, and are attacked only by emulsin.

An interesting fact in the case of the cell, showing physiological adaptation to environment and nutrition, is that the action depends upon the food supply; the cells forming in all probability enzymes to suit the configuration of the molecules at their disposal. Thus, *aspergillus* cultivated on a nutrient medium containing lactose or β-methyl-galactosid acquires the property of hydrolysing these, while if grown upon α-methyl-galactosid the property is acquired of attacking this substance.

The outcome of these investigations appears, then, to be that by specific action must be understood *entire* conformity between the particular enzyme and a corresponding molecular grouping or structural arrangement in the molecule attacked, and not that a single substance only is attacked by the same enzyme.

It may perhaps, in conclusion, be pointed out that this may serve to explain what sometimes seems a most fantastic distribution of certain enzymes in nature. Thus the stomach of the fish contains a milk-curdling enzyme, similar to that of the mammalia, although such an enzyme never comes in contact with milk, and never has in the development of the fishes. The presence of such an enzyme cannot certainly be regarded as a prevision of Nature for the coming mammalia, and points to the fact that the milk-curdling enzyme must have other functions than the coagulation of milk. Since such ferments are also found in plants, it follows that they must, like the saccharolastic enzymes, be adapted to some definite molecular grouping upon which they act, and that milk coagulation can be but one example of their activity.

The Chemical and Physical Nature of Enzymes

Little is known regarding the chemical nature of enzymes, because all attempts to isolate them in a state of purity have hitherto failed. In fact, there is nothing to give certainty that at the end of any process the product in the case of such complicated substances is pure, a remark which applies equally to ordinary proteids. None of the criteria of purity in the case of an ordinary crystalloid apply in the case of a colloid of complex constitution, except constancy of percentage composition. It does not crystallise out,[1] it has no definite melting-point, it does not affect the freezing-point or boiling-point,[2] it cannot be synthesised by reactions which can be followed in their course, and it is probably polymerised to a high degree. Hence it would be more correct to say that we do not know whether proteids and enzymes have ever been prepared in a state of purity, rather, than is usually done, that they never have been so prepared.

One of the great difficulties in freeing enzymes from other substances, such as proteids, is that they share the common property of colloids, of being easily thrown mechanically out of solution, by electrolytes or organic precipitants of proteids.

The methods of attempted separation have differed in the case of different enzymes and cannot all be gone into in detail here.

One general method is that of allowing the ferment to digest out any substratum present naturally with it as much as possible where it can be made to do this, as in the case of the proteoclastic enzymes, and then to precipitate it by means of some indifferent precipitate such as calcium phosphate in the case of pepsin, or by the addition of collodion or cholesterin dissolved in a mixture of alcohol and ether. Another method when the ferment does not rapidly undergo alcohol coagulation is to remove the accompanying proteid by allowing the mixture to stand for some weeks under alcohol, and then dissolve the ferment by means of water, as in the case of fibrin ferment (thrombase). Another method is to allow the strong solution to remain standing at the freezing-point for some days, when the enzyme falls out in granular

[1] Unless when in combination with other bodies which confer crystalloid properties.

[2] At least to such an extent that measurements can be made.

form, as in the case of fibrin ferment precursor (pro-thrombase) in oxalated plasma, or of pepsin from pure gastric juice.

The investigations have shown that enzymes are not in all cases proteids. Thus the purer preparations of pepsin and invertase do not give the proteid colour tests.[1] In elementary composition the enzymes do, however, resemble the proteids more than any other class of bodies. In addition to being salted out, or precipitated out like colloids, the enzymes are further shown to be colloids by the fact that they do not diffuse through parchment paper, or diffuse with great slowness.

The so-called Inorganic Enzymes or Metal-Sols

It has already been repeatedly stated that the enzymes are a particular class of the bodies known as catalysts, which modify the conditions of a reaction. The enzymes differ from most of the inorganic catalysts, however, in that they are colloids, and to this certain of the differences in action between inorganic catalysts and enzymes are due.

The method of Bredig for obtaining, in the case of certain metals, catalysts of inorganic nature in colloidal solution as metal-sols is hence of high interest. These metal-sols have been termed inorganic enzymes by Bredig from their close resemblance in many respects to the enzymes produced by living cells; but it is questionable whether the use of such a term is justifiable, since the properties of such colloidal solutions are only exaggerations, probably on account of increased surface, of the action of the finely divided metals when not in solution, and most of the reactions destroying or removing the properties of such solutions may be ascribed to the throwing of the metal out of solution or to alteration of the active surface.

We have no proof that the similar actions in the case of the enzymes are due to similar causes. Also, we have no case where these colloids act upon another colloid as in the case of enzymes, nor of any hydrolytic action upon organic bodies caused by their

[1] In other cases it appears almost certain that either the enzyme is a compound proteid or closely attached to one, for example, trypsin and thrombase. Also the precipitate from gastric juice in the cold appears to be a compound proteid according to recent observations of the Pawlow school.

agency as in the case of enzymes. In fact, their action is confined to simple reactions upon inorganic bodies, such as the catalysis of hydrogen-peroxide into water and oxygen, or of hydrogen and oxygen to form water. Such reactions are shown by the metals concerned in finely divided form apart from solution, and the increased activity is merely due to finer division, and is removed by anything which interferes with the action of the large surface. Accordingly, it appears to the writer that the term "inorganic enzyme" is not a very appropriate name for a colloidal solution of platinum, since it indicates that we possess more knowledge of the mode of action of enzymes than we really do.[1] The same applies to the term "poisoning" as applied to the action of hydrocyanic acid and sulphuretted hydrogen in stopping the action of such metal-sols when added in minute traces, for this term is one which has hitherto been applied to the destruction of life by reagents, and until we know that the stopping of the action of colloidal platinum by hydrocyanic acid arises from the same causes as the poisoning of a living cell by that reagent, it is dangerous to apply the same term to both processes. By a strange process of reasoning this same "poisoning" of the colloidal platinum by hydrocyanic acid has been put forward as supporting the view that the platin-sol is an "inorganic enzyme." Now, if the process of "poisoning" proves anything at all, it proves that the colloidal platinum is a living cell, for it is living cells and not enzymes that are destroyed by the action of hydrocyanic acid, by which in such minute concentrations most enzymes are entirely unaffected. The only action related to enzymes which is stopped in the same degree by hydrocyanic acid is the identical one to that stopped in the case of colloidal platinum, namely, the action upon hydrogen-peroxide, which is found not only in practically every enzyme but in nearly all tissue extracts. This action is ascribed usually to enzymes called "oxydases" or "peroxidases," occurring generally in all tissues where oxidative processes occur; but our knowledge of the subject is scarcely sufficient to state whether it is to be ascribed to any

[1] The suggestion of Oppenheimer (*Die Fermente*, p. 46), that it would be better to call the enzymes "organic catalysts," is much better if any change must be made. For it is much better to retain the name catalyst as a general class name, including both inorganic and organic, and the enzymes now form a well-defined group of organic catalysts for which the name ought to be retained.

particular enzyme or group of enzymes, or is a general property attaching to all enzymes. The action, as has been stated, is stopped by a trace of hydrocyanic acid without interfering with the specific action of the enzyme accompanying the "peroxidase," it is also stopped by the action of heat similarly to that of an enzyme, but it has not clearly been shown that the substance causing the oxidation is not altered in the process, nor that there is no stochiometric relationship between the quantity of "peroxydase" and the amount of hydrogen-peroxide changed. In fact, it appears that the "peroxydases" are very sensitive, and that after conversion of a certain amount of peroxide the action ceases.

Whether the "peroxidases" be specific enzymes or not, the action of hydrocyanic acid in stopping or "poisoning," both in their case and that of colloidal platinum, is confined to one reaction, that of the conversion of hydrogen-peroxide into water and oxygen, and it appears to the writer that this is a narrow basis on which to lay any weight as a proof of identity in mode of action between enzymes and colloidal platinum. It has not even been proved, for example, that hydrocyanic acid does not act directly as a negative catalyst to the reaction concerned.

While it cannot be admitted, therefore, that the terms "inorganic enzyme" and "poisoning" can be legitimately employed in connection with these metallic catalysts, the effects obtained with them are of high interest in regard to the manner in which a colloid in solution can act as a catalyst.

It was already known to Faraday that all porous bodies, and especially certain metals, such as platinum, possessed the property of absorbing large amounts of gases. The velocity with which the gases are absorbed increases with the state of sub-division of the metal, and is best seen in the case of platinum when this is used in the finely divided form of platinum black. Faraday pointed out also that this sub-division favoured the action of the platinum black as a catalyst by increasing the local concentration of the substances to be catalysed, and even showed that the action of catalysis by platinum black of an explosive mixture of hydrogen and oxygen was stopped by the presence of traces of carbon bisulphide or sulphuretted hydrogen. It is wonderful how closely this view of Faraday comes to the modern view with regard to the mode of action of a colloidal catalyst or enzyme.

The catalytic action of finely divided metals was taken up by Bredig and Müller von Berneck, and tested in the case of platinum and hydrogen-peroxide. For this purpose, finding platinum black difficult to subdivide and suspend in solution, they evolved the ingenious method of obtaining the platinum in colloidal solution. Colloidal solutions of metals had already been prepared by Carey Lea by chemical means, such as colloidal silver by reduction of silver nitrate by ferrous sulphate; but Bredig discovered the much simpler method of detaching the metal from the negative electrode by means of a high potential in distilled water.

The process consists in establishing an electric arc between stout metallic electrodes of the metal of which it is desired to make a colloidal solution, in as pure as possible distilled water, which must be maintained at a low temperature. The conductivity of the water must be low, or otherwise electrolytic conductivity comes in and destroys the process, and, further, the presence of electrolytes tends to precipitate the colloidal metal.

The process can be best carried out by utilising the electric lighting mains (the usual 110 volt, constant current, circuit), and placing in the circuit an ammeter, a flat crystalling dish containing about 100 c.c. of pure distilled water which has previously been boiled to expel dissolved carbon-dioxide, and a variable fluid resistance which is regulated to give a current of 4 or 5 ampères after the electric arc has been established under the distilled water. The electrodes consist of stout platinum, gold, or silver wire from 1 to 2 millimetres in diameter, and can either be passed through two glass tubes so as to be easily handled, or sealed into two glass tubes which are filled with mercury by means of which electric contact is made with the platinum.

In using the apparatus a short circuit is made between the two electrodes under the distilled water, and then separated, when an electric arc is established, and glows beneath the water; minute particles of metal are now detached from the negative pole, some of which pass to the positive pole, but others remain in the distilled water and form an exceedingly fine suspension or colloidal solution of the metal.[1]

The electric arc frequently breaks down on account of variations in resistance, when it must be established as before.

[1] That the metal comes from the kathode was shown by Bredig by weighing the electrodes before and after use, when a loss was found in the kathode, and a gain of less amount in the anode.

The colloidal suspension begins at once, and in a few minutes a fluid of a deep dark colour is obtained, which in the case of platinum resembles the colour of reduced osmic acid solutions. Accompanying the colloidal particles there is always a certain amount of metal in coarser suspension, which may be removed by allowing to stand, filtering, or centrifugalising. The dark-coloured solution remaining is perfectly clear, it may deposit a little more platinum in the first day or two, but the rest remains in solution for months, and apparently indefinitely.

In the case of gold the colloidal solution has a deep dark-red colour, while the silver-sol varies in colour from dark reddish brown to olive green, according to the dilution and fineness of the subdivision.

All the metal-sols are extremely sensitive to the presence of electrolytes; they cannot be prepared in normal saline, and even addition of normal saline to them when prepared precipitates them entirely from solution.

This somewhat militates against their employment for intravenous injection as germicides in septicæmia, as has been recommended in the case of silver-sol, for example, in septic endocarditis; for it is probable that the sol will be precipitated by the saline of the plasma, and so its effectiveness diminished or destroyed.

The writer has found that platin-sol in distilled water can be injected in animals without any untoward results and apparently without affecting the animal in any way. It was found that the addition of 0·7 per cent. saline to the platin-sol in distilled water had the effect even at this dilution of completely precipitating it. An attempt to prepare the platin-sol in isotonic glycerine solution was unsuccessful; more success attended the addition of glycerine to make an isotonic solution after first preparing in distilled water, for some of the platinum remained in colloidal solution, but even here there was considerable initial precipitation. It must be remembered, however, that one colloid has often a great effect in preserving another against precipitation by electrolytes, and hence it is possible that on intravenous injection of the platin-sol in distilled water, the proteids of the plasma may assist in retaining the platinum in colloidal solution against the precipitating action of the plasma saline.

Thus Lobry de Bruyn has shown that when two salts which form a colloidal precipitate, such as potassium chromate and silver nitrate, &c.,[1] are mixed in a warm 10 per cent. gelatine solution, the

[1] A similar action occurs in preparing a silver gelatine mass, as for silver staining of mammalian lung epithelium, and is connected with the success of such an operation.

mixture remains quite transparent, which is due to the colloid gelatine preserving the amorphous precipitate in colloidal solution. No such effect is obtained with crystalline precipitates.

The catalytic reactions of metal-sols which have been studied are the conversion of hydrogen-peroxide into water and oxygen, and of hydrogen and oxygen to water. These have been most studied in the case of the platin-sol by Bredig and his pupils.

The action of platin-sol upon peroxide was found to follow approximately the logarithmic law for a mono-molecular reaction, but in the case of hydrogen and oxygen Ernst found that the velocity was proportional to the absolute amount of platinum present, and in the peroxide experiments it was found that the activity did not vary in simple direct proportionality to the concentration in platinum, but was expressed by the empirical law, $\frac{k_1}{k_2} = \left(\frac{C_1}{C_2}\right)^b$, in which k_1 and k_2 are the velocity constants corresponding to concentrations in platinum of C_1 and C_2, and b, instead of being unity, is a constant, the value of which lies between 1·6 and 1·3. Hence we see that there are abnormalities in the action of these inorganic catalysts, similar to those already described in the case of the enzymes. The abnormalities are explained upon the basis of intermediate compounds between catalyst and substratum, or products of reaction.

Even equal platinum concentrations do not always lead to the same velocity of reaction, this depending upon variations, in fineness of subdivision of the platinum, and upon the previous history of the solution, such as its age, the temperature at which it has been preserved, &c.; to this variation van Bemmelen has in analogy with similar phenomena in magnetisation applied the term "hysteresis."

The dilutions in which these colloidal platinum solutions exert their catalytic effects are enormous. Thus a solution containing only 0·000,01 grm., that is, $\frac{1}{100}$ milligram, per c.c., acting upon a solution of hydrogen peroxide containing 0·06 grm. per c.c., converted more than the half in forty minutes. The action of $\frac{1}{100000}$ to $\frac{1}{3000000}$ milligram of colloidal platinum upon more than a million times its weight of hydrogen peroxide could still be detected.

Ernst also found that $\frac{1}{10}$ milligram of colloidal platinum

catalysed 70,000 times its own weight of hydrogen and oxygen to water at ordinary temperature without losing its activity in the least.

The retarding action (or so-called "poisoning" action) of certain substances upon the catalytic action of these inorganic colloidal catalysts is also remarkable upon account of the minute quantities required to stop the reaction.

For example, the addition of 0·000,000,014 grm. per c.c. of hydrocyanic acid, to the above-mentioned experiment, in which 0·000,01 grm. per c.c. of platinum was acting upon 0·06 grm. per c.c. of peroxide, served to decrease the rate of change by one-half, and the addition of 0·000,000,001 grm. per c.c. of hydrocyanic acid in another experiment had an equal effect upon the velocity of conversion by a colloidal platinum solution in concentration of 0·000,006 grm. per litre. It is to be noted that the hydrocyanic acid produces its marked effect in amounts of $\frac{1}{200}$ to $\frac{1}{700}$ of that of the platinum in these experiments, so that there cannot be an ordinary chemical compound between the catalyst and the "paralysator." It must be remembered, however, that even at these dilutions the platinum is present not in molecular form but in suspended particles, and that to stop the reaction it is only necessary for the hydrocyanic acid to combine with the surface layer of each platinum particle, which may explain the small amount necessary. Such combination might be either of a chemical or physical nature, but the latter is the more probable.

Before leaving the subject of the action of hydrocyanic acid it may be recalled that it possesses a similar action in minute traces, as shown by Schönbein in the case of the peroxidases accompanying ferments. Now if these peroxidases are responsible in the tissue cells for the uptake of oxygen by the protoplasm, it may well be that the poisonous action of hydrocyanic acid in such minute doses is due to interference with the action of the peroxidases.

A similar effect is probably found in what has been termed the "oligo-dynamic" property of heavy metals, as a result of which a trace of certain metals in distilled water, too minute for all chemical analysis, leads to the death of living organisms. Thus mere immersion of a strip of clean copper in a vessel of distilled water containing a number of tadpoles, which would other-

wise live therein for weeks, is sufficient to kill the animals in a few hours.

A large number of other substances were also quantitatively tested by Bredig and his co-workers as to their action upon the catalytic power of colloidal platinum. Thus so little as one part of sulphuretted hydrogen in 300,000,000 parts showed clearly a retarding effect upon the catalysis. The colloidal platinum is capable of recovery from the action of some of the reagents employed and not from others. Thus if a stream of air be passed for some time through a solution of colloidal platinum which has been rendered inert by addition of a trace of hydrocyanic acid, so as to remove the latter, the activity is again restored.

Bredig states that recovery can take place from hydrocyanic acid, carbon monoxide, phosphorus, hydroxylamin, but not from cyan-iodide, iodine, sublimate, or arseniuretted hydrogen.

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CHAPTER V

THEORIES AS TO MODE OF ACTION OF CATALYSTS AND ENZYMES—CORRELATION OF THEORIES—MEANS OTHER THAN CATALYSIS BY WHICH CHEMICAL REACTIONS CAN BE INDUCED, OR REACTION VELOCITY VARIED

1. *The Theory of Molecular Vibrations.*—Theories having a bearing upon catalytic action first began to be formulated for the purpose of giving some explanation of the processes of fermentation which at the time occupied the attention of the chemist more than of the biologist. The brilliant work of Pasteur had not as yet illuminated the action of micro-organisms in these processes, when Liebig, searching for some explanation of the action of yeast upon glucose, evolved the theory that such action arose because the yeast was itself involved in a process of chemical activity or decay and provoked as a result chemical activity in the sugar. In support of his theory Liebig adduced many examples from inorganic chemistry of where the chemical activity of one body influences that of another, and of cases where even mechanical vibration causes changes, as in explosive bodies, such as the iodide or chloride of nitrogen.

It is not to be wondered at that Liebig in those early days looked upon those activities which are now known to be due to micro-organisms as due to chemical action entirely.

Many of the experiments carried out by Liebig and others of his school of thought, to attempt to exclude the possibility of micro-organisms being present, were well and scientifically thought out, and if they led the distinguished chemist into error, it was only because of failure to realise at that early date the difficulty not only of maintaining but of preserving complete sterility. When one reads the experiments by which it was sought to prove that it was the access of air and not the presence of micro-organisms which conditioned fermentation, one is forced to admit that they

were made with great ingenuity, and to acknowledge that the errors to which they led were due to the stage of advancement of scientific knowledge at the time, and not to want of care on the part of the experimenter.

The indisputable proof by Pasteur and others that fermentation is due to the life processes of micro-organisms and the strong position which Liebig took in combating this led to his theory that catalytic activity was due to the activity of one body being transferred to another, and so to the catalysis of the latter, fell into disrepute, until it was revived by Nägeli in the form of the theory that the decomposition by a ferment is due to a transference of "molecular vibration" from the ferment to the substratum. The molecules of the substance undergoing the fermentation were supposed to be already in a condition of vibration, which became increased by the sympathetic swingings or vibrations in the ferment to such an extent that the vibrations passed the point of equilibrium, and the substance accordingly underwent decomposition. This revival of a part of Liebig's view by Nageli was only applied by him to the organised ferments, and he stated that it was the living protoplasm which acted directly in increasing the molecular vibrations. He maintained the view that the living cell and the enzyme were different both in mode of action and in their physiological rôle, the enzymes preparing the food-stuffs for use in physiological activity, and the organised ferment or living cell making use of the material so prepared.

It is now clear that Liebig was wrong in regarding the yeast as being in a process of decay and giving rise to accompanying chemical changes in the substratum which lay apart entirely from any life processes.

But if we strip Liebig's statements free from this error there is much in them which even to-day demands attention.

We shall see later that no single theory which has ever been put forward is capable of accounting for all cases or classes of catalytic action, and it is quite probable that catalysis is due in one case to one cause or factor, and in another case to quite a different one, so that different theories may by no means be incompatible.

It seems to the writer that for those cases of almost instantaneous reaction due to mechanical vibration or friction, or to

contact with another chemical substance, Liebig's view of the *vis inertiæ* to reaction, or, as we would term it, of the resistance being overcome by an enhancing of molecular vibration, fits the facts better than any other theory.

A concise account of the view can perhaps best be put in Liebig's own words:—

"It is well known that there are chemical compounds of so unstable a nature that changes in temperature and electrical condition, or even simple mechanical friction, or contact with bodies apparently totally indifferent, cause such a disturbance in the attraction of their constituents, that the latter enter into new forms, without any of them combining with the acting body. These compounds appear to stand but just within the limits of chemical combination, and agents exercise a powerful influence on them which are completely devoid of action on compounds of a stronger affinity. Thus, by a slight increase of temperature the elements of hypochlorous acid separate from one another with evolution of heat and light; chloride of nitrogen explodes by contact with many bodies which combine neither with chlorine or nitrogen at common temperatures; and the contact of any solid substance is sufficient to cause the explosion of iodide of nitrogen, or of fulminating silver.

"It is evident that the active state of the atoms of one body has an influence upon the atoms of a body in contact with it; and if these atoms are capable of the same change as the former, they likewise undergo that change; and combinations and decomposition are the consequence. But when the atoms of the second body are not of themselves capable of such an action, any further disposition to change ceases from the moment at which the atoms of the first body assume the state of rest, that is, when the changes or transformations of this body are quite completed."

It is worthy of note, in view of modern opinion upon the subject, that Liebig was well aware of the fact (and actually deprecates the use of the term *catalytic force* for this very reason), that a catalytic agent need not necessarily start a reaction, but only increase the rapidity of one already in progress; instancing as an example the catalysis of hydrogen peroxide by platinum or silver.

There is no other theory which appears to the writer to cover the case of detonating substances in which the mechanical percussion acts as a catalyst in inducing chemical reaction; similar

action can in many instances be induced by contact with a chemical substance, and by analogy it is probable that in a certain number of cases the explanation of catalysis may lie in molecular kinetics.

In any case the theory does not deserve dismissal in the cavalier fashion in which it is often treated by modern physical chemists.

Other instances of mechanical movement affecting chemical or physical change are the effects of motion in causing crystallisation from supersaturated solutions. While this action is not marked in some cases, it is conspicuous in others, as in the two cases quoted by Liebig, of acid potassium tartrate on shaking mixed solutions of a potassium salt and tartaric acid, or on stirring with a glass rod the sides of a beaker containing ammonio-magnesium phosphate, when the crystals separate out on the parts rubbed by the rod. Similar effects are seen in the crystallisation out from amorphous solids (or solid solutions) of sugar, glass, or sulphur on mechanical stimulation.

II. *The Theory of Intermediate Reactions.*—This theory of catalytic action, in its application at least to one classical reaction, viz. that of production of sulphur trioxide from sulphur dioxide and oxygen by the catalytic action of nitric oxide, was introduced even before the previous one, although it does not appear to have occurred to any one to invoke it as a general theory of catalysis until long after Liebig's theory had been propagated. As early as 1806 Clement and Désormes described this process in the leaden chamber sulphuric acid process as arising from an alternating reduction and oxidation of the nitrogen oxides. Another classical example is the formation of the intermediate ethyl-hydrogen sulphate, shown by Williamson to occur in the continual etherification process for the production of ethylic ether from ethyl alcohol. Although bodies corresponding to such intermediate reactions have been isolated in some cases by slightly varying the conditions of reaction, it has frequently been disputed whether or not they actually occur in the reaction as it takes place under usual conditions. Further, Ostwald has rightly pointed out that the mere fact of the occurrence of such a body is by no means a proof that it is the cause, or a step in the process, of the quicker catalytic reaction. It may not be an intermediate product, it may merely be a bye-product. In order that such a body can be shown to

be a part of the catalytic reaction, it must be shown not only that it occurs, but that the *sum* of the times, for its formation and its breaking up again to form the final products of the reaction, is less than the time of the direct non-catalysed reaction. Otherwise it is clear that the reaction will run more quickly without the intermediate body being formed.

It is clear that this condition is satisfied in those cases in which the reaction does not go at all in the absence of the catalyser, or does not go within appreciable experimental limits, as in the case of sulphur dioxide and atmospheric oxygen mentioned above. Here it is obvious that the reaction without the catalyst runs more slowly than the sum of the formation of nitrosyl-sulphuric acid, and the decomposition of this to form sulphuric acid and nitric oxide, without any quantitative work on the subject. Also in one case of a reaction which does run at a measurable rate by itself, viz. that between hydriodic acid and hydrogen peroxide in which water and iodine are formed, but which is catalysed into much greater velocity by molybdic acid, it has been shown by Brode that an intermediate compound per-molybdic acid can be isolated, and also that the *sum* of the times of formation of (1) per-molybdic acid from molybdic acid and peroxide, and (2) the oxidation of hydriodic acid by per-molybdic acid to iodine with regeneration of molybdic acid, is less than the time of direct oxidation of hydriodic acid by peroxide in the absence of molybdic acid.

Although a proof that the sum of the times was *greater* than the direct time would rule out the intermediate compound, it must be clearly pointed out, however, that the times being *less* does not necessarily prove that the intermediate compound formation actually occurs and is the cause of the catalyses. What it does prove is that the formation of the intermediate compound would increase the velocity of the reaction by a definite amount; but such increase might be due to other causes, and the increase by such other means might be greater than that by the path of the intermediate reaction.

Still, an experimental proof that the path by intermediate reaction is quicker, is presumptive evidence that the reaction probably occurs by such intermediate reaction.

It is only in a comparatively few of the known catalytic reactions, however, that such intermediate compounds have been shown

to exist, and in a few others they have been introduced as an hypothesis to explain irregularities of reaction. In the majority of cases there is no experimental proof for the existence of such compounds. Further, as Ostwald has pointed out, the theory of intermediate reaction fails entirely to account for the action of negative catalysts, for here the indirect action *must* go more slowly than the direct one. Accordingly the direct reaction would run at its own undiminished rate by itself, for the quantity of catalyst is too small to take all the substratum at the beginning and compel its conversion by the slower rate.

The conclusion may accordingly be drawn that although intermediate reaction may and probably does occur, and increase reaction velocity in a considerable number of catalytic reactions, it is evident that this does not furnish a universal explanation of catalysis applicable to all cases.

III. *Theory of Altered Solubility and Different Reaction-velocity of the Substratum in the Catalyst.*—The outline of this theory was first given by Faraday, who showed that all porous and finely divided bodies, such as porcelain clay, wood charcoal, animal charcoal, and some metals, notably platinum, possessed the property of taking up different gases and condensing them, and ascribed to this property the power such bodies were known to possess of inducing or favouring chemical reaction, as, for example, in causing the union of a mixture of hydrogen and oxygen at ordinary temperatures in the case of spongy platinum. Such an effect can obviously only obtain in a heterogeneous system, that is, a system in which there is discontinuity of structure and chemical composition, for in a homogeneous system there is no opportunity for variation in concentration of the components of the system. For example, in the above case of spongy platinum, there are portions of the system where on account of the presence of the platinum within molecular distance of action, the hydrogen and oxygen can become condensed in the metal or on its surface, and the action can therefore proceed more rapidly than in those parts of the system outside the range of action of the platinum where the reaction, if it proceeds at all, can only proceed at the rate at which it takes place in the absence of platinum.

Similarly, in the case of a colloidal catalyst such as most enzymes are supposed to be, it may be supposed that the catalyst is present in the form of ultra-microscopic particles suspended in the

solution, and that the substratum possesses a higher solubility in the particles of the ultra-microscopic emulsion, or by surface tension is attracted in increased concentration to the surface of the particles. In either case a portion of the system is obtained in a state of higher local concentration than in the absence of the catalyst, and accordingly the reaction will proceed more rapidly in this portion of the system. If now the relative solubilities of the substratum and products of reaction in the colloidal catalyst portion and the remainder of the solution are such as to give sufficient rapidity of diffusion, of the substratum into, and of the products of reaction out of, the colloidal particles, then the reaction as a whole will be hastened by the condensation within or upon the colloidal particles.

This view as to the mode of action of colloidal catalysts has in recent times been prominently put forward, and the chemical kinetics of reactions in such heterogeneous systems studied by Bodenstein, Bredig, Goldschmidt, Findlay, and von Ernst and others.

It has been shown by Menschutkin that the nature of the solvent exercises an enormous influence upon the velocity of the reaction, so that the same reaction running at the same temperature but in different media may occur at excessively different rates. Thus the velocity of reaction between tri-ethyl amine and ethyl iodide was tested in eight different organic solvents and found to be different in all of them, the extreme variation between acetophenon in which it was greatest, and hexan in which it was least, being no less than 720 times.

Accordingly, if we may regard the colloidal enzyme and the solution in which it is present as a heterogeneous system with two distinct phases in which the substratum to be acted upon possesses different reaction velocities, and also different solubilities, a scheme is at hand by which the catalytic reaction can receive an explanation *in such cases*.

It may be pointed out, as an extension of such a theory, that it is not necessary that the catalyst should be colloidal *provided that the substratum is in such a case colloidal*. All that is necessary is that there shall be a heterogeneous system with two phases present, in one of which the velocity can be increased by the presence of the catalyst. Thus in the case of a colloidal solution, such as starch, undergoing catalysis by a non-colloidal catalyst,

such as dilute acid, the starch particles may be regarded as a phase to which the hydrogen ions are attracted and become concentrated, and in which they increase the velocity of reaction, the products of reaction then rapidly diffusing out, through the large surface of starch particle compared to its mass, and so fresh starch molecules undergoing attack.[1]

It must be admitted, however, that such differences in velocity of reaction in colloidal catalyst or colloidal substratum as compared with the other phase of the solution have never been experimentally demonstrated. Also all attempts to demonstrate, as a model, an artificial emulsion consisting of two phases by the operation of which a reaction took place more rapidly than in a homogeneous solution of one of the phases only, have hitherto failed.

Bredig has postulated such an emulsion somewhat as follows: it should consist of two media, A and B, of which B should form a suspension menstruum for A, the reaction to be tested should run more quickly in A than in B, and the reacting substance should be more soluble in A than in B, and if possible the products of reaction should be more soluble in B than in A. Could such an emulsion be constructed, there is no doubt that the reaction would run more quickly in it than in a homogeneous solution in B alone, and it would illustrate the foregoing theory as to the mode of action of colloidal enzymes.

In conclusion regarding this most ingenious theory, which in all probability does hold in certain cases of colloidal catalysts, particularly those in which a concentration or condensation of the reacting substance occurs in or upon the catalyst, it must be emphasised, however, that it cannot form an explanation of all catalytic action, because it is a necessary premise of the theory that either the catalyst or substratum must be colloidal.

[1] Attention may be drawn to the fact that in cases where the substratum is colloidal, we have experimental evidence of an attraction between this phase of the system and the catalyst, similar to that seen between the colloidal catalyst and reacting substance in the case of platinum and gases. Thus, the strong attraction of fibrin for pepsin is analogous to the attraction of the reacting gases by the platinum. In one case the catalyst is attracted and concentrated in the substratum; in the other the substratum is attracted and concentrated in the catalyst.

Summary regarding Theories of Catalytic Action and Correlation of these Theories

A review of the theories of catalysis such as is given above appears to the author to indicate that the explanation lies not in any one of them but in a correlation of all. Each of them supplies a different path which may be used in different special cases to reach the same end. The end to be reached, in order that the reaction velocity may be increased, is to diminish the resistance to reaction. The potential factor or *vis a tergo* to the reaction is dependent upon the energy freed in the reaction, and this in turn upon the initial and final chemical energies of the reacting substances, hence this factor may be regarded as constant. But the velocity with which equilibrium will be reached, and in fact whether it will be reached at all, depends, as pointed out previously, upon what the value of the resistance is to the reaction. Now all the above theories are explanations of various means by which the resistance may be altered, and so the reaction velocity varied, and which of these means is taken must vary with the circumstances of each case.[1]

Thus, increase of molecular vibration, as in chemical reactions induced by detonation, or by mere contact with a chemical catalyst, may decrease the molecular stability, and so increase the velocity, and by analogy this may also occur in less easily provoked reactions; again, the formation of intermediate products may break up by intermediate stages the molecular stability, producing compounds in which the resistance to reaction is lessened; change in the properties of the solvent may induce or hasten reaction by bringing new attractions to bear upon the constituent groups of the molecule, so that the resistance to these parting company may be increased or diminished, and the reaction velocity correspondingly varied; this change may be occasioned by the presence of a heterogeneous system with different reaction velocities due to different solvents in its two phases; varying concentration may alter the relationships and attractions between solvent and substratum and so vary resistance and reaction velocity.

[1] It is not meant here that the means by which chemical resistance may be studied are not of the highest value, and call for most careful study, but only to point out that all these must be correlated and lead to the same end.

There is hence nothing incompatible between the different theories of catalytic action, and these must be regarded as theories of the means by which the one common factor of chemical resistance to reaction can be affected.

Means other than Catalysis by which Chemical Reactions can be induced, or Reaction Velocity varied

Catalysis is not the only means by which a reaction may be induced, or the resistance to a reaction be reduced. We have seen that provided any energy-transformer is included in the resisting system whereby energy can be added to the reaction, the reaction need not run towards the equilibrium point, but instead the chemical energy may be increased, and complex chemical substances possessing more chemical energy may be built up from simple and completely oxidised inorganic substances, and we have instanced the chlorophyll-containing cell of the plant as an example of such an energy-transformer which is capable of converting radiant solar energy into chemical energy. In all living cells we have, however, examples of such reaction in which energy set free from one reaction is utilised to run another.

To consider, accordingly, that all the reactions of cell life are catalytic is to take a narrow and incomplete view of the problem of the chemical life of the cell. Important to the cell economy as are those reactions induced by enzymes, they form but one portion of the whole, and if the chemistry of the cell included only such reactions as could be induced by enzymes, there would be no building up of compounds with greater chemical potential such as actually occurs, the whole process would become katabolic.

It is the linkage of one reaction with another, and the using of the free energy of one to run another, which specially characterises the cell and differentiates the cell from the enzyme.

In such a connection of two reactions there is something therefore superadded to the action of a catalyst. The catalyst can only alter the resistance to the induced reaction. An inducing reaction can in addition add energy to the reaction induced because it itself gives out free energy.

Hence the old idea of Liebig, that a body which is itself in a state of reaction may induce reaction in another body, although

not a necessary part of a theory of catalysis, because both bodies are altered, must not be lost sight of, since it is of high importance to chemical kinetics, and more especially for the chemistry of the cell.

It may be noted that an induced reaction by such a body may either be similar to that produced by a catalyst, in that it also runs down hill like the reaction inducing it with energy being set free, or may run in the opposite direction away from its equilibrium point, taking up the energy set free in the inducing reaction.

Even in the first case where both inducing and induced reaction run towards their respective equilibrium points, and energy is set free in both, it is evident that the inducing reaction, other things being equal, will be a more powerful agent in increasing velocity of reaction than a catalyst. For, if the catalyst, which can add no energy to the reaction because it remains unchanged itself, can diminish the resistance so as to start or increase the velocity of a reaction, still more can a substance undergoing change and giving out free energy start or hasten a reaction.

This is not mere theory or hypothesis, but experimental fact, verified by many chemical experiments in everyday laboratory use: the subject seems, however, to be often forgotten or lost sight of from the preponderating attention given to catalytic action.

No better example can be given than that quoted by Liebig in 1840, of the action of nitric acid upon silver in inducing a similar action upon platinum.

"Platinum, for example, does not decompose nitric acid, it may be boiled with this acid without being oxidised by it, even when in a state of such fine division that it no longer reflects light (black spongy platinum). But an alloy of silver and platinum dissolves with great ease in nitric acid: the oxidation which the silver suffers causes the platinum to undergo the same change; or, in other words, the latter body, from its contact with the oxidising silver, acquires the property of decomposing nitric acid."

"Electrical action ought to have retarded or prevented the oxidation of the platinum in contact with silver, but, as experience shows, this action is more than counterbalanced by chemical action."

Examples are also not wanting in inorganic chemistry of where

the energy set free in one reaction is used to cause another reaction to proceed away from the direction of its equilibrium point, as in the reduction actions often induced by oxidising agents. For example, in the action of hydrogen-peroxide upon certain metallic oxides, such as silver, gold, and peroxide of lead. Here the reaction of formation of water and oxygen from hydrogen-peroxide which goes on slowly by itself, and gives out free energy, is actually enormously increased in velocity[1] by another reaction which absorbs energy in the process. The induced reaction runs the inducing reaction backwards away from its equilibrium point by means of the energy which would be otherwise set free. The reason for the increased velocity is the same as in the case of catalytic action; although free energy from the induced reaction is taken up by the inducing reaction, the resistance in the process of the intermediate stage due to setting free of nascent oxygen is removed, and in this respect, although undergoing alteration itself with absorption of energy, the metallic oxide acts as does a catalyst in catalysed reactions.

But it is in the metabolism of the living cell that we meet with examples of such linked and induced reactions in greatest numbers. Even in the animal cell, although the balance-sheet of metabolism is in favour of oxidation with liberation of free energy, it is a mistake to suppose that there are no reactions running in the reversed direction. We have seen earlier, that with the varying conditions of concentration in the cell the equilibrium point may alter so that syntheses forming the reversals of simple hydrolytic cleavages may readily occur in the cell, simply by the action of enzymes. Such, for example, as maltose formation from glucose, of glycogen formation from glucose, of proteid from albumose, or even of neutral fat from fatty acids and glycerine. Such syntheses demand little or no energy, because the chemical energy of the substances upon one side of the equation is practically identical with that of the substances upon the other, and hence variations in osmotic energy with changes in concentration may easily make the balance, so that an enzyme which adds no energy may affect the conversion. But in such cases of metabolic change, as, for example, the conversion of carbohydrates to fats, where,

[1] We cannot say catalysed, because the inducing substance does not remain unaltered but takes up energy, but the difference is only in definition, for, as far as chemical kinetics go, the action is virtually catalysed.

weight for weight, the energy is almost double, or in the conversion of carbon-dioxide and water into organic compounds, as in the green leaf, where energy is also taken up, such energy must be provided from other sources and a more complex mechanism than that of the enzyme, capable of linking together different chemical reactions, or of acting as a transformer of other energy forms into chemical energy must be present.

This is the part taken by the living cell, which in one oxidising action obtains free energy, and in an accompanying reducing action stores this energy up, at least in part, in a new synthesised body at a higher potential of chemical energy than that from which it came. In this process enzymes may freely be used by the cell, but they are co-ordinated and regulated in the process.

Further, in the process, a set of energy manifestations peculiar to life appear, which cannot be reproduced elsewhere than in living cells, and as this is the sole criterion which differentiates one form of energy from another in the inorganic world, it may justly be maintained that we are here dealing with a peculiar type of energy, although this arises from, and ultimately passes back again into, inorganic forms.

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CHAPTER VI

SECRETION AND GLANDULAR MECHANISMS

Correlation of Secretion, Absorption, and Excretion, and their Relationship to Osmotic Energy.—The processes of secretion, absorption, and excretion are distinguished from one another only by their object or physiological function, and not by anything intrinsically different in their nature or in the mechanism by which these processes are carried out in the body. The purpose of secretion is to prepare an active substance in solution for use in assisting a process which is of service to the organism in some other part, such as a digestive secretion; a secretion of a substance which has a guiding influence upon chemical change in other tissues, and hence affects the state of activity of those tissues, as, for example, the internal secretions, adrenalin, secretin, &c.; or a secretion which acts by mechanical means, such as the secretion of the tears in the lachrymal gland, the mucous secretions on the mucous surfaces, and the serous and synovial secretions of the serous and synovial cavities. The purpose of absorption is to take up for the service of the body generally and of the absorbing cells the materials in solution which have been prepared and modified by the secretions. Lastly, the purpose of excretion is to remove from the body materials which have passed through, or been formed in, the cycle of metabolism in the body, and have become waste products for which the body has no further use. In addition, the purpose of excretion is to maintain in normal amount and concentration in the circulating fluid of the body, the blood, those products which *are* of service, for in abnormal concentrations these useful bodies become as injurious to the living cells as effete products of metabolism, or foreign substances of actively poisonous nature.

Respiration also is essentially identical in its nature with these three processes, being a combination of absorption, secretion, and excretion, the only difference being that the products concerned in respiration exist in the form of gases before being taken

into the body and after being removed from it, but in the process of respiration itself the substances concerned, oxygen and carbon-dioxide, are as truly in aqueous solution as are the substances involved in secretion, absorption, and excretion. It is clear, then, that the purposes served in the processes of secretion, absorption, excretion, and respiration differ, but we shall see that in so far as the intrinsic nature of these processes and the mechanisms by which they are carried out are concerned, they are closely similar or identical, and are governed by the same laws.

At the outset it may be pointed out that not only in respiration, where the differentiation of the process into two parts—an external respiration and an internal or tissue respiration—has been clearly recognised, but also in the other processes of secretion, absorption, and excretion, there are two parts to the process, viz. (1) an internal or cellular part, in which chemical changes, and processes involving energy changes within the cells active in the process, occur, and (2) an external or mechanical part in which the products acted upon are brought to or carried away from the cell and transferred to other parts of the organism, and by means of which, through the activity of mechanisms external to the cells concerned in the active process, the internal or cellular part is modified and regulated.

Thus, in the case of secretion, we may point out as the internal or cellular part of the process: (1) the formation and storage in the cell of the intrinsic organic constituents of the secretion, as zymogens, &c., in which process the cell acts as an energy-transformer upon the chemical energy supplied by the organic constituents of the plasma, and builds up its own special products from these constituents; (2) the formation from the inorganic constituents of the plasma of the inorganic constituents of the secretion against the laws of diffusion and osmosis, so that the osmotic energy is increased by the separation of a secretion containing substances in greater concentration than they possess in the plasma, the cell here again acting as a transformer, and converting chemical energy derived from its absorbed and oxidised food into osmotic energy.

But we have also in secretion the external part of the process in which agencies outside the secreting cell come into operation, and either modify the action of the cell, or produce an effect apart from the cell entirely.

The agents which come into operation in the external part of secretion may be classified as follows: —

(1) The alteration in the supply of fluid or solvent and of dissolved and nutrient matter to the cell, such as variations of the blood supply to the secreting cells; or alterations in the concentration of the dissolved substances in the blood supply, for example, alteration of percentage of glucose in the blood which influences the glycogen-secreting power of the liver cells.

(2) Alterations in the secretory activity of the cells due to external causes, when the supply of solvent and nutrient remain constant or do not change proportionately to the change in secretory activity, such as the stimulation of the special secretory nervous mechanism of the secretory cells, or the effects upon secretion of chemical substances —for example, *secretin* upon the pancreatic cells, or *gastrin* upon the gastric cells, or drugs such as pilocarpin or atropin upon secretory cells in general.

(3) Most external in character of all are the mechanisms by which the secretions in certain cases are carried away from the secreting cells, diverted into different channels so as to be carried away to different parts of the body, or by certain muscular arrangements in the different ducts, are retained ready and already secreted for use at intermittent periods. Examples of such external mechanisms of secretion are found in the ducts of secreting glands, often, when of an appreciable length, supplied with muscular walls which by means of peristaltic contractions pass on the secretion, or by sphincters at definite parts along their course, provide for its retention until a reflex stimulus causes it to be discharged when there is physiological occasion for its use. Storage sacs for the secretion are found in the gall-bladder, in the organs of generation, in the dilated ducts of the mammary gland, and in the poison glands, &c., of many animals.

Exactly the same division into an intrinsic indispensable cellular or internal part is seen in the processes of excretion and absorption, together with a more or less expanded and varying accessory or external part.

Thus, in absorption, we have in the intestinal columnar cells an active cellular absorption, with accompanying chemical change, and work done against osmotic pressure, and the external part of carriage of the products to the tissues, where again cellular

processes of absorption occur modified by the operation of the nervous system, and new products are given out which are carried by an extra cellular process to other cells. Similarly in excretion, we have cellular activity in which the excretory products are formed in the various cells; external processes by which these are carried to the liver; cellular processes, again, in which the excretory products are chemically modified; external processes by which the products are carried to the excretory organ, such as the kidney; and, lastly, in the special excretory organ itself, we have cellular processes again in which the excreting cell provides energy for work to be done against osmotic pressure with corresponding increase in osmotic energy, at the expense of chemical energy obtained from oxidation of nutrient matter. Throughout the processes, in addition to external carriage in the blood stream, there is also the play from without upon the active cells of the external agencies (*a*) of the nervous system directly, or indirectly through the vaso-motors, and (*b*) the stimulus of chemical substances in the circulation, which may also act upon the cells, or intermediately through varying the blood supply.

It is in the external parts of the processes that the chief differences in their mechanisms are to be found, and this statement holds not only in contrasting the processes of respiration, secretion, absorption, and excretion with one another, but in regard to the variations between one type or case of secretion, &c., and another. The variations in the external mechanisms are manifold between one process and another, and from one animal species to another with regard to how the same fundamental process is carried out; but in all cases the essential cellular process is very much alike, and the same types of phenomena are to be found. There is in every case a living cell involved in the process, and by this living cell the processes of diffusion and osmosis are profoundly modified. Substances are separated often at higher concentrations than in the bathing fluid, which can only take place on the condition that energy is transformed by the cell and converted into osmotic energy. New substances are produced in many cases which are typical of the action of the cell involved, and can only be produced as a result of energy transformations induced by the cell. Even where the concentrations of every single instance in the secretion may be less than in the bathing fluid, and no new substance is

produced in the passage through the cell,[1] the rate of secretion or transmission is so much subject to variation apart from purely physical factors, that the cell must be regarded as something more than simply an inert membrane, because its permeability for different dissolved substances, and for the solvent, vary from time to time as the cell is acted upon either by the nervous system or by substances in solution in the plasma. Such specific substances dissolved in the plasma possess the power of affecting permeability for other and quite different substances in a manner never seen in the case of non-living membranes or apart from living cells.

The consideration of the mechanism of secretion may accordingly be divided into two parts, viz. (1) the intrinsic activity of the cells concerned in secretion, and (2) the accessory mechanisms by which the rate of secretion is varied and controlled.

Intrinsic Activity of Secreting Cells

That secreting cells do not act in a passive inert manner as filtering mechanisms, or as membranes possessing different and constant permeabilities for different dissolved substances in the plasma, or as media in which different substances possess different solubilities, is proven by many experimental observations. Thus that the rate of secretion is not merely passively dependent upon blood pressure and blood supply (although under normal conditions it is subject to variations corresponding to changes in these physical factors) is shown by the observation of Ludwig that the secretion pressure in the submaxillary salivary gland, when the outflow is resisted by fluid in a manometer, may rise much above the arterial pressure; and also by the observation that after administration of a drug, such as atropin, the blood supply may be increased as much as before administration of the drug on stimulation of the secretory nerve, without however calling forth any flow of secretion. In other cases, such as the kidney, where the secretion pressure cannot be raised above arterial pressure, this is due to the nature of the minute anatomical structure, as a result of which all supply of fluid is cut off from the secreting cells

[1] It is improbable that this condition ever is completely realised in the action of living cells.

before the pressure in the ductules can exceed that in the blood vessels, and so the stoppage of secretion is a purely mechanical effect.

These experiments prove that, although secretion under normal conditions may be aided by filtration, yet the process in its nature is not one of passive filtration.

That it is not passively dependent upon osmotic pressure is shown (1) by the fact that the total osmotic pressure of the secretion, as shown by depression of freezing point, is in many cases greater than that of the plasma; (2) that even in cases, such, for example, as the saliva, where the total osmotic pressure is less than that of the plasma, the osmotic pressure of certain constituents is higher than their pressure in the plasma—for example, in the saliva, the pressure of dissolved carbon-dioxide, of calcium salts, and of the sulphocyanide; (3) that new constituents appear in the secretion as a result of chemical activity in the cell which are entirely absent in the plasma, and are not sent into the plasma, but into the gland duct, by means of cellular activity and in opposition to the operation of osmotic energy; (4) the alteration in many cases of chemical reaction by concentration of hydrogen or of hydroxyl ions in the secretion high above the concentration which they possess in the plasma, may be quoted as an example of cellular activity producing an effect in opposition to osmotic pressure.

Now it is clear that while the source of energy residing in the blood pressure might separate a secretion, with concentration possessing any value up to that of the same dissolved constituent in the plasma, it cannot produce a concentration in even a single constituent exceeding the value of the concentration of that same constituent in the plasma, and certainly cannot produce a new constituent not present in the plasma. When the results of experiment are taken in conjunction with this statement, it is found that in every secretion in the body cellular activity must be brought into action, in other words, the secreting cell must furnish energy in the process of secretion, and this not only holds obviously for the new constituents, but also for all those crystallised and inorganic constituents which are found in the secretion at a higher concentration than in the plasma, and hence possess more osmotic energy.

Not only does the increase in concentration of certain constituents in the secretion above their concentration in the plasma

rule out, as far as these constituents are concerned, the [illegible] of filtration and osmosis, but it also rules out any [illegible] of the secretion whatever, which does not involve [illegible] the cell as an active energy-transformer.

If any single substance is [illegible] above the concentration which it possesses in the [illegible] increase in concentration in [illegible] osmotic pressure,[1] *and in consequence [illegible] by the secreting cell, and the secretion of such substances cannot be explained by any theory which does not take into account the work of the cell as an energy-transformer.*

A recognition of this principle would have saved much error in not recognising the limitations of [illegible] theories which have been put forward in explanation of [illegible] and secretion by the cell.

In the first place may be mentioned the selective absorption theory. Overton's explanation of the selective uptake by the cell of different salts, erythrol and other nutrient matter, and the retention of [illegible] in the cell, such, for example, as potassium salts [illegible] concentration than in the plasma.

Overton supposes that the [illegible] enclosing the cell or separating off from some [illegible] its protoplasmic constituents from the plasma, a thin envelope or layer of [illegible] substances, chiefly lecithin, which possesses selective permeability for different substances and ions, a solution being impermeable entirely for some, easily permeable for others and in other cases permeable with difficulty. This lipöid membrane or "plasma haut" is supposed in this way to determine the [illegible] and output of the cell, and its osmotic behaviour [illegible] to different substances, and has also been applied [illegible] to the toxicity or otherwise of different substances [illegible] cell, and of the effects of anæsthetics.

Taking this theory first from the experimental point of view, although it must be admitted that "lipöids" (if by this term is meant [illegible] substances soluble in ether) are present in all [illegible] and have been found in all of those in which it has been experimentally [illegible] although often only in small traces: yet it has never been shown experimentally that this forms a separating membrane

[1] This does not mean that the osmotic pressure is balanced or overcome by hydrostatic pressure in the cell, but that osmotic or volume energy must be replaced by energy in another form by the agency of the cell. (See p. 161.)

between plasma and cell protoplasm, as has been assumed by Overton. Further, it has never been shown that this hypothetical membrane possesses for different ions and crystalloids the permeabilities and impermeabilities ascribed to it.

In order to attempt to test the Overton theory with regard to salt solutions, the writer has prepared a lecithin membrane by thoroughly impregnating a membrane of parchment paper with lecithin, so that the pores of the paper were thoroughly soaked with the lecithin, and there was a continuous layer of lecithin on both sides of the paper, and using this as a membrane between sodium chloride solution and water, or between two sodium chloride solutions of different strengths, so as to avoid action of the water upon the lecithin.

According to Overton's view, the lecithin membrane, like the cell, ought to be impermeable to the sodium chloride, and in solutions of different strength an osmotic pressure effect ought to have been obtainable with such a membrane.

It was found, however, when the membrane was used in an osmometer (1) that no osmotic pressure whatever developed on the sodium chloride solution side, or on the side of the stronger solution, and (2) that sodium chloride did pass through the membrane.

Accordingly, the presence of a lecithin membrane, even were such shown experimentally to exist, would not explain the osmotic phenomena of the cell or the impermeability of the cell for the sodium ion.

Taking next the membrane hypothesis from the theoretical point of view, the following arguments may be urged, which apply not only to the lipöid membrane but to any other conceivable membrane by which an attempt may be made to explain upon such a passive basis the active work of the cell in maintaining a different composition and concentration of the crystalloids and ions within it to that which obtains in the medium in which the cell lives. It is on account of this general applicability against an explanation by any passive membrane theory that the arguments are here given at length.

Take, first of all, the position that the cells are *entirely* impermeable to certain ions (and to other non-dissociated organic crystalloids), and that it is on account of such perfect impermeability that these are found only within or only without the cell,

or in such very different and fixed concentrations within and without the cell. For example, that potassium salts are found in the cell in excess, and sodium salts in the plasma in excess, and that this is due to a membrane refusing passage entirely to sodium and potassium ions. Then this excludes all exchange of such ions between cells and plasma, and there is neither any explanation on such a basis of how the present state of affairs with such an unequal distribution of potassium and sodium ions occurred in the first instance when the cells were formed and growing; nor any explanation of how more potassium ion is taken in and sodium ion excluded when cell division takes place and new cells are growing and causing increased volume of cellular tissue without any drop in potassium ion concentration. The explanation on the ground of complete impermeability can obviously only hold so long as the cell is in complete equilibrium with the plasma as regards the inner and outer level in potassium and sodium salts. But there is no explanation whatever of how that equilibrium was attained initially, nor how it is maintained when the cell volume increases as cell multiplication occurs. Are we to suppose that the original fertilised ovum contained all the potassium salts of the adult organism? Obviously such a conclusion is absurd, and it must be admitted that the cells must have at some time taken up potassium and continued to reject sodium ions.

In fact, it is quite clear that so far from being impermeable to potassium ions, up to the period at which the cell attained its maximum saturation, it must have greedily taken up potassium ions, from an exceedingly low concentration in the plasma, by an *active* process of selective absorption against osmotic pressure [1] and with corresponding expenditure of energy by the cell, in the same fashion as a diatom concentrates the silica for its skeleton from the trace present in sea-water, or as the bone-forming cells take up the calcium salts from the circulating plasma. Once the cell has attained its normal level of potassium ion concentration this action ceases and equilibrium is attained; but this condition is preserved only so long as the cell is resting in size. There is no evidence that there is an impermeable membrane formed, or that the cell is really impermeable to potassium salts, because it does not give them out or take them up any more in appreci-

[1] See footnote on p. 145, and also p. 164.

able quantity; all this means is, that there is a balance being maintained dependent upon the nature and active properties of the particular cell protoplasm involved. When such a cell is immersed in a solution of a potassium salt it takes practically none up, because it has already attained its balance in potassium ions, and *actively* preserves this. Did it behave as an inert membrane, as when it is killed, it would take up more potassium ions in a strong solution; but the living cell does not do so to any appreciable extent; it actively preserves itself against osmotic invasion. On the other hand, when such a cell is placed in a solution not containing potassium salts, such as a solution of sodium chloride, it does not part with its potassium salts in appreciable amount; but this need not be because it is surrounded by a membrane impermeable to potassium ions but because it actively retains its potassium ions on account of that affinity or activity by which it originally took them up when they were present in traces only in the plasma.

Thus the balance of concentration for each individual ion and salt and dissolved substance within and without the cell is maintained, and readjusted when it changes, not by means of any hypothetical inert impassable membrane stopping any reaction between the cell contents and the constituents in solution within and without, but by the play of the cell's activities upon the medium in which it lives.

This, it may be remarked, is not theory but experimental fact; we *see* that the growing cell takes up certain definite constituents from the medium and rejects others, that the constituents taken up are often taken up in opposition to osmotic pressure, and hence only possible by the expenditure of energy by the cell. Why, then, when the cell comes into a position of *labile* equilibrium with its medium should the basis of explanation be changed, and it be supposed that instead of those forms of energy which brought the cell to that state, being still active in maintaining it in that state, the mechanism of a hypothetical membrane or permeability be invoked?

The condition is analogous to that of a chemical reaction which has come into equilibrium; here we do not suppose that the reaction is frozen rigid, so to speak, at the equilibrium point, or that membranes of an impermeable type are formed around the molecules which keep them from reacting. No, the reaction is pre-

served by the balance of opposing factors, reactions still occur between the molecules, but these are equal and opposite.

So also in the case of the living cell in equilibrium, the case is not that of an impenetrable membrane through which an ion of potassium or sodium never passes, but a labile equilibrium with both potassium and sodium ions passing in and out all the time, but the numbers passing in and out are equal, so that the concentrations are preserved unaltered.

That this is the true state of affairs there is abundant experimental proof. For let the resting cell divide, and the two daughter cells commence to grow, then the supposed impermeable membrane for potassium ions quits the scene of action, and the growing cell readily takes up potassium ions.

Further proof of the existence of a labile balance of equilibrium is seen in the physiological behaviour of the cell when the appropriate ions are absent from its circulating fluids.

As we have seen in the preceding chapter in describing the effects of inorganic salts upon living cells, in order that the physiological properties of tissues may be maintained in a normal condition, it is necessary that normal amounts of different ions shall be present in the circulating medium. Thus the normal heart-beat cannot be retained unless a certain definite low concentration of potassium ion is maintained in the perfusing fluid. What explanation of this can be given on the basis that the active cells are impermeable to potassium ions? If the cells of the heart muscle are impermeable to potassium ions, how can the presence, or absence, or variation in concentration, of such ions in the circulating fluid affect the physiological activity of the cells? Obviously the cells *are* permeable to the potassium ions and in both directions; for when there is no potassium ion in the circulating fluid, the balance, for potassium ion between circulating fluid and cell contents, becomes upset and corresponding to the low pressure in potassium ion in the circulating fluid, potassium ion must be given out by the cell until a new equilibrium is reached. On the other hand, if potassium ion is present in the circulating fluid at the proper concentration to correspond to and balance the concentration in the cell, then exchange will be equal, the concentration of potassium ion in the cell will not change, and the cell will preserve its normal activities. Finally, if the concentration of the potassium ion in the circulating fluid be greater

than that required to balance the concentration within the cell, then more potassium ion must enter the cell than leaves it, and the effect becomes evident in a change in the action of the cell.

But how, it may be asked, is such a statement to be correlated with that upon which the supposed impermeability of the cell for potassium ions is based, with the fact, namely, that the cell does not appear, as far as chemical investigations go, to take up, for example, potassium ion from a solution of a potassium salt in which it is immersed? The correlation of the two sets of experimental facts is not, however, a difficult task. The explanation lies in the fact that the cell possesses different affinities for the different ions and other dissolved constituents of its circulating fluids, so that at the equilibrium point for normal conditions, the concentrations for each constituent within and without the cell are never equal but bear a definite ratio to each other, and further that these constituents enter into unstable physical or chemical relations with the protoplasm, so that there is a more or less definite minimal concentration for each constituent ion or dissolved substance in the plasma, which might be termed the "dissociation pressure or concentration" for that particular ion or substance at which the protoplasm becomes combined with it. There is an unstable chemical or physical combination formed between the protoplasm and each of the active constituents of the plasma, the existence of which depends upon the osmotic pressure or concentration of the particular constituent in the plasma; just as the existence of the compound oxy-hæmoglobin in the red blood-corpuscles depends upon the partial osmotic pressure of oxygen in the plasma.

Just as in the case of oxy-hæmoglobin but little oxygen is given off until the pressure of oxygen in the plasma has fallen to the level of commencing dissociation of oxy-hæmoglobin, so in the case of the tissue cells in general but little potassium ion is given off until the osmotic pressure of that ion has fallen in the plasma below a certain limit, when the range of dissociation of potassium ion commences.[1] Accordingly it is only at this limit that the change in physiological action of the cell due to diminution of potassium ionic pressure in the plasma begins to become evident.

[1] The concentration of potassium ion in Ringer's solution lies above this limit.

On the other hand, with increasing osmotic pressure of oxygen in the plasma above the point at which oxy-h·emoglobin has been completely formed, there is but little further uptake of oxygen by the red blood-corpuscle; and similarly in the case of the potassium ion, or any other active ion, in the plasma above the concentration at which the protoplasm of the tissue cell has been saturated, the uptake of potassium ion by the cell will be small and inappreciable to chemical investigation, so that even in an isotonic or somewhat hypertonic solution *of po'assium salt alone* the amount of potassium ion taken up by the cell will not be appreciably greater to ordinary chemical analysis than that taken up from normal plasma where the osmotic pressure of potassium ion is many times lower, but still sufficiently high to cause almost complete association between the protoplasm and the potassium ion. Although the difference in uptake of either oxygen or potassium ion is so small as to escape chemical determination, it may, however, produce in both cases profound physiological effects, probably from the rapid increase in osmotic pressure of the constituent concerned in the cell after the saturation point has been passed. Thus, although at two atmospheres of oxygen pressure the amount of oxygen dissolved in corpuscles, plasma, and tissue cells is not very appreciably higher than when the oxygen pressure is about 100 mm., yet the activities of the cells become affected and the animal dies in convulsions. So although the uptake of potassium ion by the cell may not be appreciably affected quantitatively when the concentration in the plasma is increased compared to the uptake at a lower concentration, yet the physiological action of the small additional amount upon the cell may be enormous.

It must be remembered that just as in the case of oxy-h·emoglobin there is no absolutely definite pressure which can be spoken of as the dissociation *point*, but rather a short range of pressure, during which association of the oxygen and hæmoglobin occurs, so in the case of other dissolved constituents (ions, organic crystalloids, and anæsthetics) and the tissue cells, there will not be a sharp point, but a range of association with increasing pressure, and the curve of osmotic pressure and association will also vary with each dissolved constituent and each type of tissue cell.

In the case of every active drug, and every active constituent

of the plasma, some such association must occur as the pressure of such constituent in the plasma rises, and dissociation (with recovery in the case of a drug) take place as the pressure falls. No drug or other substance can be active unless it either enters the cell, and forms some combination with the protoplasm, or else prevents in some manner association and dissociation of a like type in the case of some other important constituent necessary to normal protoplasmic activity.

The action of different drugs, their rapidity of action and their dosage, will depend on the nature and extent of the association between them and the cell protoplasm. If the saturation point of the drug and protoplasm is attained at a low pressure and with a low amount of drug, then the amount of the drug necessary to produce the full effect will be small, and in all probability the cell will take up but little of the drug, so that to chemical analysis the uptake may appear to be zero, and yet physiological methods of examination may show that the effect is very profound.

For example, in the case of salts of iron, the saturation pressure must be excessively low, and a proteid substance fully combined with iron contains but a very low percentage of iron, hence the physiological effect of iron may be very large, although the uptake is infinitesimal, and the time required for uptake is large. Thus, in an individual weighing, say, 60 kilograms, the weight of blood would be approximately 4 kilos, that of hæmoglobin about 500 grm., and in this the iron would be about 0·4 per cent., or 2 grm. Therefore in a course of iron treatment lasting over some weeks the amount of iron necessary to be taken up in order to produce a marked effect would be so small as to be entirely beyond the bounds of determination under the conditions of experiment.

Nor does the view of varying permeability of the cell to different dissolved substances, of high permeability for some and low permeability for others, give any better solution to the real problems of secretion and absorption than that of complete impermeability. For the simple reason that variations in permeability form a passive factor like the variation of a resistance, and hence can at most alter the time relations of the process, and not the end results, and so there can on such a basis be no explanation of the fact that work is done by the secreting cell in the process, as when a constituent dissolved substance is secreted at higher concentration

and pressure than in the plasma. Thus if a cell is immersed in a fluid containing any given constituent in solution, it will, if it possesses any degree of permeability whatever for that constituent, become ultimately saturated to the equilibrium point with the constituent, and the point of equilibrium will not vary with the permeability, the only thing which will vary with the permeability will be the time in which equilibrium is attained. In considering the effects of change in permeability upon the time relationships of absorption and secretion, the factors to be borne in mind are the thickness of the layer through which diffusion has to occur, the difference in concentration of the diffusing dissolved substance or ion at the two surfaces bounding the layer through which diffusion is taking place, and the coefficient of diffusion for the particular substance through the layer. The rate of diffusion, regarded as a purely physical process unaided by the cellular activity (and dependent only upon the difference in osmotic pressure at the two sides of the layer or membrane, the thickness and the coefficient of permeability or diffusion), may be said to be directly proportional to the difference in osmotic pressure and to the coefficient of permeability, and inversely proportional to the thickness of the layer or membrane, that is, the length of the absorbing or secreting cell. Hence diffusion can only occur so long as there is a fall in osmotic pressure in the direction in which diffusion is taking place; when the two pressures become equal diffusion must stop, and if by any chance the pressure became greater in the direction in which diffusion had been taking place, then the purely physical process of diffusion would carry out or tend to carry out the process in the opposite direction. Accordingly any separation of a constituent at a higher osmotic pressure must be carried out against diffusion, with increase in osmotic energy, and heaping up of difference in osmotic pressure or increase in the potential factor of osmotic energy.

It is, then, only when the concentration of a substance, either secreted or passing through as an absorption product to the other side of the active cell, is diminished that diffusion due to osmotic pressure can be regarded as a factor in the process, and it is here only that we have to consider the possible effects of changes in the permeability of the cell. If the secreted or absorbed product is carried rapidly away from the other side of the cell after having passed through, so that it does not tend to approach in concentration, as a

result of stagnation, that concentration it possesses in the fluid from which secretion or absorption is occurring, then the rapidity of secretion or absorption of the substance will be greater the thinner the secreting or absorbing cell and the higher its coefficient of permeability. In other words, accordingly, as the cell grows thinner and more permeable, the more nearly will the secretion approach in concentration of its constituents to the fluid from which the secretion has been formed.

In so far as the cell has a lower permeability than the plasma or lymph, it will form a resistance of varying amount upon the rate of secretion, and in so far as the cell has a greater permeability than these fluids it will form a less resistance than a layer of equal thickness of these fluids, and to this extent the increased permeability will aid the rate of secretion. But it must clearly be pointed out that change in permeability can only act as a variation in resistance, and hence the concentration can never be increased, nor the dissolved substance be expedited through the cell at a greater rate than if the cell did not exist on the path, that is, than if the resistance for the length of the cell were zero—in other words, as far as diffusion is concerned the cell can have no positive effect, such as is seen for some constituent or other in every secretion.

Further, it may be pointed out that the extent of the secreting or absorbing surface is in all cases so large, and the thickness of the layer so small, amounting to the length of a single cell, that increase in permeability *above* the value for a layer of lymph or plasma of equal thickness can possess but a very secondary value in determining rate of secretion or absorption. If we imagine the layer of secreting or absorbing cells spread out so as to form a huge plane lamina, the thickness of which is that of a single secreting cell, and the area of the side that of the total secreting area of cells, and that this lamina forms a membrane between lymph upon the one side and secretion upon the other. Then if this lamina were supposed to have the same resistance to diffusion through it as a lamina of lymph of equal thickness, such resistance would be excessively low, and with a rapid removal of fluid from the secretion side the concentration of each constituent upon the secretion side would be practically the same as upon the lymph side of the lamina. Hence the supposition of a higher permeability or selective permeability of the secreting cell above that of the lymph (or water) can have but an infinitesimal effect,

since it cannot increase, as we have seen, the concentration above the value in the lymph, and if it had the value of the lymph (or water) in permeability, the concentrations would be practically the same. It is when the permeability changes in the opposite direction, and the degree of permeability of the secreting or absorbing cell becomes progressively less and less than that of a layer of lymph or water of equal thickness, that the only and indeed an important effect of cell permeability becomes obvious, in slowing, never in hastening, the rate of secretion of constituents. For as the permeability of our imaginary secreting or absorbing lamina to any constituent becomes less and less, its resistance to the passage of that constituent becomes greater and greater, and the concentration of the constituent in the secretion or absorbed fluid less and less, until in the limit none may pass through at all.

It is in such a resisting action that the value of differences in permeability comes in, by causing the retention of substances in the lymph, and not in a high degree of permeability causing increased rate of passage, and increased concentration of substances in secretion. Examples are the retention of the plasma proteids in the glomerular secretion or filtration, and the prevention of ingress of poisonous substances in many cases to the tissue cells. But the greater concentration of substances and ions in the secretions cannot be explained by the application of the principle of altered permeability. Diffusion and permeability can accordingly explain the passage of such substances as are already contained in the plasma up to the concentrations at which they are contained in the plasma, but furnish no means for obtaining substances not present in the plasma, or for concentrating crystalloids or ions in solution to osmotic pressure higher than in the plasma. The latter effects, which are universal in processes of secretion and absorption, can only be obtained from expenditure of energy by the cell.

An attempt has recently been made by Overton and Meyer and by Friedenthal to explain the secretion and absorption of substances by the cell on the basis of varying solvent powers of the cell or certain of its constituents for such substances.

Thus Overton would explain the effects of anæsthetics as arising from the high solubility of the anæsthetic in the *lipoids* or lecithin of the cell, and also the absorption or non-absorption of other substances by the cell as dependent upon whether they dissolve

or not in the lipöid membrane, and hence can obtain ingress to the cell. The author does not state in the case of the anæsthetics whether the action is to be ascribed to the physical action upon the lipöids themselves, or whether it is due to a passage through the lipöids afterwards to the cell protoplasm.

Friedenthal has evolved a similar theory for the absorption of fats in the intestine, which he ascribes to the high solubility of the fats in the protoplasm of the absorbing cells. The theory is also extended to other substances taken up in solution for absorption or secretion, so that these processes are placed in dependence upon the peculiar and selective properties of the cell as a solvent.

The two theories of the lipöid membrane acting as a selective solvent and of the cell protoplasm playing a similar rôle may be taken together, as the same arguments apply to both views.

Neither of these theories furnish any basis of explanation of how energy is expended in concentrating any secreted or absorbed substance. For the fact that a substance, such as the lipöids or cell protoplasm, is a good solvent for a given constituent does not give any power to the solvent to pass that substance through the cell in more concentrated solution, or indeed to alter the concentration of the dissolved substance anywhere *save in the solvent itself*. Further, increased concentration in the solvent has no effect whatever upon rate of passage of the substance through the solvent or through the cell, and will indeed delay passage through the cell until the lipöid or cell protoplasm has become saturated with the dissolved substance, and after that will behave in an inert manner, without any effect either upon uptake of dissolved substance, rate of passage of dissolved substance through the cell, or output of dissolved substance at the other side.

In the statement of the two theories there is a complete confusion of solubility and permeability, which are quite distinct processes.

The matter may perhaps be most easily made clear by means of a diagram.

Suppose we have a sphere of fluid C, surrounded by a continuous layer of a different fluid B, and immersed in a vessel containing a quantity of fluid A, and that A contains a substance *x* in solution. Further, that the substance is also soluble in the fluids B and C,

and that the coefficient of distribution of the substance x is such between the three fluids, that the concentrations of βx in B and of γx in C correspond to the concentration αx in A, so that there is equilibrium when the ratio of concentrations is $\alpha x : \beta x : \gamma x$ in the three fluids A, B, and C. Now if at the commencement there is none of x in B or C but x is present in A, diffusion into B will take place, and as soon as the concentration of x in B commences to rise there will be diffusion from B into C. Also, the higher the value of the ratio $\beta : \alpha$, the more rapid, other things being equal, will be the rate of entry of x into B; *but* if β be increased so as to increase the ratio $\beta : \alpha$, the ratio of $\gamma : \beta$ which determines the rate of output into C will be correspondingly *diminished*, and hence the rate of passage from A to C is entirely independent of the solubility in B, and depends only upon the rate of *transmission* or diffusion through B.[1] Also the final condition of equilibrium is independent of the solubility in B, for A and C are each in equilibrium with B, and hence are in equilibrium with one another, so that when the final concentrations are attained the ratio of concentrations in A and C must be the same as if B were left out and A and C had been placed in contact and allowed to come into equilibrium. The only factor which affects the result apart from equilibrium is the rate of diffusion of the substance x through the layer B, and this may not bear any constant relationship to the solubility of x in B. If the solubility in B is very low, so also in all probability will be the rate of diffusion through B; but here, as pointed out above, the extreme thinness of the layer B, in the case of the cell or any cell membrane, and the extent of surface, renders any such factor in most cases of but secondary importance. Certainly, however, solubility in B is not the determining factor with regard to the distribution of the substance *except in B itself*, and in no case can a high solubility in B determine a higher concentration in C than is given by the coefficient of distribution between A and C.

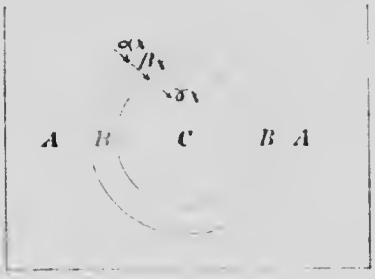

FIG. 4

[1] The matter may also be put thus—the rate of solution from A into B is given by $k_1 \frac{\beta}{\alpha}$, that of solution from B into C by $k_2 \frac{\gamma}{\beta}$, therefore that from A into C is given by $k_1 \frac{\beta}{\alpha} \cdot k_2 \frac{\gamma}{\beta}$, or $K \frac{\gamma}{\alpha}$.

We see, therefore, that a high solubility of any dissolved substance in a supposed lipöid membrane or in the cell protoplasm, will cause a corresponding accumulation of that substance in the lipöid membrane or in the cell protoplasm, but cannot act as an engine or energy producer for sending the substance through the cell as a secretion or an absorbed product.

The substance taken up as a result of higher solubility, such as an anæsthetic absorbed by lipöids, or by fat in ordinary adipose tissue, is hence imprisoned to that same extent in the fat or lipöid, and kept from attacking or combining with the protoplasm; and accordingly the presence of such bodies, instead of aiding or causing anæsthesia, act in the opposite sense by forming a reservoir for the anæsthetic where it is inert so far as the cell protoplasm is concerned, which is its real objective so far as production of anæsthesia is concerned.

The view expressed above, that those substances which are actively absorbed and retained by the tissue cells form unstable physical or chemical compounds with the cell protoplasm dependent upon the osmotic pressure of such substances, cannot any more than the others which have been criticised be put forward as an explanation of the active work of the cell in secretion and absorption, when the product is not to be retained in the cell, but is to be turned out in greater concentration than that at which it entered. For substances in such unstable combination, although subject to different laws of relationship between concentration and osmotic pressure, obviously come into equilibrium also at a given point of concentration and osmotic pressure, and hence their formation cannot be turned into a continuous source of energy for the performance of work by the cell, such as is required to fit the case of secretion.

The formation of such unstable compounds is capable of explaining the selective uptake and retention of constituents by the cell, just as the different solubilities of different constituents by the cell protoplasm or lipöids may explain such uptake or retention, but neither view can explain more than this. Before passing on to a consideration of the energy changes involved in secretion, and the possible explanation of such changes, it may be well, however, to point out that the view of formation of unstable chemical combinations between cell protoplasm and selectively absorbed and retained constituents, fits the observed facts much

better than the alternative view of solution in cell protoplasm or in cell lipöids. For if the explanation were solution, then the osmotic pressure and amount of substance taken up must be in simple ratio to each other. On doubling the osmotic pressure of any constituent in the lymph, the amount taken up or secreted by the cell ought to be doubled, since for simple solution the coefficient of distribution between cell and lymph must be constant, or, in other words, the relationship between osmotic pressure and amount absorbed by the cell should be a linear one.

This is not found, however, experimentally to be the case: the absorption at first rises very rapidly with increasing osmotic pressure, then later the rise in amount absorbed for equal increments of osmotic pressure is much decreased, an almost maximum value is later reached, after which there is hardly any appreciable absorption. This sequence of events is precisely what would occur if formation of an unstable or reversible chemical combination took place at a certain range of pressure, and is seen, for example, typically in the combination between haemoglobin and oxygen. Hence it is most probable that such a type of combination exists in the case of those ions and other cell constituents which are selectively absorbed and retained.

The Energy Changes Involved in Secretion

The work done by the secreting cell in the process of secretion may be considered as divided into two fractions, viz. (1) the work done in increasing the volume energy, or work done against osmotic pressure in increasing the concentration of dissolved substances already present in the lymph, and (2) the work done in increasing chemical energy by the formation in the cell of new substances not present in the lymph from other substances and by means of the chemical energy supplied by other substances present in the lymph.

It is only for the first of these types of energy production by the cell that accurate quantitative estimations can be made; because for the second type the chemical energy and amounts of the organic substances formed in the cell, and the chemical energy and amounts of substances used by the cell in their formation, are at present unknown to us.

Method of Estimating the Work done against Osmotic Pressure in separating each Constituent of a Secretion.—The amount of work done in separating each constituent of a secretion can easily be deduced when the pressure of the substance in the lymph and in the secretion are known, and the total volume of the secretion. But such estimation must be made for each constituent of the secretion separately, and the total work done is the sum of the work done in the separation of each constituent. It leads to quite a fallacious result to merely take the two depressions of freezing point of the lymph and secretion respectively, calculate the total osmotic pressure of lymph and secretion from these two values, and then assume that the work done is the product of the volume of the solution and the difference in pressure. For the amount of volume energy change, as has been pointed out in a previous chapter, depends upon the two pressures for each constituent between which pressure has varied for that particular constituent, and since in the formation of a secretion the same ratio is not preserved between the pressures of the various constituents as exists in the lymph, but one constituent is far more compressed or concentrated than another, it cannot be taken that the lymph is compressed or concentrated as a whole as it were by a piston impermeable to all the dissolved constituents, and the work done obtained from the total initial and final osmotic pressures and the change in volume, but instead the work done upon each pressure giving constituent must be taken separately, and the total work calculated as the sum of all these fractions.

As demonstrated in a previous chapter, the work done when a grm. molecule of substance is compressed from pressure p_1 to pressure p_2 is given by the expression RT $\log \frac{p_2}{p_1}$, and if Q be any other weight in grm. of the substance and M the molecular weight, then the number of grm. molecules will be $\frac{Q}{M}$, and the expression for the amount of work done in changing the pressure of the quantity Q grm. in solution at pressure p_1 to pressure p_2 will be $RT \frac{Q}{M} \log \frac{p_2}{p_1}$.

If now there are any number of substances A, B, C.....N in solution in the secretion in quantities Q_a, Q_b, Q_c.....Q_n, and the molecular weights of the substances be M_a, M_b, M_c.....M_n,

and the pressures of the substances in the lymph be represented by p_a, p_b, p_c.....p_n, and the corresponding pressures in the secretion by p'_a, p'_b, p'_c.....p'_n; then the expression for the work done upon each substance in its production from the pressure in the lymph to the pressure in the plasma will be the same as that given above for a single substance; for example, for substance A the expression will be $\frac{Q_a}{M_a} RT \log \frac{p'_a}{p_a}$. Accordingly the value of the total amount of work done against osmotic pressure (W) will be given by: —

$$W = RT\left(\frac{Q_a}{M_a}\log\frac{p'_a}{p_a} + \frac{Q_b}{M_b}.\log\frac{p'_b}{p_b} + \frac{Q_c}{M_c}\log\frac{p'_c}{p_c} \cdots \frac{Q_n}{M_n}\log\frac{p'_n}{p_n}\right)$$

or

$$W = RT\Sigma\frac{Q_a}{M_a}\log\frac{p'_a}{p_a}.$$

If any of the constituents is electrolytically dissociated, then Q in the expression for the work done in separating it must be multiplied by the dissociation factor, because the osmotic pressure will be higher on account of the dissociation, and correspondingly more work will be done in the separation; thus in the case of the sodium chloride of the urine, for example, at the concentration at which that salt is there separated, it is almost completely dissociated, and Q must be multiplied by the factor 1·9 approximately in order to obtain the amount of work done.

The above investigation of an expression for the amount of work done against osmotic energy in separating a secretion is entirely different from that usually given, which is quite erroneous in that it supposes all the constituents of the secretion to be equally concentrated in the process of separation from the plasma. Such a supposition is wrong in fact, and leads to quite a wrong expression for the total amount of work done, as well as for the work done upon each constituent. For example, while the concentrations of urea in plasma and urine are respectively 0·04 and 2·0 per cent. respectively in human urine, the similar concentrations of sodium chloride are 0·55 and 1·10 per cent.; and hence in the expressions for the work done in secreting urea and sodium chloride respectively the factor $\log_e \frac{p_2}{p_1}$ has quite a different value in the two cases, being $\log_e 50$ in the case of the urea and $\log_e 2$ in

the case of the sodium chloride. As a result, taking the average daily quantities to be 30 grm. in the case of urea, and 16 grm. in the case of sodium chloride, and correcting for the almost complete dissociation of the sodium chloride, a calculation of the work done in the separation in the two cases shows that the amount of work done in separating the urea is nearly six times as great as that done in separating the sodium chloride.

This is quite different from the usual type of treatment, in which it is taken in calculating the work done merely from the lowerings of freezing point of serum and of urine respectively, that the calculation may be based on the supposition that the secretion may roughly be regarded as a concentration of sodium chloride.

The reason of the fallacy is not far to seek, the urea solution is, roughly speaking, concentrated 50 times in the process of secretion, while the concentration of the sodium chloride is barely doubled. If then we imagine the urea and sodium chloride as being separately removed from the plasma by the action of a semi-permeable piston, in the first case impermeable to urea and in the second case impermeable to sodium and chlorine ions and to sodium chloride; then to separate in each case 1500 c.c. of secretion containing in one case 2 per cent. of urea, and in the other case 1·1 per cent. of sodium chloride, from a plasma containing 0·04 per cent. of urea and 0·55 per cent. of sodium chloride, we should require to take in the case of the urea $1500 \times \frac{2}{0{\cdot}04} = 75{,}000$ c.c. of plasma and compress down to 1500 c.c., while in the case of the sodium chloride we should only have to take $1500 \times \frac{1{\cdot}1}{0{\cdot}55} = 3000$ c.c. of plasma and compress down to 1500 c.c.

Hence to get the true expression for the work done against osmotic pressure in secretion, each constituent must be treated separately, and the work done depends in large degree upon the pressures of the separated constituent in plasma and secretion respectively, and the total molecular amount separated. So that as a result, for example, in the case of the urine, the separation of the urea involves more work than the separation of all the other constituents combined.

As an example of the method of calculating the work done in secretion against osmotic pressure, we may give the calculation of the amount done in secreting the normal daily amount of urea,

viz. 30 grm. in a 2 per cent. solution, measuring accordingly 1500 c.c. The molecular weight of urea is 60, and it is not dissociated, so that there is no correction for dissociation, also the usual figure of 0·04 per cent. may be taken for the concentration in the plasma.

The expression for the work done is

$$W = RT \frac{Q}{M} \log_e \frac{p'}{p}.$$

If we express this amount of work as heat energy in small calories the value of the constant R becomes 1·98, if T be taken at 40° C., the value of T in absolute scale becomes $273 + 40 = 313$, and hence the value of RT is 620 at this temperature;[1] Q is 30 grm., and the value of M, the molecular weight in grm., is 60, so that $\frac{Q}{M}$ becomes 0·5; the value of the ratio of $\frac{p'}{p}$ is the same as that of the two concentrations of the urea in secretion and plasma respectively $= \frac{2}{0{\cdot}04} = 50$, and for $\log_e 50$, we can substitute $\log_{10} 50$, on dividing by the Briggs modulus for transference from Napierian to common logarithms, the value of $\log_{10} 50$ is very closely 1·7, and the value of the modulus is 0·434; so that we finally get on making all these substitutions in the above equation, for the value of the work done expressed in small calories:—

$$W = 620 \times 0{\cdot}5 \times 1{\cdot}7 \div 0{\cdot}434 = 1214 \text{ cal.}$$

This amount of energy may be expressed as mechanical work by remembering that the small calory is approximately equivalent

[1] The value of 5·8 rational calories or 580 small calories, given in a previous chapter, was the usual value based on a temperature of 15° C., the value 620 small calories used above is that which the expression RT has at a temperature of 40° C., the approximate temperature of secretion of the urine. The value of 1·98 for R is obtained by using the formula $PV = RT$, or $R = \frac{PV}{T}$, and then substituting the values for P, V, and T for a grm. molecule at any given values of pressure, volume, and temperature corresponding to one another. Thus a grm. molecule at 0° C. has a volume of 22,330 c.c., a pressure of 76 c.m. of mercury $76 \times 13{\cdot}4 \times 981$ dynes, and T is 273 on absolute scale. Also 1 small calory 42×10^6 dynes, and on substituting these values in the above equation we obtain for the value of R in small calories:—

$$R = \frac{22330 \times 76 \times 13{\cdot}4 \times 981}{273 \times 42 \times 10^6} = 1{\cdot}98.$$

to 0·042 kilogram-metres, and multiplying by this factor, we obtain $12{\cdot}14 \times 0{\cdot}042 = 5{\cdot}09$ kilogram-metres as the work done by the kidneys in secreting the urea against osmotic pressure. The work done in similarly secreting the sodium chloride is less than 10 kilogram-metres, as can be shown by a similar calculation, and these two form the chief amount of the work done against osmotic pressure, because the amount of the other constituents is comparatively low.

The estimate of 400 kilogram-metres would therefore be certainly above the amount of total work done by the kidneys in the twenty-four hours, and it must be pointed out that this amount is by no means large. Expressed as heat it would only, if it were all taken as heat from the urine secreted, lower the temperature between 1° and 2° C.

The osmotic pressure of a secretion expressed as a hydrostatic pressure may give a very high value; thus Dreser found in the morning urine of man a lowering of the freezing point amounting to 2·3° C., which corresponds to an osmotic pressure of 28·2 metres of water, or over 30 atmospheres of pressure.

In the urine of other animals still higher osmotic pressures are obtained; thus in the cat an osmotic pressure of 49,800 grm. per square centimetre was calculated by Dreser, and the statement is made that if the work of concentration were carried out by the cells of the kidney tubules, these results would imply that these cells can exert a force six times greater than the absolute force of human muscle (8000 grm. per square centimetre).

Such a statement and such a view as to the action of the cells of the tubules is, however, a highly absurd one. The kidney cells do carry out the work of concentration, but we have no evidence that they exert or resist the least possible pressure in the process. Although the osmotic pressure is so high, the amount of energy change, as is shown by the calculation given above, is comparatively very small, and the work of the kidney cell consists in supplying this small amount of energy, from energy in another form, by transformation of a corresponding small amount of the energy which it takes up as nutrient matter from the plasma. That anything approximating to the osmotic pressure of the separated urine develops in this process of energy transformation, or indeed that there is any pressure developed whatever, we possess not the smallest fraction of experimental evidence. All that is known

is that there is a small increase in osmotic energy, provided by the expenditure of energy by the secreting kidney cell. The view that the kidney cell is something in the nature of a semi-permeable membrane with a difference in pressure upon its two ends of many atmospheres of pressure is an entirely erroneous one; no cell in the body could withstand such a difference in pressure for a moment; there is no evidence that such a pressure exists in the kidney tubules; in fact, it most certainly does not exist. Finally, no arrangement in the nature of a semi-permeable membrane, could form the secretion with accompanying concentration of dissolved substances. In the first instance, because for such an operation, as has already been pointed out, energy is required which a semi-permeable membrane cannot yield. Since an energy machine such as the cell must be utilised for producing the secretion, we at once lose on the introduction of such a machine all necessity for the maintenance of hydrostatic pressure in opposition to osmotic pressure, and there is no more reason why the kidney cells should be supposed exposed to the osmotic pressure than there is to suppose that the walls of the bladder should have to withstand the osmotic pressure of the urine after it has been secreted and passed into the bladder.

In an exactly similar manner, the work done in the secretion of any constituent of any secretion can be calculated if the pressures or concentrations in plasma and secretion, and the amount of secreted substance and volume of secretion are known.

As to the mechanism or type of energy transformation by which the cell does its work nothing is known; similar phenomena of concentration of ions and of dissolved colloids by means of movement in the electric field have long been known, and it is probable that it may be the case that the living secreting cell, by developing differences in electrical potential at its two ends, or by developing differences in energy potential of some other form of energy such as that which intrinsically belongs to the living cell, may establish a directive influence upon substances in solution, as a result of which, and of energy potentials upon the dissolved molecules themselves, they may be caused to move in a definite direction and at a definite speed through the cell, different from that of the water in which they are dissolved. A similar directive movement, in fact, to that seen in the case of dissolved ions and colloidal molecules in the electric field may occur.

Or the energy changes may be brought about by chemical combinations and dissociations in the cell.

But whatever view be taken as to the mode of operation, it is clear from the experimental study of the selective rates of passage of dissolved substances through the cell that what might be termed "polar" properties must be ascribed to the cell in its phenomena of secretion and absorption. This is not theory but experimental fact. It is seen that many substances pass through the cell several times more rapidly than the solvent, while others pass through more slowly. In the case of those which pass through more rapidly, work in giving velocity to these molecules or ions and in increasing osmotic energy must be done by the cell. It is clear from this that the amount of solvent in which any given quantity of a constituent is dissolved need never enter the cell, but instead the dissolved substance be attracted and moved through the solvent toward and into the cell by the energy of the cell; just as independent velocities are given to the ions towards the electrodes by the electric potentials on the electrodes without the solvent between the electrodes moving at the same rate towards either electrode. Thus there may only be a slow current of water through the cell, with a slow uptake of water from the lymph, and a much more rapid current of dissolved substances and corresponding increase in concentration of these in the secretion or absorbed fluid.

If the water containing the dissolved substances were taken up at the same rate by the secreting cell, then in order that the secretion could become more concentrated in any constituent it would be necessary that at intervals water should be returned or pressed out again at the side of the cell at which it entered, containing the constituent which was to be concentrated in more dilute solution. It hence appears more probable that instead of such a to and fro movement of water, the dissolved substances are taken up upon the entrance side of the cell more rapidly than if they passively moved in with a corresponding amount of water.

That this view is probable is seen from the enormous amount of water which would have to pass into and out of the cell alternately if only passive absorption of water and its dissolved substances formed the first stage in the process of secretion. Thus in the secretion of hydrochloric acid in the gastric juice, the con-

centration of the hydrogen ion has to be increased from an almost immeasurably low concentration in the plasma up to about the strength of a deci-normal solution, and to do this by passive absorption an enormous amount of water must enter the secreting cell and be again rejected at the same side at which it entered. Again, in the secretion of urea in the urine 75 kilograms would have to enter and pass through the kidney cells and be reabsorbed in order to concentrate and separate the daily output of urea. Also, in absorption from the intestine, to take up a meal of 150 grm. of carbohydrate or fat in one per cent. solution, which is probably in excess of the concentration at which these food-stuffs are normally absorbed, it would be necessary for 15 kilograms of water to be taken up by the absorbing cells, and either returned by alternating back streams into the intestine free from carbohydrate or fat, or else poured into the blood stream. Such an amount is probably much in excess of the sum of the water taken with the food and the combined digestive secretions.

Hence we must suppose that the cell, whether absorbing or secreting, does not undergo passive infiltration by the fluids in contact with it, and allow these, or even the water, to stream through passively, but is an active energy machine, and takes up the various constituents and their solvent in definite and well-regulated proportions.

For the reason stated at the outset, the amounts of energy involved in the formation by the cell of the new organic constituents of the secretion not present in the plasma cannot at present be estimated, and so we pass to a consideration of the extrinsic mechanisms of secretion.

The Extrinsic Mechanisms of Secretion

Alterations in the Blood Supply to the Secreting Gland.—Accompanying the increased amount of physiological work which the secreting cells have to perform, there is always during secretion an increase in the amount of blood supplied. This increase was estimated by Chauveau and Kaufmann in the case of the submaxillary salivary gland as amounting to three times the blood supply in the resting condition of that gland, but according to more recent experiments by Barcroft, it may in the dog be set

down as amounting to more nearly six times the blood supply in the resting condition.

Comparative analyses of the blood gases in the arterial blood, and the venous blood passing from the gland show also, according to Barcroft's experiments, that the amount of oxygen used by the gland, and also the amount of carbon-dioxide formed, as shown by the sum of the increase in the venous blood and the amount in the saliva formed, both increase during activity much above the amounts similarly determined in the case of the resting gland, pointing to increased chemical activity during secretion.

Thus, Barcroft found that during secretion of saliva by the submaxillary gland, induced by stimulation of the chorda tympani, the oxygen taken from the blood was increased to an amount which was three to four times that taken up by the resting gland. The carbon-dioxide given out by the gland was also increased under the same circumstances to an equal or even greater amount. While after an injection of atropin sufficient to cause paralysis of the secretion the intake of oxygen was not increased by stimulation of the chorda tympani, on the other hand the output of carbon-dioxide was increased, at least for a time.

In the case of the pancreas Barcroft and Starling found that the secretion was also accompanied by an increased oxygen absorption from the blood, and that this increased oxidation took place irrespective of increased blood flow through the organ. These observers also found that the normal oxidation in the pancreas was much greater than in the body generally, and about the same as that of the submaxillary gland.

In experiments upon the metabolism in the kidney, Barcroft and Brodie found that the production of diuresis was accompanied by a marked increase in the absorption of oxygen, although there was no direct proportionality in oxygen absorption and degree of diuresis. The authors found no definite relation between the oxygen taken in and the carbon-dioxide given out, and also that the onset of diuresis was not necessarily accompanied by an increase in the rate of blood flow through the kidney, and even where an increased flow was found it was never proportional to the acceleration of the urinary flow.

Influence of the Nervous System upon Secretion

The profound influence of the nervous system upon secretion is in the case of certain glandular structures a matter of common experience. Thus it is well known to us that the sight or smell of food often provokes salivary secretion, or causes the mouth to water, in every-day parlance; but the effect of the nervous system is in the case of other glands most difficult to prove, and may be said in certain cases not even yet to have been unequivocally demonstrated. Certain it is in some cases from the recent experiments of Bayliss and Starling that this nervous stimulation cannot be regarded as the sole, if indeed the most fundamental and important factor; and we shall see in the next section that it must be regarded as supplemented or replaced by the important action of chemical stimulation and the production of specific secretory substances which act upon the secreting cells after having been absorbed by the circulating blood.

As has been well pointed out by Pawlow, it is dangerous, in the case of the nervous mechanisms of secretion, to generalise from the somewhat simple mechanism of salivary secretion, for the secretory innervation of the whole secreting system of glands, for in the case of other glands, such as the gastric glands and probably the pancreas, the influence of inhibitory nervous mechanisms comes into play and complicates the problem. Hence we are forced to consider the nervous mechanisms in the case of each important secreting gland separately.

Before proceeding to the separate accounts, however, it may be well to point out the general resemblances.

In each case where an influence of the nervous system upon secretion has been clearly demonstrated, it has been shown that a complete reflex arc exists. The nervous stimulation is excited at the peripheral endings of afferent fibres, which excite nerve-cells in the central nervous system and cause stimuli to be discharged along efferent paths to the secreting cells. In the case of the salivary glands the afferent channels are nerves of special sense, either the optic or ophthalmic nerves, or the endings of the gustatory nerves in the mucous membrane of the mouth. In the case of the gastric secretion the afferent impulses arise at the mucous membrane of the stomach by the stimulation of peripheral

nerve-endings through the medium of digestible substances present in the stomach, or through nerves of special sense by the sight of appetising food, as has been shown by the experiments of Pawlow.

In all cases, the efferent nervous impulses by which secretion is excited pass along one of two paths, one coming directly from the central nervous system and the other indirectly through the sympathetic nervous system.

In the case of the salivary glands, our knowledge of the efferent paths belongs to classical and well-established physiological history, while in the case of the gastric and pancreatic secretions the efferent channels may be said still to be in dispute, and indeed in the case of the pancreatic secretion the influence of the nervous system at all must be regarded as *sub judice*.

The Innervation of the Salivary Glands.—Each of the important paired salivary glands receives efferent fibres from two sources, viz., directly from a cephalic nerve, and indirectly from the sympathetic system. Over fifty years ago Carl Ludwig showed in the case of the submaxillary gland that the gland possessed a special secretory nerve, the *chorda tympani*, which on stimulation called into activity a copious secretion of saliva. The flow of saliva was large in quantity but poor in organic constituents and in the specific ferment. About twenty-five years later Heidenhain demonstrated that the gland also received secretory fibres from the cervical sympathetic, which evoked a flow of saliva small in total quantity but rich in organic constituents and in the specific ferment produced by the gland.

As a result of his experiments, Heidenhain evolved the theory that the salivary glands possessed two sets of secretory fibres, one obtained from the cephalic nerve and possessing the property of evoking a flow of water and saline, and the other obtained from the sympathetic system and responsible for stimulating the secretion of organic substances and the specific ferment which he termed the "trophic" or "anabolic" nerve.

This view of Heidenhain's was subsequently generalised for secretion in general without adequate experimental proof. In the case of the submaxillary gland, however, it must be admitted, from the clear experimental evidence, that of the two efferent sets of fibres which govern the secretion of the gland, one induces a free flow of dilute saliva poor in organic constituents, while the other causes a scanty flow of a richer saliva. Also, as shown by

Langley, alternate stimulation of the two nerves causes an increase in the amount of saliva which would arise from stimulation of the sympathetic only, and indicates that in normal secretion there is a conjoint action of the two efferent nerves, giving rise to the usual secretion. Hence we must regard the chorda tympani as largely responsible for the flow of water and saline constituents, and the sympathetic as responsible for the stimulation of the gland cells to the production of the organic constituents and ferment.

The Innervation of the Gastric Glands.—The gastric glands, like all the other digestive glands, are supplied by two sets of nerve-fibres, one cerebro-spinal and the other sympathetic. In the case of the stomach the cerebro-spinal fibres are supplied by the vagus and the sympathetic fibres are derived from the solar plexus.

The proof that these fibres possess an effect upon the process of secretion by the gastric glands has, however, been exceedingly difficult to obtain unequivocally by experiment, mainly on account of the important nerve supplies to other organs and regions which accompany the gastric nerve-fibres in the vagus. As a result of this, section or stimulation of the vagus gives rise to profound effects other than those upon the secreting cells of the gastric mucous membrane, which obscure and mask, or interfere with, the effects upon secretion; and hence it was only by ingenious methods of avoiding such results that Pawlow and his co-workers were able to demonstrate that the vagus contained excitatory fibres for the secreting cells. Several of the earlier workers upon the subject found that section of both vagi in the neck led to suppression of the gastric secretion; but this double operation performed at one time leads to such profound disturbances that, as Pawlow points out, it had little effect in encouraging a belief in a causal connection between the vagus-fibres and gastric secretion, since it is not to be wondered at that an operation which in a short time brings the whole functions of the organism to a standstill should amongst other things disturb the action of the gastric glands. An attitude of caution towards the results of an experiment with such drastic consequence was suggested by the further experiment of Schiff, of dividing the vagi beneath the diaphragm in dogs, when, especially in young animals, there was good recovery and the animals lived in excellent health after the operation. Also Rutherford found that gastric secretion could be formed after section of both vagi, or of both splanchnics. Similarly Pawlow

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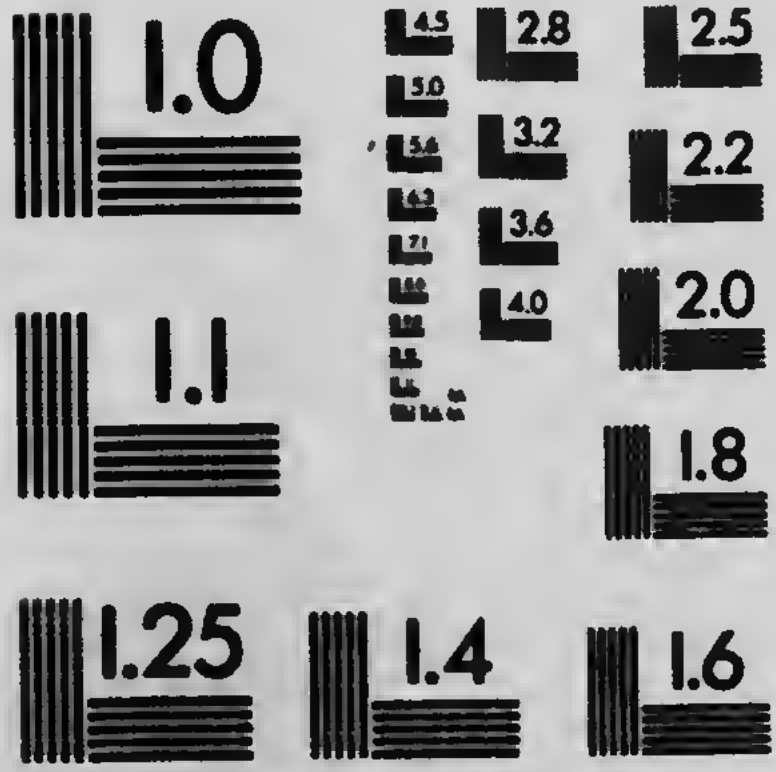

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found much more recently that even after double vagotomy the stomach is capable of preparing its specific secretion in the absence of vagal influence.

But, as Pawlow points out, this does not settle the problem as to whether the vagus contains fibres which influence the secretion, and he adduces evidence going to show that the vagus probably contains both excitatory and inhibitory fibres for the secreting cells of the stomach.

It is only by careful comparison of the secretory activity of the stomach before and after vagotomy, and by stimulation of the peripheral end of the nerve in such a manner, or after such procedures, that other effects upon the heart, &c., are not excited, that we can judge as to any possible effect upon secretion.

Previous to Pawlow's more detailed experiments as to the paths along which efferent stimuli pass to the secreting cells, it had been shown fairly clearly that the gastric secretion could be called forth by reflex nervous mechanism. Thus Richet showed in the case of a boy with an inoperable and complete stricture of the œsophagus occasioned by swallowing caustic alkali, upon whom a gastrotomy had been performed, that soon after taking anything sweet or acid into the mouth a secretion of pure gastric juice occurred, which could accordingly only be excited by a reflex nervous stimulus. Bidder and Schmidt also showed that the sight of food in a dog with a gastric fistula led to a flow of gastric juice. The experiment of Richet was, however, an isolated one, and in those of Bidder and Schmidt the stimulus might have been a direct one due to swallowed saliva.

To Pawlow belongs the credit of having devised most ingenious methods for studying the secretion of the gastric juice; the reflex influence of the nervous system upon the secretion, and the efferent path by which the reflex travelled; as also the effects of different forms of food upon the amount and properties of the secretion.

A method for studying the secretion, apart from any influence of the contact of saliva or swallowed food, was obtained by making a fistula of the œsophagus in the neck in addition to the usual gastric fistula. After the double operation the effect of "*psychical*" stimulation could be studied by *showing* appetising food to the animal but not allowing it to chew or swallow it, when a copious flow of gastric juice resulted after a latent period of about five minutes. Also the effect of *sham* feeding was investigated, in which

the animal, in addition to being shown the food, was allowed to chew and swallow it; but the food dropped out at the œsophageal fistula, and did not enter the stomach and so excite it by direct contact. In this method of sham feeding the flow of gastric juice was somewhat greater in most cases than where the afferent stimulus occurred from the sight of food only; but the increase was never very marked, and in the case of some foods which greatly excited the appetite, the psychical juice or "appetite juice" was as great or even exceeded slightly that from sham feeding.

Again, Pawlow was able to study by this method the effect of stimulation of the gastric mucous membrane by means of direct contact of the food, which was introduced into the stomach through the gastric fistula without the knowledge of the animal, when it was asleep, or when its attention was strongly excited in some other direction. In this case there is an absence of the ordinary excitants of appetite in the sight and smell of food, and in the operations of chewing and tasting it. Here it was found that the effect upon secretion varied with the character of the food, and that contrary to what might perhaps have been expected, digestible proteid food did not always prove to be a strong excitant to a flow of gastric juice. Thus milk or a solution of white of egg introduced into the stomach gave rise to scarcely any secretion, not any more than a quantity of water or dilute saline solution. But meat broth, meat juice, or solutions of meat extracts gave rise in all cases after a latent period of a few minutes to a considerable flow of gastric juice. The number of such direct excitants of a flow of juice was, however, found by Pawlow to be small, being almost confined to certain constituents of flesh food, which are also found in meat extracts. Thus, fats, carbohydrates, and ordinary proteids were without any effect.

In the case of flesh food, Pawlow showed that the amounts of secretion obtained by sham feeding and by direct introduction of the flesh into the stomach, when added together approximately, equalled the amount of secretion when the animal ate the food, and the food which dropped out at the œsophageal fistula was placed in the stomach.

Accordingly the excitation to secretion through the nervous system may be divided into three fractions: viz. (1) that due to the sight and smell of the food; (2) that due to the taste, mastication, and swallowing of the food; and (3) that due to the contact

of the food with the stomach. And of these three the first, according to Pawlow's experiments, is responsible for the greater portion of the flow of secretion.

The statement that mere mechanical irritation of the gastric mucous membrane by contact with foreign bodies is an efficient stimulus to provoke a flow of gastric juice is so often made in physiological text-books, that it may be well to state that Pawlow entirely denies such an influence, and states that the most thorough and prolonged irritation of the mucous surface, with a glass rod or feather, or by the blowing of sand into the stomach, is incapable of causing a single drop of secretion.

Another experimental method of great importance devised by Pawlow, both for investigating the effect upon secretion of various forms of foods and for studying the innervation of the glands, was that of forming a miniature stomach completely lined by mucous membrane, and possessing its nerve supply intact, yet completely shut off from the main stomach.[1] Different foods could be introduced into the main stomach by the usual process of feeding, or, in certain other animals in which the method of operation above described of œsophagotomy and ordinary gastrotomy had been performed in addition to the formation of the miniature stomach, the food could be introduced directly, into the stomach, or psychical or sham feeding could be carried on.

Since the mucous membrane is not injured in the operation, and the nerve supply is left intact, the small pouch of mucous membrane isolated becomes a faithful mirror or index of what is occurring in the main stomach. Accordingly the rate of secretion and the quality of the secretion can be studied throughout the whole process of digestion of a meal of any type, and also the innervation of the glands can be tested by observing the effects of section and stimulation of the nerves supplying the stomach.

We may now return, after the above short sketch of the methods by which Pawlow prepared the stomach for experimentation, and observed the reflex effects of the nervous system upon gastric secretion, to the experiments by which the same observer studied the efferent path in the vagus of the reflex excitation of the secretion.

[1] For the details of this ingenious operation the reader is referred to that most interesting book, "The Work of the Digestive Glands," by J. P. Pawlow, English translation by W. H. Thompson; Griffin, London, 1902.

As has already been stated, the other functions of the vagus are so important that the effects of the nerve upon gastric secretion cannot be observed by the usual simple methods of section and of stimulation of the nerve, without certain preliminary operations which allow of section and stimulation without calling forth an interference at the same time with other important functions. The procedures differ somewhat according to whether the effects of section or of stimulation of the vagus are to be tested, and hence it is better to describe each experiment separately.

Effects upon Gastric Secretion of Section of the Vagal Fibres.— The operation is carried out upon a dog in two stages. In the first stage an ordinary permanent gastric fistula fitted with a metallic cannula is made, and in addition an œsophageal fistula, so that the mouth is cut off from all communication with the cavity of the stomach. At the same operation the right vagus nerve is divided below the point of exit of the recurrent laryngeal and cardiac branches, so that on any subsequent section at a later stage of the left vagus the vagal control of the larynx and heart will still be left in action. If at some time after recovery from the operation food is offered to the animal and is eaten, it of course drops out by the œsophageal fistula and nothing reaches the stomach. Under such circumstances, however, and although the gastric fibres of the vagus on the right side have been completely severed, a copious flow of gastric juice is obtained which starts about five minutes after the commencement of the sham feeding. If now the left vagus be dissected out and severed there is no profound general disturbance of functions, because, although the pulmonary and abdominal vagal fibres on both sides are paralysed, the laryngeal and cardiac fibres on the right side are still intact. If a process of sham feeding be now commenced, although the dog takes and swallows the food greedily, no secretion of gastric juice is evoked by the process, not a single drop flowing from the gastric fistula.

In the same animal in which the above procedures had been carried out the right vagus was at a later period divided *in the neck*, yet the animal continued in perfect health and enjoyed its life to the full, although both cervical vagi were now severed. Double cervical vagotomy was also carried out in similar fashion upon a second dog, which survived the double operation for months. In both these animals after the severance of the second vagus,

sham feeding was found never to give rise to a secretion, although often tested.

In addition to demonstrating that the vagus is at any rate the most important efferent channel for reflex stimulation of the gastric secretion, these experiments clearly demonstrate that the profound and fatal effects of double cervical vagotomy carried out at one operation are due to the sudden shock of complete removal of vagal control from the heart, respiratory, and alimentary systems, and that compensation can occur and prevent the fatal result, if the operation be carried out piecemeal.

Although sham feeding calls forth reflexly no flow of secretion after the vagal fibres have been completely severed, it must not, however, be hastily assumed that no secretion can occur under any circumstances after the vagal fibres have been so thrown out of action, for both Pawlow and other observers, as already mentioned, have observed secretion under such conditions. Whether such secretion is due to stimulation through other nervous channels such as the sympathetic fibres, or to absorption of chemical substances which cause direct chemical stimulation of the gland cells, is still doubtful, but recent work goes to show that such direct chemical action upon the cells is a very probable cause of secretion.

Effects on Gastric Secretion of Stimulation of the Peripheral End of the Severed Cervical Vagus.—The experiment of vagus stimulation yields results entirely confirmatory of those obtained by section of the nerve, but similar preliminary precautions are necessary.

After gastrotomy and œsophagotomy have been previously carried out as before described, one vagus (the right) is cut through as before below the cardiac and laryngeal branches, then the other vagus is cut through in the neck, and after a length has been dissected out and attached to a ligature it is left *in situ*, and the wound closed up for a period of three to four days. The stitches are then carefully removed, exposing the nerve for stimulation, and this is stimulated with slow rhythmic induction shocks at intervals of one to two seconds. A secretion of juice is invariably obtained from the empty stomach as a result of such stimulation. The object of waiting for three or four days after section of the vagus is to allow time for the cardiac fibres to degenerate, which process appears to occur earlier than the degeneration of the secretory fibres to the stomach.

After obtaining positive results regarding the efferent function

of the vagus in gastric secretion by this so-called "chronic" method, Pawlow and his co-workers returned to the attempt to obtain evidence by the so-called "acute" method of stimulation of the peripheral vagus immediately after section. The experimental procedure was to perform tracheotomy, so that artificial respiration could be carried on, to cut the spinal cord beneath the medulla so as to throw out reflex action upon the gastric glands, to sever the vagi, keeping the peripheral ends attached to ligatures for stimulation, to establish an ordinary gastric fistula, and to ligature off the stomach from the œsophagus and pylorus. The results of stimulation of the vagus in these acute experiments were not, however, invariably the same; in more than half of the experiments a flow of secretion was obtained, but the latent period was prolonged from the usual five minutes to from fifteen minutes to an hour or more, and the causal connection of a secretion occurring an hour after stimulation has commenced is, to say the least of it, very doubtful. After the nerve had once commenced to work, however, the dependence of the secretion upon the stimulus became more apparent, for on removal of the stimulus the process of secretion gradually stopped, and on renewal of the stimulus, secretion now appeared with greater rapidity. Administration of atropin stopped the secretion. Pawlow explains the long latent period on the assumption that the vagus contains inhibitory fibres as well as excitatory fibres for the gastric glands.

Nothing is known worth recording regarding the action upon the secretion of the sympathetic fibres which run to the stomach. It is almost impossible to find and stimulate these after they leave the solar plexus. It has been stated that gastric secretion still persists after section of the splanchnics; but this fact alone proves nothing as to the possible effect of these nerves in initiating, inhibiting, or controlling gastric secretion.

Innervation of the Pancreas.—The study of the influence of its nerve supply upon the secretory activity of the pancreas has proved one of the most difficult and perplexing of the problems of gland innervation, and we cannot yet be said to be in possession of clear and complete information as to the influence of its nerves upon the physiological activity of this most important gland. But the study of the subject has indirectly led to most important results in the discovery of the fact that gland activity may be called out,

apart from nervous activity, by the chemical action directly upon the gland cells of substances which are formed in cells in other regions away from the gland, and are carried by the blood stream directly to the gland cells.

The subject of chemical stimulation will be treated in a subsequent section, and we shall here deal with the subject of pancreatic innervation which properly leads up to it.

The nerve supply of the pancreas is similar in plan to that of the stomach, being provided by cerebro-spinal fibres coming from the vagi, and sympathetic fibres derived from the solar plexus.

The methods for studying the effect o ves upon the secretion of pancreatic juice devised by Paw and his co-workers closely resemble those employed in the case of the gastric secretion, with one important exception, namely, that no means was devised similar to the œsophageal fistula for preventing escape of material from the stomach into the duodenum, as a result of, or accompanying, the stimulation of the nerves. This difference is of importance, because it was shown by the workers of the St. Petersburg school themselves that the application of dilute acid solutions or of acid chyme to the duodenal wall gives rise to a copious and long-continued flow of pancreatic juice. This flow was ascribed by these experimenters to a stimulation by the acid of afferent nerve-endings of a local reflex nervous mechanism in the duodenal wall; but it was later shown by Bayliss and Starling, as we shall see in detail in the next section, that the flow of pancreatic juice so obtained was not due to a direct action of the acid upon afferent nerve-endings in the duodenum, nor indeed to nervous mechanism at all, but to a chemical action of the acid upon a substance formed in the duodenal mucous membrane cells which they named *prosecretin*. This pro-secretin is formed during rest in the duodenal cells, and when acid arrives from the stomach is converted into an active substance called *secretin*, which enters the blood stream, is carried to the pancreatic cells, and excites these cells to secretion by acting as a direct chemical stimulus.

For the present, what concerns us here as a preliminary to the description of the experiments of the Pawlow school upon the effect of stimulation of the vagus and sympathetic nerves on the process of pancreatic secretion, is the experimental fact that passage of acid chyme from the stomach to the duodenum

is capable of calling forth a secretion of pancreatic juice. It is accordingly just as important, in experimenting upon the effects of nerve stimulation upon secretion by the pancreas, to make certain that no chyme passes from the stomach to the duodenum, as it is in similar experiments upon gastric secretion to make certain that no food or saliva passes from the mouth to the stomach, upon the importance of which Pawlow himself lays great stress and against which the œsophageal fistula was intended to guard.

The absence of such a precautionary measure seriously invalidates the result of many of the earlier experiments of Pawlow and his collaborators on the effects of vagal stimulation upon pancreatic secretion. Thus, while Pawlow invariably obtained a positive effect upon pancreatic secretion as a result of vagus stimulation after certain preliminary procedures, which will presently be described, had been carried out, Bayliss and Starling were quite unable to find any result upon pancreatic secretion from stimulation of the peripheral end of the vagus. It must hence be regarded as a possibility that in Pawlow's experiments, as a result perhaps of stimulation of movement of the stomach by the vagal excitation, that acid which had escaped from the stomach set free secretin from the duodenal mucous membrane, and this in turn directly stimulated the pancreatic cells. Bayliss and Starling, while not explicitly denying a control of secretion by the vagus, state that they have not in several experiments been able experimentally to demonstrate the fact, and certainly regard the chemical stimulus as the adequate and efficient one.

Hence judgment must be reserved regarding the control of the pancreatic secretion upon the nervous side, and it must be remembered that this serious defect exists in the experiments hitherto made; still the methods used may here be described from their interest as leading up to the discovery of the chemical control, and as the experimental basis of any future attempts at a study of the influence of the gland nerves, when the additional safeguard has been provided of a fistula between pylorus and duodenum, or the separation of these by ligation.

In preparing the permanent pancreatic fistula in the dogs used for the experiments, Pawlow employed a slight modification of the method used by Heidenhain. Heidenhain, in preparing his fistulæ, had completely resected the intestine in order to obtain

a short piece of the intestine into which the pancreatic duct opened, and which was then, after splitting open, attached to the abdominal wall, the continuity of the intestine having of course been restored by suturing together the two ends of the intestine after the removal of the short piece containing the opening of the pancreatic duct. Pawlow improved upon this by merely cutting out a small oval patch of the intestinal wall around the entrance of the pancreatic duct, and then reclosing the intestine, which was only slightly narrowed by this procedure. The small oval patch so removed was then implanted on the outside of the muscular abdominal wall, so that the pancreatic secretion was now poured out to the exterior.

By careful nursing, adaptation of the food, and administration of sodium bicarbonate so as to make good the loss of alkali due to the flowing away of the secretion to the exterior, dogs with such fistulæ could be preserved alive and in good health for a long time, and after recovery could be used for the study of the effect of alterations in the nature of the food upon the amount and quality of the pancreatic secretion, and for the investigation of the effects of the gland nerves upon the process of secretion.

In such an animal, the preliminary procedure to studying the effect of stimulation of the vagus upon the secretory process is to dissect out a portion of the nerve in the neck and cut it, attaching a ligature to the peripheral end. The nerve is then preserved under the skin for a period of four days, in order to allow time for the cardiac fibres to degenerate. After the lapse of this time, the stitches are removed and the nerve stimulated with slow induction shocks. As a result of stimulating, it is found that after a latent period of about three minutes a flow of pancreatic juice commences and gradually increases in quantity. On stopping the stimulus the flow does not instantly stop, but continues in decreasing quantity for a period of four or five minutes from the cessation of the stimulus. Positive results were also obtained by Pawlow by the use of the so-called "acute" method, provided the spinal cord was cut to avoid reflex inhibition from the operative procedures, and the vagus was stimulated below the cardiac nerves so as not to produce disturbance of the heart and circulation. The order of procedure being tracheotomy, severance of cervical spinal cord below medulla, artificial respiration, opening of thorax

and preparation of vagus below the heart, insertion of pancreatic cannula, and slow rhythmic excitation of the nerve.

In using the acute method, evidence of the presence of inhibitory fibres was obtained similar to that mentioned above in the case of the gastric secretion. Thus it was found that after a steady flow of secretion had been set up by stimulation of one vagus, similar and simultaneous excitation of the other vagus often led after a latent period to a suppression of the flow.

An inhibitory influence of the sympathetic fibres was also demonstrated in the case of the acute experiment. The first effect of stimulation of the sympathetic by an induced current was a slight increase in flow; this, however, lasted only for a few seconds, and was followed later, and especially after stopping the stimulus, by a suppression of the secretion. Similar inhibitory results were obtained as a result of mechanical stimulation with the tetanometer, and in a nerve in which degeneration had been allowed to proceed for three or four days a purely inhibitory effect was obtained as a result of electrical stimulation.

The presence of inhibitory fibres in the vagus was also shown by Popielski, by the employment of another method. This observer caused a continuous flow of pancreatic secretion by injection of dilute hydrochloric acid into the duodenum, and then strongly stimulated the vagus, when a slowing of the secretion was always obtained, often to a complete standstill. Excitation of the sympathetic under like circumstances did not produce such a marked effect, but usually gave rise to decrease in rate of secretion after a long latent period.

The important fact that the presence of acids in the duodenum gives rise to a copious and long-continued flow of pancreatic juice was established by Dolinski in Pawlow's laboratory in 1891. The whole mental aspect of the workers in Pawlow's laboratory at that period was directed towards the discovery of the innervation of the pancreas, and hence naturally the flow of pancreatic secretion caused by the presence of the acid in the duodenum was looked upon as a reflex act in which the stimulation of the acid upon peripheral nerve-endings in the duodenal mucous membrane give rise to the afferent impulses.

The secretion set up by the presence of acid in the duodenum was further studied by Popielski and by Wertheimer and Lepage, who showed that secretion was still evoked by the introduction

of acid into the duodenum even after section of both vagi and splanchnics, or destruction of the spinal cord, or after complete extirpation of the solar plexus.

These experiments clearly shut out the central nervous system from participation in the supposed reflex, but the observers, still clinging to the belief that the phenomena before them arose from nervous activity, accommodated their views to the additional experimental facts, by receding to the conclusion that the secretion arose from a *peripheral* reflex act. Popielski concluded, from finding that the secretion occurred after removal of the solar plexus, and also after separating the duodenum with the pylorus from the stomach, but not if the duodenum were cut across a short distance below the pylorus, that the centres for the supposed peripheral reflex were in the scattered ganglia of the pancreas of which the most important were to be found near the pylorus, and were cut off when the duodenum was cut across near to the pylorus. Wertheimer and Lepage accepted the peripheral reflex explanation, but as they found that injection of acid into the jejunum also called out a secretion diminishing in intensity as the distance from the duodenum increased, they came to the conclusion that the centre for the supposed reflex varied according to the region of intestine stimulated by the acid, and that while the secretion in the case of the duodenum might result from stimulation of pancreatic ganglia, that from the jejunum probably was set up by stimulation of the solar plexus. The experiment of injection of acid into a loop of jejunum, after extirpation of the solar plexus, or after severance of the mesenteric nerves of the loop, was not performed by these observers. They found that the secretory effect was not abolished by administration of atropin, but instead of arousing any suspicion that the secretion might not after all be of nervous origin, this fact was only correlated to the absence of effect of this drug upon the sympathetic salivary secretion.

The possibility of the secretion induced by acid in the duodenum being due to chemical action was not unthought of entirely, however, by the St. Petersburg school, and is discussed by Pawlow in his book, in which he states that the acid works either locally by exciting the peripheral end-apparatus of the centripetal nerves in the mucous membrane, or else it is absorbed into the blood and stimulates either the secretory centre or the gland cells

directly. The view that the acid produces its effect by absorption into the blood is then negatived by Pawlow, from theoretical considerations, as well as from the fact that injection of acid into the rectum was without effect upon pancreatic secretion.

It did not occur, however, to the discoverers of the secretion of the pancreas as a specific result of the presence of acids in the duodenum, that there was a third hypothesis, namely, that the acid might awaken an internal secretion in the duodenal cells, and that the substance so secreted might travel in the blood stream to the pancreatic cells and set them in activity.

This view did occur to Bayliss and Starling, who, on testing it experimentally found it to be the correct one, and so not only brilliantly supplemented the work of the St. Petersburg school on pancreatic secretion, but made a new departure in our knowledge regarding secretory processes, and opened up a new field to research which is now being explored for other secretions.

The Chemical Mechanisms of Secretion—Chemical Excitants of Secretion or Hormones

Pancreatic Secretion and Secretin.—The apparently local character of the reaction when acid was placed in the intestine, described in the preceding section, led Bayliss and Starling to experimentation upon the subject, from the view that there might here be an extension of the local reflexes, the action of which in movements of the intestinal wall these observers had already investigated. It was soon found, however, that the phenomenon was one of an entirely different order, and that the secretion of the pancreas is normally called into action not by nervous agency at all, but by a chemical substance formed in the mucous membrane of the upper parts of the small intestine under the influence of acid, and carried thence by the blood stream to the gland cells of the pancreas. To the active substance the name *secretin* has been given by the authors.

In the earlier experiments of Bayliss and Starling, dogs were used, but in a later research other animals were used (rabbit, cat, and monkey), and it was demonstrated that the reaction is a general one for all vertebrates.

The animals received an injection of morphia previous to the

experiment, and during its course were anæsthetised with A.C.E. mixture. In order to keep the condition of the animals constant during the experiment, artificial respiration was resorted to, and a constant depth of anæsthesia was attained by placing the anæsthetising bottle in the air circuit; this procedure is especially necessary when the vagi have been cut. The animals in the earlier experiments had not been fed for a period of eighteen to twenty-four hours, but in later experiments it was shown that secretin is active no matter what may be the state of digestion. In order to avoid shock and to keep up the temperature, the animal was immersed in a bath of warm physiological saline throughout the experiment, the level of the fluid was above that of the abdominal wound, so that the intestine was bathed with the warm fluid. The arterial pressure was always recorded by means of a mercurial manometer connected with the carotid artery in the usual way. The pancreatic juice was obtained by placing a cannula in the larger duct which enters the duodenum on a level with the lower border of the pancreas. To the cannula was connected a long glass tube filled at first with physiological saline; the end of this tube projected over the edge of the bath, so that the drops of the secretion fell upon a mica disc cemented to the lever of a Marey's tambour; this was in connection by means of rubber tubing with another tambour which marked each drop upon the smoked paper of the kymograph. A time tracing was taken showing intervals of ten seconds, and an injection signal was arranged to indicate the point at which acid was injected into the intestine, or a preparation of secretin into a vein, in which a venous cannula had been placed in the usual way.

The authors first confirmed the results of previous experimenters as to the effects of injection of acid into the duodenum or jejunum, and found that the result of injecting from 30 to 50 c.c. of 0·4 per cent. hydrochloric acid into the lumen of the duodenum or jejunum is to produce, after a latent period of about two minutes, a marked flow of pancreatic juice. This effect is still produced after section of both vagi, section of the spinal cord at the level of the foramen magnum, destruction of the spinal cord, section of the splanchnic nerves, extirpation of the solar plexus, or any combination of these operations.

The next step in the chain of evidence was to test the effect of injection of acid into a loop of the upper part of the intestine

after severing the mesenteric nerves. Such a procedure was impossible for anatomical reasons in the duodenum, but was successfully carried out with a positive result on a loop of jejunum.

In this crucial experiment the loop of intestine was completely cut off from all nervous connection with the pancreas, and hence the conclusion is an inevitable one that the effect must be produced by some chemical substance finding its way into the circulation, and then either directly or indirectly stimulating the pancreatic cells.

It must be admitted here that the process of severing all the network of sympathetic nerve-fibres surrounding the blood-vessels passing to the intestinal loop is a difficult one, and it is hard to make certain that it has been effectually carried out, so that it would have been well to insert in this experiment small cannulæ into the completely severed artery and vein of the loop. But, as Bayliss and Starling point out, the experiment was that which led to the discovery of *secretin*, the specific chemical excitant, or *hormone*, of the pancreatic secretion. Also the effects about to be described of injection of extracts of the duodenal or jejunal mucous membrane prepared by the action of dilute acid clearly demonstrate a local action of the secretin upon the pancreas.

The positive result obtained in the experiment with the enervated loop of intestine, taken in conjunction with the result obtained by Wertheimer and Lepage, that acid itself introduced into the circulation has no effect upon the pancreatic secretion, led Bayliss and Starling to the view that the acid must give rise to some active substance in the cells of the mucosa which is taken into the circulation and produces the specific effect. This view was then abundantly confirmed by the results of experiment. The loop of jejunum from which the positive result was obtained was cut out, the mucous membrane scraped off, rubbed up with sand and 0·4 per cent. hydrochloric acid in a mortar, filtered through cotton wool, and the extract injected into a vein. The result was a flow of pancreatic juice at more than twice the rate produced at the beginning of the experiment by introduction of acid into the duodenum. Two further results were obtained in the same experiment: first, it was shown that the acid extract could be boiled without losing its activity, so that the active substance (secretin) was shown not to be a ferment; and secondly, it was shown that the activity of extracts of portions of the small

intestine taken at different levels showed a decreasing amount of activity as the intestine was descended, corresponding to the known effects upon the pancreatic secretion of injection of acid

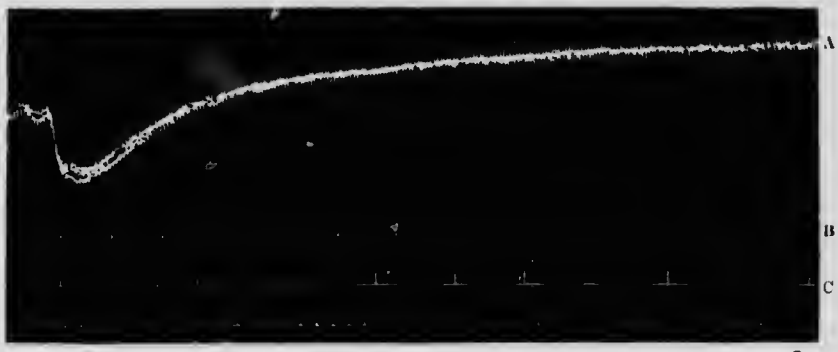

FIG. 5.—Action of Acid Extract of boiled and washed Mucous Membrane of Duodenum. A, Blood Pressure; B, Drops of Pancreatic Juice; C, Drops of Bile. (*Bayliss and Starling.*)

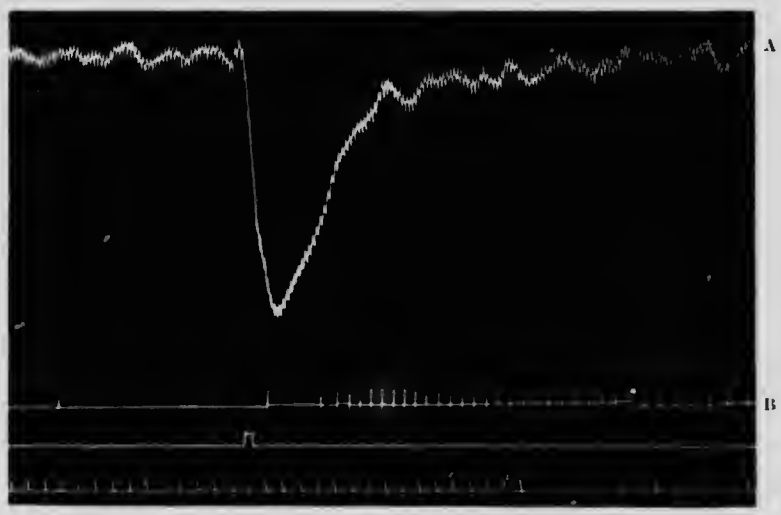

FIG. 6.—Action of Acid Extract of Mucous Membrane of Duodenum which had been dried after Dehydration by Alcohol. A, Blood Pressure; B, Drops of Pancreatic Juice. (*Bayliss and Starling.*)

into these various portions. Thus injection of acid into a loop from the lower end of the ileum gives rise to no pancreatic secretion, and corresponding to this, an acid extract from the mucous

membrane of the lower end of the ileum possesses when intravenously injected no exciting effect upon the pancreatic secretion.

With regard to the seat of action of secretin, Bayliss and Starling have traced it as far as possible towards the periphery, and conclude that it acts in all probability directly as a chemical excitant upon the secreting cells of the pancreas. It is impossible with our present experimental methods to exclude a possible action upon the nerve cells and fibres in the pancreas itself; just as it is impossible to do so in the case of tracing towards the periphery the seat of action of any drug or other active substance for example, to exclude an action of adrenalin upon nerve cells or endings upon the muscular walls of small arteries rather than upon the muscle cells directly. But it has been shown that the excitatory effect upon the pancreatic secretion is still obtained after the gland has been cut off, as far as is experimentally possible from the anatomical relationships, from connection with nervous mechanisms, both central and peripheral. The sensitiveness of the pancreas renders practically impossible the experiment of perfusion of whipped blood containing secretin through the excised gland.

Certain physical and chemical properties of secretin solution have also been investigated by Bayliss and Starling and W. A. Osborne, as well as the properties of the pancreatic juice secreted as a result of the action of secretin: the results are summarised in the following conclusions, taken from Bayliss and Starling's paper:—

1. The secretion of the pancreatic juice is normally evoked by the entrance of acid chyme into the duodenum, and is proportional to the amount of acid entering (Pawlow). This secretion does not depend on a nervous reflex, and occurs when all the nervous connections of the intestine are destroyed.

2. The contact of the acid with the epithelial cells of the duodenum causes in them the production of a body (secretin), which is absorbed from the cells by the blood current, and is carried to the pancreas, where it acts as a specific stimulus to the pancreatic cells, exciting a secretion of pancreatic juice proportional to the amount of secretin present.

3. This substance, secretin, is produced probably by a process of hydrolysis from a precursor (prosecretin) present in the cells,

which is insoluble in water and alkalies and is not destroyed by boiling alcohol.

4. Secretin is not a ferment. It withstands boiling in acid, neutral, or alkaline solutions, but is easily destroyed by active pancreatic juice or by oxidising agents. It is not precipitated from its watery solutions by tannic acid, or alcohol and ether. It is destroyed by most metallic salts. It is slightly diffusible through parchment paper.

5. The pancreatic juice obtained by secretin injection has no actions on proteids until "enterokinase" is added. It acts on starch and to some extent on fats, the action on fats being increased by the addition of succus entericus. It is, in fact, normal pancreatic juice.

6. Secretin rapidly disappears from the tissues, but cannot be detected in any of the secretions. It is apparently not absorbed from the lumen of the intestine.

7. It is not possible to obtain a body resembling secretin from any tissues of the body other than the mucous membrane of the duodenum and jejunum.

8. Secretin solutions, free from bile salts, cause some increase in the secretion of bile. They have no action on any other glands.

9. Acid extracts of the mucous membrane normally contain a body which causes a fall of blood pressure. This body is not secretin, and the latter may be prepared free from the depressor substance by acting on desquamated epithelial cells with acid.

The Chemical Mechanism of Gastric Secretion—Gastrin.—It has long been known that the introduction of certain substances into the stomach provokes a secretion of gastric juice, and the effect has been ascribed to a nervous mechanism stimulated by the effect of absorbed substances upon peripheral nerve-endings in the gastric mucosa. Quite recently, however, it has been shown by Edkins, that intravenous injection of extracts prepared in special manner from certain parts of the gastric mucous membrane leads to a flow of gastric juice. Edkins considers this action to be due to a substance which he has named *gastrin*, and which acts as a chemical excitant or "hormone" for the gastric secretion, in a similar fashion to secretin in the case of the pancreatic juice. It is hence possible that those substances shown by Pawlow to excite the gastric secretion when introduced into the stomach so as not

to call forth a psychical flow, as by the use of a sound, or better, through a gastric fistula without attracting the animal's attention, produce their effect not by exciting peripheral nerve-endings in the gastric mucosa, but by means of a chemical action upon the secreting cells. This action may either be a direct one of the substances themselves or more probably, according to Edkins's observations, an indirect action in which these substances, similarly to hydrochloric acid in the case of the duodenal mucosa, set free an active substance chiefly from the pyloric portion of the gastric mucosa. This substance, after being absorbed by the blood stream, is carried to the secreting cells lying deeper in the mucosa, and also to the secreting cells of the fundus, where it acts as a chemical stimulant, and calls forth secretion.

Edkins has studied the effects of intravenous injection of extracts made from different parts of the gastric mucosa. He placed a certain amount of saline in the stomach, and then determined the amount of acid formed in the stomach after the injection of each extract to be tested into a vein by titrating this saline for total acidity.

The results obtained were as follow

"If an extract in 5 per cent. dextran of the fundus mucous membrane be injected into the jugular vein, there is no evidence of secretion of gastric juice. If the extract be made with the pyloric mucous membrane, there is evidence of a small quantity of secretion. With dextrin by itself there is no secretion.

"Extracts of fundus mucous membrane in dextrose or maltose give no secretion; extracts of pyloric mucous membrane give marked secretion; dextrose or maltose alone bring about no secretion.

"If extracts be made with commercial peptone, it is found that no secretion occurs with the fundus mucous membrane, a marked secretion with the pyloric mucous membrane: the peptone alone gives a slight secretion.

"If the extracts be made by boiling the mucous membrane in the different media, the effect is just the same, that is to say, the active principle, which may be called 'gastrin,' is not destroyed by boiling.

"Finally, it may be pointed out that such absorption as occurs in the stomach apparently takes place at the pyloric end. In the pig's stomach, in which the cardiac region differs from the

ordinary type in only having simple glands as in the pyloric, extracts of the cardiac region in general have the same efficien v in promoting secretion as do pyloric."

The media most powerful in calling forth secretion in these experiments are hence those containing the products of advanced salivary digestion, or of peptic digestion, viz. glucose, maltose and commercial peptone, and the region from which active preparations can be prepared being the pyloric mucous membrane, which also is the region in which any slight absorption in the stomach occurs, the indication of the experiments is that the precursor of the active gastrin is formed in the pyloric mucosa, and is activated by the absorption of these digestive products, and discharged into the blood stream, whence it reaches the gastric secreting cells.

The earlier experiments of Pawlow upon those substances which excite gastric secretion on introduction into the stomach are of interest in the light of these later experiments on intravenous injection. Thus introduction of water into the stomach, *even after section of both vagi*, always gave rise to a secretion, although not a very copious one; here there is a good deal cut off from central control, as the vagi are clearly, from Pawlow's other experiments, the most important efferent nerves for gastric secretion, and it would be most interesting to know if this secretion on the introduction of water also occurred after more profound interference with the central nervous system connections, *e.g.* if it still took place after destruction of the spinal cord and extirpation of the solar plexus.

Alkaline solutions, such as sodium bicarbonate, were found by Pawlow to exercise an inhibitory effect upon gastric secretion. Fresh meat and meat extracts were found to be the most powerful excitants, and research is required to test whether this action is nervous or chemical in origin. Starch and fat were found by Pawlow not to excite secretion on direct introduction without psychical stimulation. Bread and solution of egg-albumin also were found to be non-excitants, but the fluid digestive products from the stomach of another dog which had eaten egg-albumin, when introduced without psychical effect directly into the main stomach, gave a stronger and more constant effect than a like quantity of water

The above experiments upon the effects of chemical stimulants

formed in the cells of the body itself upon the activity of the secreting cells of pancreas and stomach open up to physiological research a field of great importance, and one with practical bearing for medicine and organo-therapy. Doubtless similar actions occur elsewhere in the body which will in the future be brought to light. Bayliss and Starling in their paper briefly draw attention to what they term the *chemical sympathies* between uterus and mammary gland, and to the modifications in the composition of the pancreatic juice accompanying long-continued change in the diet, such, for example, as the production of a laccase as the result of milk feeding, and call attention to the advisability of a renewed investigation of these facts from the point of view of the production in such cases of bodies allied to secretin. There is no doubt that in many cases the stimulus to seasonal functional activity of organs may be a chemical one. In this connection also might be mentioned the occurrence of menstruation, and the seasonal recurrence of rut in cattle, also the absence of these during pregnancy accompanying the changed chemical metabolism at such a period, and the chemical changes going on in the corpus luteum of the ovary.

Thus the field of "internal secretion," which first began to be explored in the case of the ductless glands, the thyroid and suprarenal, goes on widening in scope, and we learn afresh that an organ or cell, in addition to its most conspicuous function, may possess other and no less important chemical activities.

Effects of Food upon the Production of the Digestive Secretions.—A number of most interesting and valuable observations have been published from the Pawlow school, upon the effects of different foods on the rate of secretion, and variations in this during the period of digestion, and on the alterations in the quality of the secretion resulting from the intake of different foods, and continuance upon different diets for more prolonged periods. The series of experiments upon these points are very extensive, and only a summary of results can be included in this article; a good account of the matter is contained in Pawlow's book on "The Work of the Digestive Glands" (translated by W. H. Thompson).

1. Secretion under normal conditions only commences as a result of food being taken into the alimentary canal. The miniature

stomach does not secrete during inanition, but commences a few minutes after a meal. The quantity of juice from a pancreatic fistula during hunger amounts to only two or three c.c. per hour, but some time after a meal increases to many times that amount.

2. The quantity of juice secreted in the case of the same food is directly proportional to the quantity of food taken. Thus for raw meat, for 100 grm., 26 c.c. of gastric juice were secreted; for 200 grm., 40 c.c. of juice, and for 400 grm., 106 c.c. On a mixed diet of meat (50 grm.), bread (50 grm.), and milk (300 c.c.) 42 c.c. of gastric juice were secreted, for double these quantities 83·2 c.c. were secreted.

3. The secretion is not all poured out rapidly at the beginning, but is distributed throughout the period of digestion, and the curve of quantity secreted and time varies for the different types of food. Each food possesses a modifying effect both upon the quantity and quality of the secretion. Also the presence of one food has a modifying power upon the secretion called forth by another, and on the whole course of digestion.

Thus in the case of gastric secretion of a meal of flesh, bread, or milk respectively. Each separate food corresponds to a definite hourly rate of secretion, and calls forth a characteristic alteration of the properties of the juice. Flesh and bread diet produces a maximum rate during the first hour of digestion, while milk gives the maximum rate during the second or third hour. Tested as to maximum content in ferment during the period of digestion, the greatest activity is found with flesh diet in the beginning; with bread in the second and third hours; and with milk in the last (or sixth) hour. Contrasting the digestive power of the juices for proteid at corresponding periods of digestion in the case of the three foods, the greatest power is found in the case of the flesh diet, the bread comes second, and, in the earlier stages at least, close to the meat, while that on the milk diet is much feebler in proteolytic power.

In the case of the pancreatic secretion, a similar adaptation of the secretion to the nature of the food is seen, and here the changes become more striking, because there is a ferment for each class of food-stuff, and relative variations can be contrasted.

The following table of results by Walther, quoted by Pawlow, gives the variation in secretion (quantity and ferments) of pan-

creatic juice on milk, bread, and flesh respectively. The quantities of each food given are based on the percentages of nitrogen contained in each variety :—

Diet.	Quantity of Pancreatic Juice.	Proteoclastic Strength.	Amylolastic Strength.	Steatoclastic Strength.
Milk, 600 c.c. .	18	226	9	903
Bread, 250 grm.	151	134	106	53
Flesh, 100 grm.	114	106	15	250

The adaptation of the secretion to the nature of the food requires no comment. Attention may be drawn to the high proteoclastic power of the secretion called forth by the milk, and to a contrast of this with the low proteoclastic power produced by milk in the case of the gastric secretion. In the case of flesh there is the reverse effect. It looks from the figures as if the proteid of the flesh were digested chiefly in the stomach and that of the milk in the intestine.

Similar variation in the curve of rate of secretion and time are found in the case of the pancreas as in the stomach, the curve being characteristic for each food. The form of this curve is altered by the simultaneous presence of different food-stuffs: thus the curve of gastric secretion for lean meat consisting chiefly of proteid, becomes profoundly modified if a small amount of fat or oil be also given: the rate of secretion and amount of pepsin being reduced, and the maximum point of secretion being pushed back to a later point in the period of digestion. Similarly the curve of secretion for flesh is modified by the addition of starch to the flesh meal, so as to come to resemble fairly closely that of a bread meal.

4. When an animal is kept for a long period (some weeks) upon a definite and constant diet, the ferment content of the pancreatic juice becomes adapted to the character of the food. If, for example, an animal which has been fed for some weeks entirely upon bread and milk is brought on to an exclusively meat diet, which in contrast with the other diet contains more proteid but scarcely any carbohydrate, it is found that the power of the pancreatic juice for digesting proteid increases from day to day, while the digestive power for starch progressively diminishes. On reversing the diet

again to bread and milk, similar but inverse changes are observed. The moral from this for practical medicine, which experience had already indicated, is that a sudden change from one régime to another may have a disastrous effect upon the digestive process, by subjecting the glands to a strain to which they have not been adapted. Hence changes in dietary should be brought about slowly and progressively wherever possible, and not by a sudden and sweeping change.

The physiological causes and mechanisms of this interesting adaptation in quantity and quality of the digestive fluids to the nature of the food are as yet obscure to us.

Pawlow, their chief discoverer, ascribed them to a differentiated peripheral nerve supply in the mucous membrane of the alimentary canal, whereby the absorption of different digested food-stuffs stimulated different nerve endings, fibres, and cells, and caused a discharge of correspondingly different efferent stimuli to the gland cells, as also to the variation in amount and kind of psychical stimulation by the variation to sight and smell of different sorts of food.

This explanation was given, however, before the days of Bayliss and Starling's discovery of the chemical excitants to secretion, and the question now remains an open one whether the nervous system has anything, and if so how much, to do with the adaptation of secretion to food, and with the characteristic variations above described of rate and progress of secretion with the nature of the food.

In the light of our new knowledge the whole subject of secretion stands ripe for investigation, and is rich in promise of new additions to our knowledge, of the highest value to physiology and to medicine.

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CHAPTER VII

THE ATMOSPHERE

THE conception of Laplace has been generally accepted that the solar system arose as a condensation from a vast nebula of gaseous matter, a nebula such as may be seen in various parts of the heavens at the present day. This nebula, by radiating its heat into space, cooled and contracted in volume, and in so doing threw off rings of vapour at various stages. These rings by the action of gravity and centrifugal force became moulded into planets.

The earth, intensely heated by the latent heat set free in the process of condensation, first appeared as a luminous mass surrounded by an atmosphere of metallic vapour—"vapours which condensed and fell and boiled off again on touching the hot surface." In the infinity of time the crust of the earth cooled, the metallic vapours condensed into solid earth, the atmosphere cleared, and finally, but under vastly different conditions to the present, conditions of heat, humidity, &c., with earth and atmosphere in a more intense state of chemical flux, the synthesis of protoplasm took place. As the earth cooled it ceased to appreciably affect the temperature of the atmosphere, and the latter finally came to be maintained by the sun's rays some 290° C. warmer than space. Atmosphere and water clung to the earth owing to the attraction of gravity. The moon with its smaller mass has long lost both.

The question has often been raised as to whether life exists in any of the other planets. The survey of terrestrial life shows a wide range in the scale of physical conditions under which protoplasm is capable of living. Condors circle round the peaks of the Andes miles high in realms of cold attenuated air, fishes swim in the profound depths of the ocean where the water pressure is equal to two tons to the sq. inch. Algæ have been found in hot springs living at a temperature of 55° C., and Richet discovered the sulphur organisms of Luchon living in water at 70° C. Dollinger, in a period of four years, gradually

accustomed *Flagellatia*, which normally live in water [illegible] C., to live in water at 70 C. To destroy the spo[illegible] or bacilli temperatures of 110 to 120 C. are required. On the other hand, algæ which produce red snow flourish at a temperature below 0 C., and bacteria can withstand cold exceeding −200 C. produced by the evaporation of liquid air. Some organisms only flourish in the purest sea-water, while others multiply in conditions of putrescence where there is entire absence of free oxygen. Nevertheless, in Mars, which is but half the diameter of the earth, and still more in the other planets, the conditions of heat, moisture, light, and gravity must incalculably transcend all terrestrial variations, and render the existence of protoplasm there most improbable, and certainly forbid the evolution of terrestrial forms of life. It is none the less conceivable that in the infinity of space other suns exist attended by planets where the physical conditions are similar to those on the earth, and where life exists. As the structure of the atom is now recognised to resemble a miniature universe, so our universe may be but an atom in one infinitely greater.

The chief source of kinetic energy on the earth—with the exception of the tides, which depend on the rotation of the earth on its axis—is the light and heat rays of the sun. Currents of air, winds, and storms arise from unequal warming of the different layers of the air, the ocean currents from unequal warming of the sea. By the sun's heat water vapour is formed and lifted into the higher and cold regions of the atmosphere. There the vapour condenses and [illegible] of the ethereal waves appears as the kinetic energy of the falling rain, and of the flowing brooks and streams. [illegible]es alike the ships and windmills, the water-wheels and [illegible]c electric motors at the Niagara falls, the sunlight which illuminated the earth millions of years before burns in our fires, and drives our factory machines and locomotives. The sunlight generates the energy of the whole living world.

The composition of the atmosphere and the nature of the process of combustion and of respiration have only been known for some six score years, while geological evidence points to the existence of man for years which probably number hundreds of thousands, and archæological evidence shows that the chemical handling of metals had advanced in Egypt so far as the fashion-

ing of copper and gold ornaments 6000–7000 years ago. The energy of the alchemist, misdirected in pursuit of the transmutation of metals, was wasted owing to the neglect of accurate measurement and weighing of the substances used in and the products obtained by their manipulations. "If," says Scheele in 1777, "the chemists of the preceding century had thought worthy of a more particular examination the elastic fluids resembling air which manifest themselves in so many operations, how advanced should we now be! They desired to see everything in corporeal form, and to collect everything as drops in the receiver." [1]

Leonardo da Vinci, who followed the pursuit of science with no less genius than that of art, observed that fire robbed air of its life-sustaining property. Van Helmont (1557–1664) separated a gas which was given off by the action of vinegar on shell, by burning wood and by fermentation, a gas which would not support life — in fact, carbonic acid gas. Mayow (1646–1679) recognised that there was in the atmosphere an essential part, active in supporting combustion, in calcining metals, in changing venous to arterial blood, and in sustaining fermentation. He determined that confined air loses its power to support combustion and life. Mayow, in fact, discovered oxygen under the name "spiritus nitro-aerius," but his experiments attracted little notice and were soon forgotten. Boyle (1624–1694) demonstrated to the newly-founded Royal Society the death of animals placed in the evacuated receiver of his celebrated air-pump, and recognised that the air must be renewed in sustaining life by artificial respiration. Bernouilli found that fish could not live in boiled (gas-free) water. Lower (1669) observed the colour change of venous into arterial blood during the artificial respiration of an animal. Joseph Black (1757), by his admirable researches on the analysis and synthesis of chalk, separated "fixed air" (carbonic acid), and studied the properties of this gas. In 1772, Scheele, a poor Swedish apothecary, "put an ounce of purified nitre in a glass retort for distillation, and made use of a bladder, moistened and emptied of air, in place of a receiver. As soon as the nitre began to glow it also began to boil, and at the same time the bladder was expanded by the "fire-air" (oxygen) that passed over. In 1774, Priestley, an eminent Unitarian minister of Birmingham,

[1] Perkin and Lean, "Introduction to Chemistry and Physics."

separated "dephlogisticated air" (oxygen), by heating with a burning-glass red-lead over mercury. He determined that "fixed air" (CO_2), is a product of respiration, and that plants purified confined air and made it again fit for respiration. Priestley determined that combustion, respiration, and the colour of arterial blood depended on the presence of "dephlogisticated air." But confined by the theories of Stahl, he misinterpreted his results, and supposed that respiration disembarrassed the body of "phlogiston" or fire matter introduced with the food, that arterial blood became red because it was freed from "phlogistic air" (nitrogen). Lavoisier (1743–1794), hearing of Priestley's discovery, by his memorable experiments lasting twelve days successively synthesised and analysed red oxide of mercury, showed that this is a combination of oxygen and mercury, and that the separation of the two is effected by heat. Stahl and his school supposed the metals to be compounds of earths charged with the matter of fire, impregnated with "phlogiston"; that the earths were simple bodies freed from phlogiston by calcination. In the hands of Lavoisier the process of oxidation became clear, and combustion was proved to be a case of oxidation.

Air is not a chemical compound but a mixture. Analysed in the dry state, it yields on the average by volume 20·93 per cent. O_2, 79·04 per cent. N_2, ·03 per cent. CO_2 with traces of ammonia and ozone, argon, krypton, neon, metargon. The last four gases owe their presence to their non-combining property. They are residues not employed in the building of the earth's crust. Air, when pure, is free from colour, taste, and smell. It has frequently been asserted that air varies slightly in composition at different places and seasons. Such variations are, with the exception of CO_2, due to unavoidable errors in analysis—errors which are greater, or smaller, according to the degree of perfection of the analytical apparatus and the skill of the analyst.

Air collected by means of balloon sounds at an altitude of 15,000 m. has the same composition as air at sea-level. Air taken from the ill-ventilated pit of a theatre contained 20·7 per cent. O_2 by volume. In towns the CO_2 percentage may rise to 6·9 parts per 10,000. Ozone contains three atoms of oxygen in its molecule, one being in the active state. The traces of it formed by atmospheric electricity are more abundant on mountains and at the seaside.

Gases diffuse with a velocity inversely as the sq. rt. of their densities, but diffusion is so slow a process that it would take ages to restore uniformity to a perfectly still atmosphere if this were once effectually disturbed. The ceaseless convection currents due to the sun's heat keep the atmosphere mixed and practically uniform in composition.

Deoxygenated or impure air only lurks in mines, tunnels, wells, and brewers' vats, and large public halls, and these require artificial methods of ventilation. In small rooms the ventilation which takes place through the walls is usually sufficient, but in large rooms as the air content increases as the cube while the wall surface increases as the square, the ventilation is imperfect, and stagnant air fills the central parts (Haldane).

When a mixture of gases dissolves in a liquid, each component dissolves according to its own partial pressure (Dalton's law). There are divergences from these laws in the case of very soluble gases and at great pressures. The chief gases of the atmosphere are sparingly soluble, are neutral gases forming neither acidic nor basic substances by their union with water. The higher the temperature and the less the partial pressure, the more the gas molecules by their increased mobility escape from their solvent.

The air respired by aquatic animals is that dissolved in the water, and as the respective solubilities of N_2 and O_2 at 0° C. are ·02 and ·04 respectively, water holds in proportion to nitrogen more oxygen than the atmosphere. The amount of these gases dissolved in the sea appears to be uniform at all depths thus:—

Depth.	Vol. O_2 per Litre.	Vol. N_2 per Litre.
0	6·1–7·8	11·3–14·1
3219 m.	6·7	12·9

The CO_2 in the sea is combined as bicarbonate with calcium carbonate, of which there is always an excess ($CaCO_3 + H_2CO_3$).

The composition of the atmosphere probably has been almost constant throughout the more recent geological ages. It is kept so by the metabolic antagonism of the plant and animal world. The food-stuffs of green plants, CO_2, H_2O, nitrates, and other mineral salts are saturated oxygen compounds. They possess no more potential energy than a stone lying on the earth. The energy of the sunlight activates by means of chlorophyll the plant protoplasm, and this sundering oxygen from carbon and hydrogen builds compounds which are themselves

poor in oxygen, and possessing a high affinity for oxygen, store up chemical potential energy.

As a generalisation, plants build and animals destroy organic substances. The life of the plant is synthetic, a reduction process: that of the animal analytic, an oxidation process. The plant converts kinetic into potential energy: the animal converts the potential energy of its food-stuff the plant into kinetic energy. But there is no jump in nature. There are unicellular chlorophyll-free organisms—moulds and bacteria which cannot assimilate carbon out of CO_2. They must be fed on organic combinations—on sugar, tartrates, &c. On the other hand, these organisms assimilate nitrogen from inorganic combinations salts of ammonia, nitrites—and synthesise proteid. By producing fermentation they decompose organic compounds, and like animals use up potential and develop kinetic energy—warmth and movement. Certain highly organised parasitic plants, belonging to the phanerogams, are also chlorophyll-free and feed on organic matter synthesised by other plants.

On the animal side there are certain worms and Coelenterates, such as *Hydra viridis*, which contain chloroplasts, and seek the sunlight and die when kept in the dark. The chloroplasts belong to monocellular algæ, which live symbiotically in these organisms. Within the stiff cellulose membrane of each cell of the higher plants there lies a contractile protoplasmic body which respires and shows active streaming movements, using up oxygen and giving out carbonic acid. Only in the chloroplast-holding cells is this process of oxidation covered by an intenser process of reduction which obtains during the incidence of sunlight. Similarly in every cell of the higher animals synthetic as well as analytic processes take place.

The combustion that takes place in the living organism is no such simple process as the combustion of coal in a steam-engine. The food, before it reaches its final end products, undergoes a whole row of different chemical changes, changes of varying energetic significance. In the steam-engine the heat, derived from the chemical energy of the fuel, is the motive power, and it matters little from what fuel the heat comes. In the organism the food is not only heat-producer, but tissue builder and repairer, and generator of new organisms of a similar type.

Regarded as a source of energy, the food must be converted

into varying intermediary products, the sources of muscular, nervous, and secretory activity, conversions which signify manifold chemical processes. The living cell, wonderful in its minuteness, is capable of the most extraordinary number of chemical reactions, many of which run in opposite directions. The liver cell, for example, builds glycogen out of sugar, and sugar out of glycogen, forms urea and uric acid out of amido-acids and ammonia, breaks down haemoglobin, separating iron and forming bilirubin, produces cholalic acid out of unknown precursors and links it with taurin and glycin, and binds phenol with the radicle of sulphuric acid. These are the chief known activities; in addition there are hosts of others, including the assimilation, hydration, and oxidation of food-stuffs. In the laboratory such chemical reactions can only be carried out with the different reagents separated in many vessels, with the aid of heat and other physical agencies, acids, alkalies, &c., used as activators, and many laboratory appliances. In the cell we are confronted with an astounding simplicity of structure, and a mechanism which spares space and energy to a marvellous degree.

Hofmeister suggests that the fundamental principle of this structure is the existence of innumerable enzymes, or precursors of enzymes, of a colloidal nature, which are fixed in the colloidal bioplasm owing to their non-diffusible nature. The cell is formed like a foam-structure, and the colloidal enzymes, being separated by the impermeable membranes of the foam, give a chemical organisation to the cell and allow an orderly progression of chemical reaction. If we conceive [illegible] the products of one reaction activate another enzyme, [illegible] starts a second reaction, and so on in progression, the sequence of activity is explained in a plausible way. The cells, owing to their colloidal structure, are continually permeated by a current of diffusible substances, which sets from blood to lymph, a watery solution of oxygen, salts, sugar, glycogen, &c. The reactions take place between the enzymes and the contents of this current. Nothing but a colloidal structure would allow such a complication of organisation to be confined in the smallest space, together with permeability to non-colloids. The kinetic energy of living organisms is obtained from the enzymic decomposition either of the bioplasm itself or of the non-colloidal food-stuffs that permeate the bioplasm. The quick

road that proteid takes to urea when fed in excess renders untenable the hypothesis of Pflüger that all proteid is built into bioplasm of the cells before its energy-producing decomposition. In the exchange between tissue cell and blood probably the same enzymic decomposition of the colloidal food-stuffs takes place as in the intestine. The minced organs, when allowed to undergo autolysis in aseptic conditions and at body temperature, yield ferments and products of digestion which suggest that each cell can at need dissolve parts of its corporeal mass and send into the blood stream non-colloidal food-stuffs, which there become raised to the colloidal state and carried to other organs to satisfy their needs. Such a process takes place in the formation of the substance of the generative glands of the salmon from the fat and proteid of the back muscles, during the long fast that these fish take in ascending the rivers from the sea to their breeding stations.

The products of the enzymic decomposition of the food-stuffs are in most cells brought into the final state of oxidation as CO_2, H_2O. The animal organism uses sparingly the nitrogen-holding nucleus of its bioplasm, which is only obtainable out of proteid foods, and obtains its energy from the combustion of the carbohydrate group. This group can be conceived either as permeating the cell in the non-colloidal state, or as forming a side chain to the central nitrogenous nucleus, a chain which can be easily bound and broken.

The demand for oxygen varies greatly in different organisms. In mammals and birds the supply becomes rapidly exhausted, while the lower vertebrates can survive asphyxia for many hours at low temperatures. This difference does not depend entirely on the homoio- or poikilo-thermism of the vertebrates, for it is also found among invertebrates. Thus Copepods became motionless after exposure to a current of hydrogen for thirty minutes, while leeches show active movements after three days. The streaming movements of the cytoplasm of plant cells, *e.g.* the stamen hairs of Tradescantia, the development of ova, the movements of amœbæ, and the wonderful to and fro streamings of the plasmodium of myxomycetes are arrested alike in the absence of oxygen, and finally the protoplasm in each case becomes cloudy, clots and dies. The anaerobic bacteria gain oxygen by decomposing nitrates, and using this to decompose carbohydrates, obtain energy.

The whole of the carbon of the living world, of the vast forests and myriad animal forms, comes from the ·03 per cent. CO_2 in the atmosphere.[1] The amount of this CO_2 is estimated at $2{\cdot}4 \times 10^{12}$ tons. Brown and Escombe have contrasted the rate of absorption of a solution of potash with that of the stomata of the leaves of *Catalpa bignonioides*, and found that the latter absorb CO_2 from a current of air no less than fifty times quicker than the potash. One square metre of leaf surface laid on 1 grm. of dry substance per hour corresponding to 784 c.c. CO_2, and the stomata occupied only 1 per cent. of the surface. The chloroplasts of the plant cells act as an "activator," converting by the fluorescence of the chlorophyll pigment a large part of the highly refractile rays of sunlight into red rays, and activating the chemical reactions of the protoplasm which result in the condensation of CO_2 and H_2O to hexose. It has been suggested that the formation of formaldehyde, $H.CHO$, is the first stage in the reduction of CO_2. This substance in the presence of weak alkali readily undergoes polymerisation into formose $(CH_2O)_n$, a mixture of substances some of which have the composition of the sugars. Huxley has calculated that the atmosphere, superincumbent on one square mile of country, continually holds 13,800 tons of CO_2—enough carbon to supply 7400 tons of trees. Bunge suggests that in the early and more volcanic periods of the earth's history the percentage of CO_2 stood at a higher figure, and the luscious growth of vegetation in the Carboniferous period abstracted this excess. This, buried as coal, is now being rapidly returned to the atmosphere by fire. Bunge also suggests that the percentage of CO_2 in the atmosphere is being reduced by its union with the bases of the earth's crust. The chief rocks of the crust are carbonates and silicates of calcium, magnesium, ferrous oxide, &c. In the presence of water and in the cold CO_2 has a greater affinity than silicic acid for the bases. Rain and waves containing traces of CO_2 ceaselessly corrode the silicic rocks, and from their solution the carbonates of chalk and magnesia, and the silicates clay and sand are thrown down. In the deeper strata heat arises from the mechanical effects of compression, and under the influence of heat silicic acid

[1] Bacteria are known which assimilate the minute trace of hydrocarbon in the atmosphere and cannot utilise CO_2.

gains the mastery over and expels CO_2. The latter gas is returned to the atmosphere through volcanic fissures. Bunge likewise suggests that the percentage of oxygen is lessening. Ferrous oxide arises as a product of the decomposition of certain silicates, and this combines with oxygen to form ferric oxide. The ferric oxide is decomposed in the presence of rotting organic matter and the oxygen returned to the atmosphere combined as CO_2. Plant life, either living or rotting, seems to be the sole means by which oxygen is set free, and it is doubtful whether all the oxygen returns to the atmosphere, which is combined in the processes of respiration, combustion, and in the oxidation of iron and sulphur.

As the earth cools and its crust consolidates, Bunge supposes more and more CO_2 will become fixed, and if this fixation is unbalanced, life must finally cease. There is, however, another source of CO_2—the bicarbonate of lime dissolved in sea-water. When the tension of CO_2 in the air falls, bicarbonate becomes dissociated into carbonate and CO_2. The process reverses when the tension rises. Thus the amount of CO_2 is kept constant, and Bunge's prophecy can be discarded. In the sea it is estimated there is 27 times as much CO_2 as in the atmosphere, and that an additional 0·1 per cent. CO_2 in the atmosphere would lead to the absorption by the sea of $3{\cdot}85 \times 10^{9}$ tons in one year (A. Krogh).

Without a due supply of nitrogenous food living matter cannot carry on the processes of growth—the building of skeletal structures and the storage of food-stuffs. In the higher plants nitrogen appears to be employed almost entirely for the building of organ proteid, while carbohydrate is used for skeletal structures—*e.g.* bark, store of this being easily obtained.

Saprophytes and parasites living in plenteous supplies of nitrogenous matter build with chitin in place of cellulose. Animals use both nitrogenous and carbohydrate matter as sources of kinetic energy, and in them a daily output as well as intake of nitrogen occurs. Nitrogenous matter as a source of energy is wasteful, for only the carbohydrate moiety is required. The higher plants obtain energy from the combustion of carbohydrate, and with the possible exception of the alkaloids, do not excrete nitrogen. Nitrogenous matter is used as a source of energy in the decomposition of organic nitrogenous matter by numerous kinds of

denitrifying bacteria, and those bacteria which produce the hydrolytic fermentation of urea into ammonium carbonate ($CO(NH_2)_2 + 2H_2O = CO_3(NH_4)_2$), &c.

The discovery of nitrifying bacteria, and of the fact that atmospheric nitrogen is assimilated by their means, is one of the most interesting and important of recent times.

Beijerinck discovered that a bacterium—the bacterium radicicola—lives symbiotically in the root nodules of the Leguminosæ,

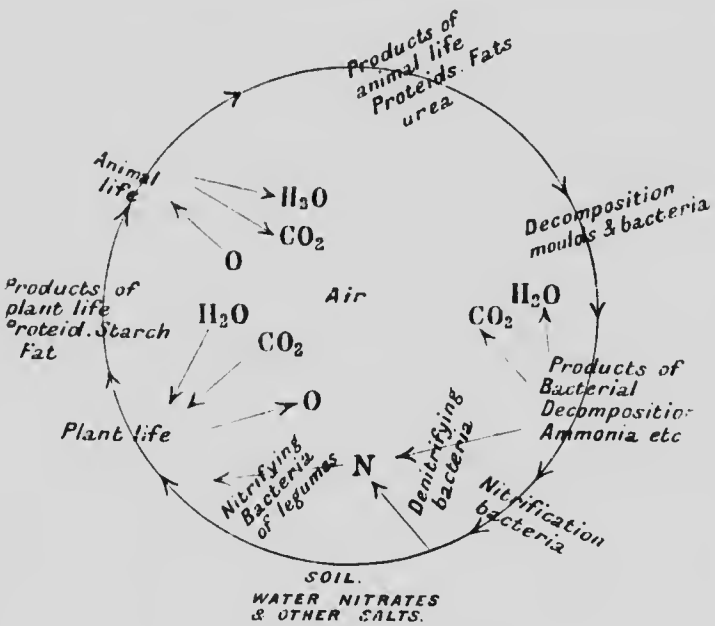


FIG. 7

such as the pea, clover, lupin, and that by means of these bacteria the peas acquire more nitrogen than there is in the test soil. Such crops may be grown and ploughed in to enrich sandy soils for wheat growing. Kühn has calculated that a hectar (about two acres) of soil may be enriched by 66 kg. of N_2 yearly by means of nitrifying bacteria. The raw nitrogenous material available for the synthesis of proteid includes (1) the atmospheric N_2 obtained by nitrifying bacteria. (2) The traces of ammonia and nitric acid in the air. Electric discharge in a moist atmosphere,

as in a thunderstorm, causes the combination of nitrogen and hydrogen to form ammonium nitrite, $2N + 2H_2O = NH_4NO_2$. It has been calculated that about 15 kg. of N_2 are thus carried down by the rain per acre per annum. (3) Ammonia salts, nitrites and nitrates in the soil and water. (4) Organic nitrogenous compounds from dead plants and animals, such as acid amides, amido-acids, amines, and other proteid derivates. Such are the supplies. On the other hand, every combustion of organic matter, whether by fire or by denitrifying bacteria, sets nitrogen free. Bunge has calculated that in 50 years 10 men die for each sq. kilometre of land of the earth's surface. If cremation becomes universal so much poorer must the land become in nitrogen. An additional loss for the present time is the vast amount of sewage thrown into the sea and the enormous mass of organic matter burnt. Already the older countries, such as England, must be manured to ensure the raising of good crops, and to-day the combined nitrogen of the virgin soils of America are poured down the English sewers into the sea.

Denitrifying bacteria are very numerous in dung heaps, &c. They act only in anaerobic conditions and in the presence of carbohydrate. These organisms have been regarded as the farmer's enemy, as destroyers of the nitrogenous food of plants. Probably the conditions are such in the soil (*e.g.* presence of oxygen) that their action is not energetic. The small amount of animal life in the Pacific Ocean has been attributed to the presence of denitrifying bacteria, to these bacteria robbing the plants of nitrates, and so preventing a sufficient growth of food supplies for animals. The process of denitrification probably consists of two stages, each stage being induced by the enzymic action of a different organism. The first stage is the reduction of nitrate to nitrite, the second the decomposition of the nitrite and evolution of nitrogen. Such a decomposition occurs in a solution of ammonium nitrite near the boiling-point — $NH_4NO_2 \rightleftarrows N_2 + 2H_2O$, and is accelerated by platinum black acting as a catalyser. The nitrifying bacteria probably reverse the action of the denitrifying bacteria.

Nitrification bacteria were discovered by Winogradsky. The *nitrosomonas* oxidises ammonia into nitrates, while the *nitrobacteria* oxidises nitrites into nitrates. These organisms only

grow in media containing neither organic nitrogen or carbohydrate. They are of great theoretical interest, because they build proteid out of inorganic salts (ammonia or nitrites, CO_2 and Na_2CO_3, are necessary) without the aid of chlorophyll. It has been demonstrated that the synthesis of plant protoplasm takes place in the green leaves, for the proteid content increases if the leaves of the sunflower are fed with a solution of sugar and potassium nitrate. (This synthesis goes on in the dark.) A purely tentative suggestion has been put forward, that oxy-fatty acids produced from the sugar may unite with ammonia nitrite to form amido-acids, *e.g.* $CH_2OH.COOH + NH_4NO_2 = CH_2NH_2.COOH + HNO_2 + H_2O$, and that the proteid is formed by the condensation of these amido-acids. The study of the hydrolytic decomposition of proteids and of the products of condensation of amido-acids, such as Grimaux's artificial colloids, has led Hofmeister to the conclusion that proteids are chiefly formed from the condensation of these acids.

The molecular constitution of the proteids has been studied by decomposing them by hydrolytic means—by boiling mineral acids or by ferments, and also by strong potash, oxidising agents, &c.

The end products of the relatively most simple proteids consist of a number of carbon nuclei, which are in chemical constitution so far apart from one another that they are regarded as existing preformed in the proteid molecule.

The nuclei constantly present, according to Hofmeist consist of—

(1) Nuclei containing C,N,H. Example, the guanidin radicle—$CNH.NH_2$. (A group which, on hydrolysis with baryta water, yields urea.)

(2) Nuclei containing C,N,H,O—

(*a*) Mono-basic, mono-amido acids, with the general formula $CnH_{2n+1}NO_2$—as leucin (C_6), glycin (C_2), alanin (C_3).

(*b*) Dibasic mono-amido acids, $C_nH_{2n-1}NO_4$—as glutaminic (C_5) and asparaginic (C_4) acids.

(*c*) Mono-basic diamido acids, $C_nH_{2n-2}N_2O_2$—as ornithin (diamido valerianic acid), arginin (guanidin-amido valerianic acid), lysin (a-ϵ diamido caproic acid).

(3) Amido-alcohols or hexosamines, which yield the carbohydrate group of proteids as chitosamin ($C_6H_{13}NO_5$). A carbohydrate group has been obtained from both serum albumin and globulin, and is probably common to most proteids.

(4) Nuclei containing CN_2H_4OS, as cystein or amido-thio lactic acid, $CH_2SH.CH(NH_2)COOH$.

(5) Aromatic nuclei. The phenyl-amido-propionyl nucleus constantly appears, as in Phenyl-alanin, Tyrosin.

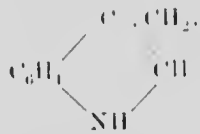
OH . . . . (1)

C_6H_4

$CH_2.CH.NH_2.COOH$ (4)

(6) Nuclei of the pyrrol group: pyrrolidin-carbonic acid.

(7) Nuclei of the indol group: indol, skatol, tryptophan (skatol amido acetic acid)



(8) Nuclei of the pyridin group; after long lasting peptic digestion of albumin, and heating the product with NaOH, a smell of pyridine results.

CHAPTER VIII

THE EFFECT ON LIFE OF LESSENING THE BAROMETRIC PRESSURE

According to the kinetic theory of gases, the molecules of a gas are in a state of constant motion, and the pressure exerted by a gas on its neighbourhood, whether solid, liquid, or gas, is measured by the number of molecular impacts per unit of time. If the gas within a chamber be compressed, the paths of the atoms are shortened, the number of impacts increased, and the pressure rises. If the temperature be raised the speed of the movement increases, the number of impacts increase in unit time, and the pressure rises. When a gas is suddenly compressed the rate of impact is increased by the push, and the temperature rises; conversely, when it is decompressed—allowed suddenly to expand—the temperature falls.

When air under constant pressure is heated it expands by 0·00367 of its volume at 0° C. for each degree Centigrade. Thus 1 litre of air at 0° C. becomes 1·00367 l. at 1° C., and 1·0367 l. at 10° C. To find what the observed volume of a gas at the observed temperature t° C. would be when reduced to 0° C. we have

$$V : V' = 1 : 1 + 0{\cdot}00367\ t^{\circ}$$

$$\therefore V = \frac{V' \cdot 1}{1 + 0{\cdot}00367\ t}$$

At a constant temperature the volume of a gas is in inverse proportion to the pressure. A litre of air at one atmosphere occupies half a litre if compressed to two atmospheres. To find what the volume of a gas measured under a pressure p will be under normal pressure 760 mm. we have—

$$V = \frac{V' \cdot p}{760}$$

These two formulæ can be combined as one—

$$V = \frac{V' \cdot p}{760\ (1 + 0{\cdot}00367)\ t}$$

and this is the formula used for reducing all gas volumes to the standard 0° C. and 760 mm. If the gases are measured wet, the

tension of water vapour at t must be deducted from p. The percentage composition of the air measured by volume is the same on hot or cold days, at high or low altitudes, but in given volume contains less air by weight on hot days or at high altitudes.

The presence of water vapour in the atmosphere lowers the tension of oxygen. For example, the barometric pressure was 756·6 and the water-vapour tension 49, and the O_2 tension on this occasion equalled $(756{\cdot}6 - 49) \times \frac{20{\cdot}93}{100} = 148{\cdot}1$ mm.

The intake of O_2 is not *directly* affected by variations in temperature and dryness of the air, for as the lung air is always saturated at 37° it does not matter whether the air is hot or cold, moist or dry. The O_2 intake is, however, *indirectly* affected by the mechanisms which regulate the body temperature.

In 1640 Torricelli invented the barometer, and eight years later Perier, on the suggestion of Pascal, showed by an ascent of the Puy de Dôme that the barometric pressure fell with the increase in altitude. The mean pressure at sea-level and latitude 45 is 760 mm. Hg. It varies slightly with the latitude in proportion to the variation of the acceleration due to gravity.

The calculations of the height of the atmosphere vary from 42,000 to 320,000 kilometres, and have been made from the fall of barometric pressure in balloon ascents, from the height of meteors which become incandescent from friction on entering the atmosphere, and from the duration of twilight, which is caused by the scattering of light by the dust and vapour particles in the atmosphere. As the atmosphere is not homogeneous in composition, and the centrifugal force and the acceleration due to gravity which act upon it vary with the distance from the earth, a complicated formula (Laplace) is required to deduce an altitude from the barometric pressure.

At 5000 m. the pressure is roughly 400 mm. Hg, and the oxygen by weight 11 per cent., or almost half that at sea-level. For each 300 ft. of ascent the temperature falls about 1° F., and thus the refugee from the hot plains of India finds at a height of 6000 ft. on the Himalayan slopes the mean temperature of the Riviera.

The higher strata of the air are very cold (−52° C. at 10,500 m.) —owing to the diathermancy of pure air, the absorption of the heat radiated from the earth by the dust and water vapour with which

the lower strata are laden, and the propinquity of the higher strata to space. The radiant heat of the sun, on the other hand, is much greater at high altitudes owing to the diathermancy of air, and the absence of dust and water particles which scatter the rays in the lower strata. The velocity of the wind increases in the higher regions of the atmosphere as the friction against earth and the denser lower strata decreases, while the slope increases down which the air raised by the tropical heat flows towards the poles. These factors, together with the dryness of the atmosphere and the lessened CO_2 and O_2 tension, have great influence on the existence of life in high altitudes. The freedom of the air from bacteria is complete—there are only 150 dust particles per c.c. of air on Mont Blanc against 150,000 in cities under the worst conditions. "I had forgotten a wallet on the Matterhorn," said a guide, "in which there was some bread and cheese, and last year I found it again and ate up the contents, which were not at all musty." Ropes and ladders last for years which would have rotted in the valleys.

Water boils on Mont Blanc at 84·3° C. owing to the diminished barometric pressure. In Pike's Peak Observatory (14,147 ft.) a pan full of loose snow was set on the hot stove to melt, and in a very short time the water in the bottom of the pan began to boil, while the snow on the top of it was yet 3 to 4 inches deep. Fuel burns with difficulty owing to the diminished pressure of oxygen. Thus the oil burnt in an asbestos wick lamp in a given time was 2·193 grm. at 760 mm. Hg and 1·9119 grm. at 360 mm. Hg. The electrical potential in the region of lofty peaks is often very high and produces striking effects.

The air is ordinarily charged with a certain amount of positive electricity while the earth is usually negative. Increase in electrical potential is caused by the masses of water vapour which, rising from the sea or snow-fields, become condensed. As the tiny droplets unite in the clouds to form larger drops, the electrical charges which always exist on their surfaces become added together. Since the surface of the drop is far smaller than the surfaces of the combined droplets, the electrical potential increases with the condensation. Such condensation takes place, particularly when warm air from the plains is forced up mountain slopes. Darwin, speaking of an experience in the Andes, says,

"My flannel waistcoat when rubbed in the dark appeared as if it had been washed with phosphorus; every hair on the dog's back crackled, even the linen sheets, and leathern straps of the saddle, when handled emitted sparks."

An observer stationed at Pike's Peak Observatory records that the thunderstorms are tremendous; on one occasion "his hair stood erect, crackled, and the pricking sensation to the scalp was extremely painful. The peculiar electrical odour was strongly recognised. To protect his head, he put on his black felt hat and returned to the roof. But a few seconds elapsed before he was fairly lifted off his feet by the electrical fluid piercing through the top of his hat, giving him such a sudden and fiery thrust that he nearly fell from the roof in his excitement. Instantly snatching the hat from his head, he observed a beam of light, as thick as a lead pencil, which seemed to pass through the hat, projecting to about an inch on either side, and which remained visible for several seconds. The top of his hat was at least two inches from his head when this fiery lance pierced him. When the fluid began to thrust its fiery tongues into other parts of his body, he was spurred to a hasty, but 'brilliant' retreat." "The cups of the anemometer, which were revolving rapidly, appeared as one solid ring of fire from which issued a loud rushing and hissing sound. The observer on placing his hands over the cups did not discover the slightest sensation of heat, but his hands became instantly aflame. On raising them and spreading his fingers, each of them became tipped with one or more cones of light, nearly three inches in length."

The highest dwelling-place continuously occupied is the Observatory El Misti in the Andes, at 5880 m. The Observatory of Arequipa is at 6100 m. Thok djalung is a village in the Himalayas at 4980 m. In Peru, Bolivia, and northern Chile a very large part of the population live above 3000 m. Potosi, which has numbered 100,000 inhabitants, is at 4165 m., Cerro de Pasco at 4350 m., the mines of Villacota at 5042, the railway from Callao to Oroya culminates in a tunnel at 4760 m., almost the height of Mont Blanc. Such works are sufficient evidences of the energy of man at altitudes of 13,000–15,000 ft. An annual fair is held at Gartok at 4598 m. in the Himalayas, to which thousands annually come. Jourdanet says the inhabitants of the high altitudes in America are anæmic and of poor physique: Mosso says the same

of the shepherds of the high Alps. This is probably an effect of poor food rather than lessened barometric pressure.

	Altitude.	Barometric Pressure.
	Metres.	Mm. Hg.
The sea	0	760
Davos Platz	1,560	620
Observatory of Mont Blanc	4,810	420
Chimborazo	6,420	340
Aconcagua, scaled by Zurbriggen and Fitzgerald	7,320	305
Mount Everest	8,840	248
Berson in balloon	10,500	202
A. Mosso exposed himself in a pneumatic cabinet to a pressure equivalent to—	11,650	192

Altitude.		Barometric Pressure.
Metres.	Feet.	Mm. Hg.
0	0	760
1000	3,280	670
2000	6,560	592
3000	9,840	522
4000	12,290	460
5000	16,405	406
6000	19,850	358
7000	22,965	316
8000	26,245	279

No cats, it is said, live at altitudes above 3500 m. They sicken in the villages of the Cordillera, become dejected, have convulsions of an epileptic character, and finally die. On the other hand, the condors fly from the sea-level to the tops of the peaks of the Andes in a few minutes. From the average stretch of wings (8–14 ft.) and the visual angle Humboldt calculated the altitude of these birds. To him, stationed at an altitude of some 13,000 ft. on Cotopaxi, they appeared as mere black specks. Now Humboldt found he could detect the long white poncho of a rider in the clear air of the Cordillera at a distance of some 80,000 ft. Irradiation would lessen the size of the image of the birds in the sunlit air. Taking this into account, it seems possible that these birds attain to a height of half this, say five miles!

Plants cultivated on mountains become coriaceous and spinous like desert plants; there is an hypertrophy of the roots and an atrophy of the parts above ground. The cells of the leaves thicken, the palissade tissue hypertrophies, and the chlorophyll becomes very abundant. These changes resist the increased evaporation and compensate for the diminished tension of CO_2.

At 410 mm. Hg ordinary plants wither up however wet the earth is kept. At 500 mm. Hg germinating cress seeds grow half as quickly as at 760 mm. Hg, while at 70 mm. Hg they refuse to germinate. In the Alps the forests end at 1800 m.; in the Andes the vine grows even at 3000 m.; in the Himalayas the apricot at 3000 m., and the poplar at 4000 m.

Mountain Sickness

The travellers who followed the conquerors of South America first recorded the peculiar effects of high altitudes. Neither in the history of Cortez, the conqueror of Mexico, who sent an expedition up the crater of Popocatepetl (5420 m.), nor in that of Pizarro, who with 62 horse and 102 foot soldiers penetrated the Andes to the heart of the empire of the Incas, is there any definite chronicle of this sickness among the general record of suffering from cold and hunger.

A Jesuit, Acosta, gave in 1590 the first clear description of the symptoms of mountain sickness. These symptoms are shortness of breath, palpitation of the heart, nausea, loss of appetite, injection of and bleeding from the mucous membranes, vertigo, faintness, and in particular the difficulty of making any muscular exertion. Many are affected at 2000–3000 m., every one suffers at 4000 m. from shortness of breath and fatigue, while more serious symptoms generally occur at 5000 m. Training and acclimatisation have a great influence. At Quito and Potosi girls dance half the night and toreadors display their skill in the bull-ring, while de Saussure and his companions were so overcome at their first ascent of Mont Blanc that every movement became a difficulty. So is it with new-comers at Potosi.

Conway, when out of training for hill-climbing, suffered from shortness of breath at 7000 ft. in the Alps, a symptom which he had not experienced, when trained, at 19,000 ft. in the Karakorams. While building the Matterhorn hut at 4114 m.

the guides had to take breath every time a few blows were struck. At 6000 m. in the Himalayas Zurbriggen found he could not strike more than five blows with an ice-axe without having to pause for one minute to regain his breath. V. Mosso at Turin could execute 3·48 kg. metres of work with the ergograph, while 2·828 kg. metres produced fatigue in the Monte Rosa hut at 4560 m. Whymper found he could walk a level mile in 11′ 4″ at London, while at Quito he took 11′ 58″. Mountain sickness decreases in the Alps every year as the training of the Alpinists becomes more complete and as the refuge-huts increase in number and comfort. Training gets rid of superfluous fat and water, increases weight of muscle and muscular power, develops the economy of the nerve-muscular mechanism, the breathing and the heart's action. New movement complexes are established, fewer muscles are used, and the needless tension of antagonists corrected. The greater concentration of the tissue fluids produced by training must favour osmotic change and cell activity, and lightens the load which is lifted. Manca after seventy days' training could with dumb-bells do five times the work of the first day. The output of CO_2 was at first increased fourfold by the ascent of Berne Cathedral tower, but after training only threefold (Kronecker and Grube). The body temperature may rise from 37 to 39·5° (103° F.) in the untrained, while an Alpine soldier of splendid physique carried a pack of 40 kg. up the glacier of the Gnifetti peak with a rise of temperature not greater than a few points of a degree (Mosso). Owing to the diathermancy of the air the sun's rays have a most powerful effect, and Conway recommends that peaks should be attacked from a north-south valley to win shade, and in bad weather and by night as much as possible. Windy ridges rather than gullies should be chosen. The adaptation of the nervous system to danger is most important. Feelings of insecurity and anxiety which arise in snow-storms, fog, and darkness rapidly exhaust the Alpinist. Bert became dizzy at 420 mm. Hg in his pneumatic chamber, while Mosso by practice and aided by oxygen inhalations had the resolution to expose himself to 192 mm. Hg when his hand covered the height of the barometric column. A debauch makes a great difference in the capacity of a man to climb. At 4560 m. a soldier, under Mosso's observation, lifted 5 kg. dumb-bells at 4″ intervals 104 times. His pulse rose from 80 to 100, his respiration from 20 to 28. After a

drinking debauch he executed only 67 lifts, and his pulse rose from 56 to 110, his respiration from 18 to 32.

To show the influence of fatigue on the induction of mountain sickness, P. Regnard placed two guinea-pigs under the bell of the air-pump, one at rest and the other in a tread-wheel which was rotated by an electric motor. This guinea-pig was compelled to run up the wheel, and became affected at 3000 m. and tumbled on its back at 4600 m., while the resting animal was not affected until the barometric pressure corresponded to 8000 m.

To explain the effect of muscular work, we must turn to the study of the respiratory exchange. Zuntz and Schumberg have compared the consumption of oxygen in walking on the flat and in climbing. The subject carried a gas-meter on his back connected with a mouthpiece which was provided with inspiratory and expiratory valves. A sample of the expired air was collected by the rotation of the meter by a special device, while the total amount was measured by the meter. The percentage composition of the inspired air was calculated from readings of barometer, temperature, and humidity. Analysis of the expired air and the readings of the meter together gave the CO_2 output and the O_2 intake, and the respiratory quotient was calculated from these. The estimation of the urinary nitrogen excretion gave the proteid metabolised, and the respiratory quotient indicated the share which fat or carbohydrate respectively took in the metabolism. The nitrogen-holding nucleus of the muscle substance is not used except under conditions of insufficient food or overstrain. Zuntz calculates that 1 litre O_2 is used in the combustion of 1 grm. proteid and yields 4·476 Cal.; of fat, 4·686 Cal.; of starch, 5·047 Cal. After a meal carbohydrate is burnt, and the Caloric worth is found by multiplying the litres of O_2 consumed by 5. Before breakfast or fasting body fat is used, and the Caloric worth is obtained by multiplying by 4·8.

For walking on level ground the average consumption of O_2 per kg. body weight per km. of march averages 100–110 c.c. O_2. On increasing the pace from 58 to 140 m. per min. the O_2 use was doubled. Climbing up 100 m. in a walk covering 1 km. likewise doubled the O_2 use. Difficulties in the way, steep ground, unpractised movements, greatly increase the O_2 consumption. A sore foot increased it by 18 per cent. Fatigue likewise increased

the consumption, but only for the particular combination of muscles used in walking—hence the advantage of change of work. The volume of air breathed increases from 8 l. per min. resting, to 16 l. walking on the flat, and to 26 l. climbing. In steep climbing the volume may even be fivefold that resting. Fatigue from over-walking renders the respiration shallow and the pulse frequent. Over-strain causes a marked rise of body temperature, increased N_2 output, dilatation of the heart, sinking of the vascular tone. The kidneys secrete a dilute urine, and albumin may appear owing to congestion of the blood-vessels. Poisonous products of metabolism are produced in fatigued muscles, and extracts of these appear to be extremely toxic to normal animals. Immunity can be established by giving small doses of these extracts, and it has been suggested that training partly consists in the immunisation of the body against fatigue products.

Zuntz and Schumberg observed soldiers marching, and found they could carry a load of 22 kg., and march 15–20 km. without noticeable rise of body temperature. After a birthday drinking bout, however, the body temperature rose to 39·3, and 40·5 during the march. A load of 22 kg. has a significant effect on the untrained, while the trained soldier is hard put to it under a load of 31·5 kg. About 20 kg. is the limit of load which should be put upon the soldier, and this should be distributed so as not to disturb the equilibrium of the body. The vital capacity is lessened by the load hampering the movements of respiration, and the systole of the heart is prolonged even to 30 per cent. by a load of 18 kg.

When the breathing becomes as frequent as 35 per min. or more and shallow from fatigue, the phenomena of cardiac dilatation and venous congestion, such as increased cardiac dulness and enlargement of the liver, became marked. The frequency of respiration should not increase more than 75 per cent. during the march, and should not be more than 30 per cent. above normal after a 15 min. rest. This is an easy test for a man to apply to himself.

Over-work leads to destruction of muscle substance and long lasting diminution of functional power. Fatigue leads to a less economical use of the muscles, and increases the Calories expended in work by 5–9 per cent.

In climbing much energy is spent in maintaining the internal as well as the external work of the body, as the heart and respiration are both greatly enforced and accelerated. Owing to this more *external* work can be done in a day on level ground than on the mountains. On the other hand, judicious training in climbing does much to strengthen the heart and respiratory mechanism. The working capacity of some mountaineers who act as carriers to the Alpine huts is most astonishing.

Hueppe records a man who carried 110 kg. up 1500 m. in six hours! He calculated his work at 290,400 kgm. in 4½ hours. Hueppe gives the following calculations to show the economy of the energy[1] spent in climbing by the trained and untrained man respectively:—

	Energy Spent while Resting.	Energy Spent in Climbing (External Work).	Energy Spent in Heat Production while Climbing.	Percentage of Energy Spent in Work.
	Calories.	Calories.	Calories.	Per Cent.
Town dweller, untrained	1500	449 (6 hours' climb)	1000	15
Town dweller, partly trained	"	584 (7 hours' climb)	865	20
Alpine carrier	"	884 (10-12 hours' climb)	565	30

To study the effect of altitude apart from that of fatigue, we must consider those who ascend by rail as to Potosi, or by balloon, or the observations of those scientists like Mosso and Zuntz who have spent many days in the Alpine huts, and made therein exact observations.

Balloon Sickness

The symptoms which have been observed in high balloon ascents are a gradual decrease of mental energy, vertigo, loss of muscular power, and finally swooning. In the celebrated ascent of Glaisher and Coxwell to 29,000 ft. (barometer 9¾ inches), the former found he could no longer see to read his instruments, his arms became helpless, and his head fell on to his shoulders.

[1] The energy required to raise 1 l. of water 1° C. 1 Calorie, and equals the work done in lifting 1 kg. 425 m. high.

Everything became dark before him, and he lost consciousness for a minute or two, till aroused by Coxwell. In the fatal ascent of the "Zenith" to 8600 m. Croce Spinelli and Sivel died, while Tissandier became unconscious.

"At 7500 m.," Tissandier wrote, "the numbness which comes over one is extraordinary. Body and mind grew gradually, imperceptibly weaker without our becoming aware of the change." "Presently I wished to take hold of the oxygen tube, but found that I could not raise my arm. My mind was, however, still quite lucid. I kept my eyes fixed on the barometer. I tried to call out 'We are 8000 m. high,' but my tongue was as though paralysed. Suddenly my eyes closed and I fell down like a log, completely losing consciousness. It was about 1.30 P.M. At 3.30 P.M. I reopened my eyes, feeling dazed and exhausted, but gradually my mind grew clear. The balloon was descending with frightful rapidity. My two companions were cowering down in the car, their heads hidden under their travelling rugs. I gathered all my strength together and tried to raise them. Sivel's face was cyanosed, his eyes dull, his mouth open and filled with blood. Croce's eyes were half closed and his mouth covered with blood. . . ."

Zuntz and v. Schrötter have twice ascended to about 5000 m. In their first ascent Schrötter suffered from headache and a peculiar vertigo, and found it difficult to read the experimental observations. A few respirations of oxygen restored him. Aeronauts do not, as a rule, suffer much before an altitude of 6000–7000 m. is reached, because they are not called upon to execute strenuous muscular work in the car.

It is probable that aeronauts have often suffered from CO poisoning due to the coal-gas used to inflate the balloon. The dissociation curves of CO and O11b show that as little as 0·01 per cent. CO (measured at 0° C. and 760 mm.[1]) in the air would affect a shallow breather whose alveolar O_2 tension may sink to 40 mm. at 4000 m., while a deep breather would be affected by this amount at 6000 m. An equal weight of coal gas escaping in a dwelling in a town on the Andes must be more dangerous than on the plains.

[1] Since the CO tension goes down as well as the O_2 tension, a given percentage of CO, measured at say 400 mm., would be no more poisonous than the same percentage measured at 760 mm. (Haldane.)

The Results obtained by the Use of Pneumatic Chambers

Boyle observed a bubble of gas in the aqueous humour of a viper produced by the rapid evacuation of his air-pump. Such an evolution can never take place in the comparatively slow ascent of the balloonist. Laghi (1757) placed a bird, a cat, and a candle under a bell-glass—the animals lived long after the candle flickered out. Cigna (1760) put a sparrow under the bell of an air pump and lowered the pressure to about 229 mm. Hg (8840 m.). He renewed the air occasionally. The bird was none the worse after 30′. When an animal is killed under the air-pump it gives some signs of restlessness, then falls over and dies with a few convulsive movements. Death takes place inevitably, when the oxygen is lowered to 3 per cent. of an atmosphere. In the deoxygenated air of foul wells men suddenly, and without warning, fall unconscious.

In a pneumatic chamber enriched with oxygen Bert exposed himself to 240 mm. Hg (the height of Mount Everest). Mosso exposed himself in 25′ to 310 mm. Hg (6400 m.) and felt a heaviness of the head, and found difficulty in counting his pulse, which rose from 70 to 88. Recovering in 20′ from these symptoms he exposed himself to 292 mm. Hg (7167 m.), and again became heavy and apathetic. Oxygen was then let into the chamber, and his pulse rate dropped to 64, but became too thready to feel at the wrist. After the pressure had been lowered further to 192 mm. Hg, he became too apathetic to pick up the pencil which he had dropped. The air at this stage enriched by oxygen yielded 8·11 per cent. atm. O_2.

U. Mosso lowered himself to 310 mm. Hg. His mental faculties became blurred, he experienced difficulty in reading his watch, was twice unable to count his pulse, his handwriting altered, and his memory weakened. His eyes became dull and apathetic.

At 330 mm. Hg monkeys observed by Mosso vomited and became apathetic and unsteady on their legs. Warm-blooded animals are, owing to their quick metabolism, far more sensitive than cold, and a low temperature and rapid decompression favour the onset of symptoms (Bert). Lack of O_2 only produces dyspnœa if sudden and intense. Loewy found his breathing volume per min. to be 4·027 l. at 750 mm.; 4·497 l. at 435 mm.; and 5·556 l. at 360 mm., a trifling increase compared to that produced by CO_2 retention.

The Cause of the Symptoms of Mountain Sickness, &c.

Analysing the blood-gases, Bert found the amount of oxygen in the blood diminished by $\frac{1}{3}$ when the barometric pressure was lowered to $\frac{1}{3}$. He rightly attributed all the symptoms to want of

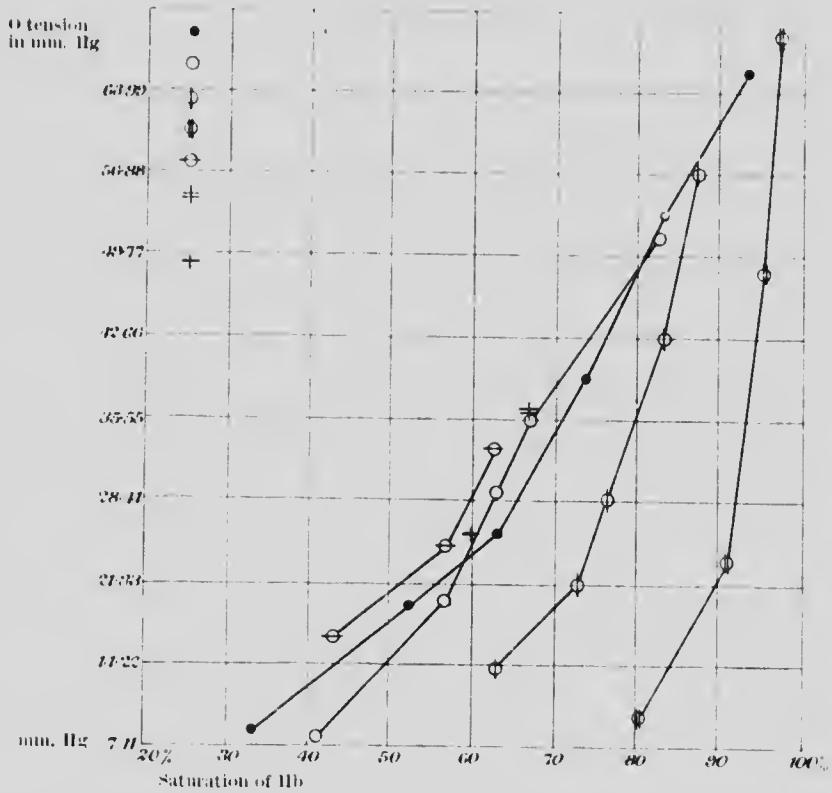


Fig. 8.—Comparison of Dissociation Curves of OHb. ● Loewy (men); ○ Paul Bert; Φ Hufner (new); ⋕ Hüfner (old); ≑ Loewy and Zuntz (dog); ǂ Loewy (O_2 sat. of venous blood of men); + Strassburg-Wolffberg (venous blood of dogs).

oxygen. His analyses were supported by others of Fraenkel and Geppert. The acceptance of this simple and satisfying explanation was rendered difficult by the publication of Hüfner's dissociation curve of oxygen, a curve which Hüfner worked out from solutions of purified hæmoglobin crystals. Hüfner found the Hb solution was more than 92 per cent. sat. when the O_2 tension equalled only 4 per cent. atm., and to explain the death

of animals submitted to this pressure he supposed that a marked difference in tension between the blood and the alveolar air is necessary to drive the blood through the alveolar wall. Hufner's curve has been received for many years into the text-books. Loewy and Zuntz have shown that Hufner's methods of preparation are at fault, and that the dissociation curve of fresh living blood is quite different to that of prepared haemoglobin or laked blood, and, in fact, closely agrees with that worked out by Bert.

Comparing the saturation of laked and normal blood, Durig and Zuntz obtained the following figures :—

O_2 Tension.	Sat. of Normal Blood.
24·07 mm.	58·71
	Sat. of Laked Blood.
23·10 mm.	78·84

which show the great effect produced by the mere solution of the haemoglobin. The use of alcohol in preparing crystallised Hb still further changes the dissociation curve.

In the blood-corpuscles, the haemoglobin is held in a suspended condition, and the other constituents of the corpuscles modify the dissociation of oxygen.

The following figures have been worked out by A. Loewy from human blood drawn from the median vein. He shook the blood at body temperature in a pear-shaped vessel with atmospheres of known composition, by a shaker which instantly threw the blood into foam.

O_2 Tension.	Sat. of Blood with O_2 compared with Sat. on Shaking with Air.
2 per cent. atm.	43·19
3 ,,	55·73
4 ,,	65·75
5 ,,	71·2
6 ,,	75·9
7 ,,	80·73

The Hb is 70 per cent. saturated with O_2 at $\frac{1}{4}$ atm. press., and 50 per cent. saturated at $\frac{1}{5}$ atm. It is owing to this slow increase in dissociation that we are able to endure high altitudes.

Hüfner has given us the formula $x = \frac{100}{1+kp}$ for reckoning the O_2 saturation, where x = the percentage in reduced hæmoglobin, p = the partial pressure of oxygen in mm. Hg, and k is a constant. From their results Loewy and Zuntz reckon the value of k to be 0·04.

Using this formula and constant, they calculate that normal blood

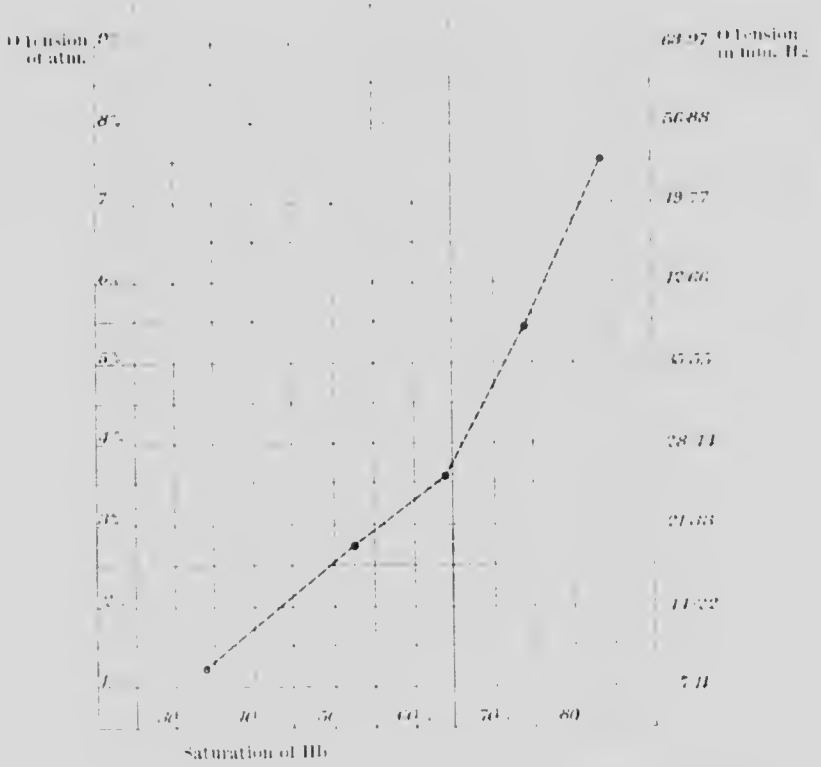


FIG. 9.—Average Dissociation Curve of OHb in Human Blood (*Loewy*).

shaken with ordinary air at body temperature is only about 88 per cent. saturated with oxygen. We must remember that part of the barometric pressure is due to vapour tension, and this lessens the tension of oxygen in the lungs where the air is saturated with moisture. Further, we must remember that the percentage of oxygen in the *alveolar air* is not that of the air but only 13–14 per cent. Taking these two factors into account, it comes out that th

partial pressure of oxygen in the alveolar air is 96·2 mm. Hg if the barometric pressure be taken at 760 and the water vapour tension at 47. Under this partial pressure of oxygen the blood is only [illegible] per cent saturated. *In other words, under ordinary conditions the blood is only ½ saturated with oxygen.* The tension of oxygen in the venous blood = 25 mm. (Strassburg and Wolffberg). The mean driving force therefore for carrying the oxygen through the alveolar wall is $\frac{96 \cdot 25}{2} = 35 \cdot 5$ mm. Zuntz and Durig have measured the rate of diffusion of CO_2, using frog's lungs and distending them with a known volume of this gas. They find that CO_2 diffuses thrice as fast through the lung as through a layer of water of equal thickness, and this is so whether the lung is fresh or killed with alcohol!

Now, the diffusion of a gas through water is directly proportional to the absorption coefficient of that gas in water and to a constant which is approximately inversely proportional to the square root of its specific weight. Thus from the diffusion velocity of CO_2 (found by experiment) that of oxygen can be calculated. Making this calculation, Zuntz and Durig conclude that a difference of oxygen tension of only 11 mm. would drive sufficient oxygen through our lungs to satisfy our needs even in times of most strenuous exertion such as climbing, while 1 mm. is enough during rest. The diffusion path might be many (25) times longer than normal, as in œdema of the lung, and yet enough O_2 pass through.

At an altitude of 4560 m. the alveolar oxygen tension of Durig fell on one occasion as low as 48·3 mm. Hg, and this makes the saturation of his blood 12 or 13 per cent. less than on the plains. The difference between the oxygen tension in the alveolar air and the venous blood was under these conditions still amply sufficient to maintain an adequate diffusion, and not this but the absorptive power of the blood fell short of the needs of Durig, who suffered from migraine and palpitation.

The alveolar O_2 tension can sink from 113 to 30 mm. without causing an absolute insufficiency, but the brain suffers before the other organs and at a higher tension of O_2.

Haldane and [illegible]rrington Smith conclude from the study of the [illegible]tive affinity of CO and O_2 of shed blood and blood in *corpore*

vivo that the pulmonary epithelium has the power of forcing O_2 into the blood at a higher tension than pertains in the alveolar air. This power, they say, continues to act when the barometric pressure is lowered, and strives to maintain tension of oxygen at the high level requisite for active life. It is weakened by fall of body temperature, acute infections, pneumonia, &c. They say that animals—whose blood is $\frac{2}{3}$ sat. with CO—suffer at the same barometric pressure as normal animals. Some of these results appear contrary to the deductions of Zuntz, Loewy, &c., which are given in the text. It remains to be seen whether their conclusions will hold.

The Effect of Increased Pulmonary Ventilation

The alveolar air is made up of the residual and reserve air, which together equal about $2\frac{1}{2}$ l.; the tidal air is 300–600 c.c. Thus, about one-eighth of the alveolar air is changed at each respiration. The mean percentage of oxygen in the expired air is 16·5. Men vary in regard to frequency of respiration from 5–10 to 18–25 per min.; in volume of tidal air from 300–900 c.c.; in volume of air breathed per min. from 3·5–8 l.

The inspired air, which occupies the larger air-tubes, is expelled by the next expiration unchanged in composition. This "dead space" equals about 140 c.c. In superficial breathers, this may equal one-half the expired air; the deeper the breathing the more closely does the alveolar air approximate to the composition of the expired air.

The composition of the alveolar air can be reckoned thus:—

In a given case the tidal air was 250 c.c. and the frequency 20. The expired air contained 3·5 per cent. CO_2 and 16 per cent. O_2. Of this, the 140 c.c. "dead space" air contained practically no CO_2.

Thus the 110 c.c. alveolar air contained 8·75 c.c. CO_2 (3·5 per cent. $CO_2 \times 250$ c.c.), that is, **7·9** per cent. CO_2.

The 140 c.c. "dead space" air contained 21 per cent. O_2, that is, 29·4 c.c. O_2 (21 per cent. $\times$ 140 c.c.). The 250 c.c. expired air contained 16 per cent. O_2 = 40 c.c. O_2 (16 per cent. $\times$ 250). Thus the 110 c.c. alveolar air contained 10·6 c.c. O_2 (40 − 29·4), that is, **9.6** per cent. On making the frequency 10 and the tidal air 500 c.c. the CO_2 tension in the alveolar became 4·9 per cent.

in place of 7·9, and the oxygen tension 14·0 per cent. in place of 9·6.

Loewy found on lowering the O_2 tension in the respired air from 12·1 per cent. to 7·81 per cent. the alveolar O_2 tension remained the same, as the breathing volume per min. increased from 5·5 l. to 11·4 l. These considerations show how a man by

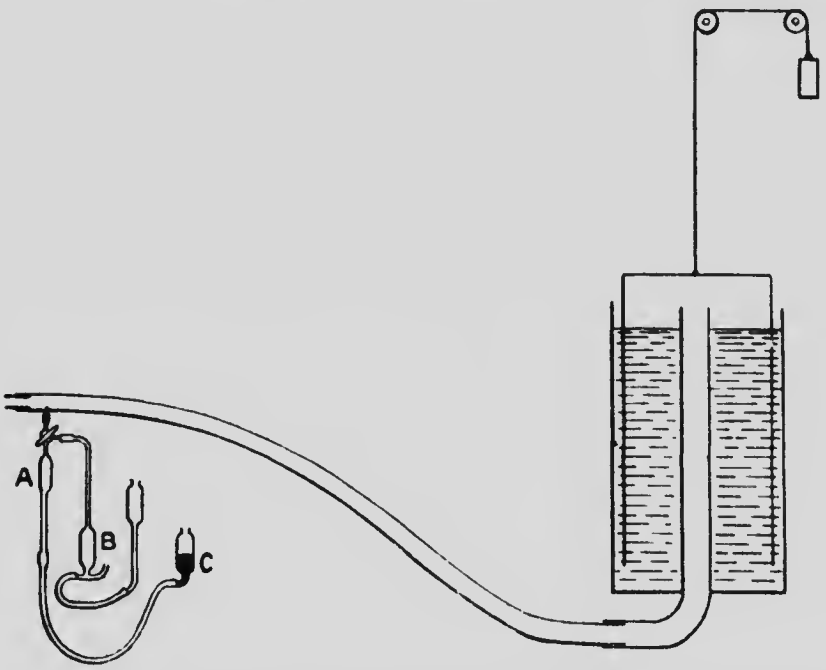


FIG. 10.—Haldane's method of estimating the Alveolar Tension of O_2 and CO_2. The subject breathes into the spirometer, and at the end of expiration closes the mouthpiece with his tongue. A sample of the air in the tube is drawn into A, the measuring tube of Haldane's air analysis apparatus. This sample is alveolar air, and its analysis yields the tension of O_2 and CO_2.

deep breathing can maintain his alveolar oxygen tension in high altitudes.

Loewy notes that a superficial breather—normally breathing 270 c.c. twenty times a minute—became affected at 500 mm. Hg = 3300 m. Loewy himself breathing 440 c.c. fourteen times a minute became affected at 360 mm. = 6000 m., while Zuntz, breathing 700 c.c. eight times a minute, was not affected at 330 mm. = 6500 m.

The efficiency of breathing depends not only on the vital capacity, but on the power of the respiratory muscles and the nervous power of the respiratory centre to maintain an ampler rate and depth of breathing, and on the power of the heart to circulate the blood rapidly. 50 per cent. of the O_2 in the arterial blood is normally used up by the tissues; in hard work the circulation may be so increased in velocity that the loss of O_2 by the arterial blood is no greater or even less.

Lewinstein and v. Schrötter found that shallow-breathing animals—rabbits, guinea-pigs—cannot live for more than three days at 350 mm. Hg = 6000 m. After death their renal tubules, liver cells, muscle-fibres of diaphragm, heart, and arteries show fatty degeneration.

Living in the Monte Rosa hut (4300 m.) for three weeks, Durig and Zuntz found that their increased breathing almost compensated for the diminished barometric pressure. The compensation was not complete on the first day—thus Zuntz increased his alveolar oxygen tension from 53·5–56 mm. Hg on the first day to 57–59·7 at the end of their visit, and Durig increased his from 48·3–53·5 to 53·6–55·5. At the same time the air-hunger, felt on effort, passed away.

By an O_2 tension of 48·3 mm. Hg the saturation of Durig's blood would have been only 65·9[1]—he suffered at this time from severe migraine, palpitation of the heart, and giddiness. Loewy found that he could endure a greater depression in the pneumatic chamber if he did moderate work, for the exercise increased his depth of breathing and raised the alveolar oxygen tension.

The mountain-climber needs a powerful heart to rapidly circulate blood through his lungs, and must be a deep breather. Differences in these respects explain the differences of altitude at which men are affected. Loewy finds that the dissociation tension of OHb varies in different men. This is another factor of importance. By respiring oxygen the balloonist and mountain climber may withstand the influence of great altitudes. A suitable portable apparatus has been contrived. It consists of a small oxygen cylinder, mask, breathing bag containing soda lime, &c., all supported by straps passing over the shoulders.

[1] Mosso found that the gases of the blood, determined by the Haldane-Barcroft method, are only slightly lessened. Further work is required on this point.

The Metabolism in High Altitudes

The observations of Loewy and Zuntz show that dwelling at lofty altitudes greatly stimulates the metabolism—at any rate of untrained town-dwellers. In Alpine soldiers inured to mountain life U. Mosso failed to find any increased CO_2 output. The increased metabolism is due neither to insolation nor to the cold and light of the snow-fields, for it persists while the subject rests within the Alpine hut.

The following figures give the respiratory exchange of Zuntz at Berlin and on Monte Rosa. His weight was 67·6 kg.:—

	Breathing Volume.	Breathing Volume red. to 0°C. and 760.	Oxygen Use per Min.	CO_2 Output per Min.	R. Q.	Alveolar Tension. O_2.	Alveolar Tension. CO_2.
			c.c.	c.c.			
Berlin, resting and fasting	4·755	4·279	232·8	182·7	0·786	103·7	36·8
Monte Rosa, resting and fasting	7·613	4·290	259·2	192·7	0·738	57·0	21·40
Climbing glacier	46·71	25·12	1329	1017·9	0·767	64	18

The respiratory exchange while resting worked out on the average 15 per cent. more on Monte Rosa than at Berlin. This increase began on the second day[1] and lasted during the three weeks' stay in the hut. In Zuntz's expedition of 1901 the stay was only one week, and the increase then was no less than 35–50 per cent. Not only the resting metabolism, but that on walking was markedly increased. A slow walk on Monte Rosa exceeded the oxygen used in Vienna during a fast walk (under the same conditions of snow, level, &c.) by one-quarter. Part of this increased metabolism must be attributed to the increased internal work of the body, the heart and respiration together striving to maintain an adequate oxygen tension in the blood. The greater rapidity of the circulation may increase the

[1] This probably explains why increased metabolism has not been observed in the pneumatic chamber experiments or balloon expeditions which only last a few hours.

metabolism of the organs. Part is probably due to the nervous exaltation produced by the bracing air, the new conditions of life, &c.[1] Loewy, however, did not find that a sojourn at a bracing seaside place had a similar influence. Some writers have attributed the increase to the electrical potential of mountain peaks, but have adduced no convincing evidence.

By lower oxygen tensions than 40 mm. Hg in the alveolar air, the metabolism of the cells becomes deranged. The respiratory quotient rises, lactic and oxalic acids appear in the urine, and the alkalescence of the blood sinks. The living protoplasm obtains its oxygen from the less organised substances in the body. Such decomposition is signalised also by the fatty degeneration of the tissues and by an increased nitrogen output. The latter rose 75 per cent. in a dog submitted for 7 hours to 230 mm. Hg (Fraenkel and Geppert).

Changes in the Circulatory and Respiratory Mechanisms occurring in High Altitudes

A diminution in the barometric pressure has no mechanical effect on the circulation or respiration. The fluids of the body equally transmit the change of pressure to all parts. The blood pressure has been measured and found to be unchanged at a pressure corresponding to an altitude of 6000–7000 m.

If the pulse is accelerated, the respiration quickened and deepened, these results are due to chemical and not mechanical causes. The pulse frequency is always increased in high altitudes, and especially when work is performed. A soldier in Turin raised 5 kg. dumb-bells at 4″ intervals 121 times, and his pulse increased from 62–68. On Monte Rosa he raised the bells 119 times, and his pulse increased from 94–120.

Cheyne-Stokes respiration and irregular depth of respiration commonly occurred in men sleeping or resting on Monte Rosa, and are probably to be ascribed to the diminished tension of CO_2 in the alveolar air. Haldane has shown that the rhythm of respiration is normally controlled by the CO_2 tension in the alveoli, and that this tension is kept remarkably constant.

[1] Aggazzotti failed to find any increased O_2 use or CO_2 output in guinea-pigs kept at high altitudes.

Pembrey and Allen, moreover, have abolished Cheyne-Stokes respiration by increasing the amount of CO_2 in the air breathed. In high altitudes the CO_2 in the air is less, and this together with the increased pulmonary ventilation produced by oxygen-hunger lowers the alveolar CO_2 tension. The respiratory centre thus lacks its normal excitant. The CO_2 in the venous blood, moreover, under ordinary conditions, raises the oxygen tension by increasing the dissociation of OHb. This enables the tissues to obtain most of the O_2 in the blood in dyspnœic conditions, produced by stenosis of air-passages, &c. (Bohr). Such action of the CO_2 is prevented by the increased ventilation. Mosso has wrongly regarded the lessened CO_2 tension as the primary cause of mountain sickness.

The Blood-Corpuscles in High Altitudes

Bert, having reached the conclusion that anoxyæmia is the cause of mountain sickness, was confirmed in this view by the observation that animals living in Mexico at 3700 m. had double the normal number of red corpuscles. Viault found 6·5 9 million corpuscles per c.mm. in Peruvians dwelling at 4392 m. In the lama he found 16 million. These results have been frequently confirmed and ascribed to many causes—to increased formation, to increased drying and concentration of the blood, to congestion of the blood in the peripheral parts and increased concentration there, and lastly, to changes induced on the blood-counting chamber by the alteration in barometric pressure. This last explanation has been negatived by exact measurements, and there can be no doubt that the change actually occurs. The change may occur in balloon ascents in less than an hour, and is to be ascribed to the increased transudation of lymph out of the peripheral vessels, which leads to a concentration of the blood in these parts. Foa has found 3 million more corpuscles in the blood taken from the vein of the ear than in that from the carotid of rabbits kept on Monte Rosa.

In animals kept at low pressures for ten days or so, there occurs an actual increase in the number of red corpuscles produced by an increase in the hæmotopoietic activity of the bone marrow. The amount of iron in the blood increases, while

in the liver it diminishes. Nucleated red cells may appear in the blood. Müntz kept rabbits on the summit of the Pic du Midi, at 2877 m., for seven years. Others he kept in the plain. On comparing the blood he found :—

	Density.	Solids.	Iron per Cent.
			Mgrms.
Pic du Midi . .	1060·1	21·88	70·2
Plain . . .	1016·2	15·75	40·3

The increase in the amount of hæmoglobin is obviously a reaction on the part of the organism, which strives to compensate for the low O_2 pressure.

It is interesting to note that diving birds which stay a minute or two under water have double as much blood as ordinary birds (Bohr).

Susceptibility to Mountain Sickness

From the previous pages it should have become evident that the susceptibility of certain individuals to mountain sickness, and the immunity of others, depends on the depth of the pulmonary ventilation and vigour of the circulation, and on the absorptive power of the blood, which may vary with both the quality and quantity of the hæmoglobin. All these factors may be modified by training. To climb to great altitudes in an untrained condition is to court disaster.

The Therapeutic Value of Mountain Air

The beneficial action of mountain climate lies in its purity from dust and bacteria, in the bracing cold and intense insolation, in the strengthening of heart and respiratory mechanism, in the increased respiratory metabolism, and increased formation of hæmoglobin. It is not by any means proved that the two last factors are due to high altitudes. Probably sea or country air with exhilarating surroundings will induce the same changes in town-dwellers.

CHAPTER IX

THE INFLUENCE OF INCREASED ATMOSPHERIC PRESSURE

NOWHERE on the surface of the world do we find aerial animals naturally exposed to increased atmospheric pressure. The deep holes in the earth's crust are filled with water, and in the valley of the Dead Sea—where the climate is so dry that the level of this sea lies below that of the ocean—in this valley the atmospheric pressure does not exceed 825 mm. Hg. Man, in his restless pursuit of a living, has driven mines into the bowels of the earth, and has sought to gather the riches of the sea by the invention of diving-gear, and to build harbours and quays, to tunnel under rivers, and establish the foundations of bridges by the construction of caissons and the employment of compressed air.

In the sixteenth century Sturmius invented the diving-bell. This bell, full of air, was lowered into the water, and at 10 metres the air occupied one-half and the water the other half of the bell, at 20 metres the air one-third, and the water two-thirds, for 10 metres of water is about equal to the pressure of 1 atmosphere. The men naturally worked under very bad conditions, and Halley tried to improve matters by sending down casks of air, which were opened in the bell, while the warm foul air escaped through a valve in the top.

In 1830, Cochrane, the famous admiral, patented a method for using compressed air to keep the water out in tunnelling under rivers. The method was first put into practical use by Triger in 1839, who successfully sunk a caisson through a layer of quicksand at Chalonnes, and thereby reached rich beds of coal. A caisson is a steel cylinder, which is sunk in water, or wet soil, and out of which the water may be kept by means of compressed air. The men in the caisson are thus enabled to work in dryness on the bed of the sea or river. The inlet of the caisson is fitted with a double set of air-tight doors opening towards the caisson and forming an air-lock. The air-lock is provided with suitable

cocks, whereby the men can be subjected to compression and decompression on entering or leaving the caisson.

Ten metres of water correspond to one atmosphere. Thus for every 10 m. or 33 ft. an air pressure of +15 lbs. to the sq. inch or 1 atm. is required to keep out the water. In the case of a diver, the conditions are the same: compressed air is delivered through a valved inlet tube to his helmet; the air escapes through a valved outlet into the water; the helmet is joined to the dress in air-tight fashion, and the water is kept out of the dress and helmet by the compressed air. The pressure of the air must always be just in excess of that of the water. At 100 ft. a diver is exposed to 4 atm. and at 200 ft. to 7 atm. pressure. It was soon found that deep divers and men working under high pressure in caissons are subject to various symptoms which occurred, not during compression, but after decompression. The minor symptoms are severe pains in the joints and muscles, popularly called "bends" or "the pressure." The severe symptoms are protean, and include epigastric pain and vomiting, respiratory embarrassment, paraplegia, auditory vertigo, sudden loss of consciousness, and death. In the caissons used to sink the piles of the St. Louis bridge the pressure equalled $4\frac{1}{4}$ atm. The number of workers was 600, of whom 14 died and 119 were more or less affected. There were 53 cases of paralysis of the lower limbs. It is recorded that the controllers of the air-lock, who were subjected to compression and decompression every few minutes, and visitors who stayed but a few minutes in the St. Louis caisson, never suffered. Among divers, paraplegia is so common a symptom that it is known as "divers' palsy." The Greek sponge and coral divers have lost as many as a score of men in a year. The men are stricken shortly after returning to the boats.

Many medical writers, knowing the effects of compression and of cupping locally applied, have ascribed the causation of compressed-air illness to the mechanical effects of the pressure. They have supposed that the blood is expressed from the outer part of the body and congested within, and that the illness arises from this congestion. They have, in particular, supposed that the central nervous system, enclosed in a bony case as it is, is protected from the pressure, and hence is especially liable to congestion. Such suppositions neglect the fact that the compressed air is

equally applied to every part of the outer surface of the body and in the lungs, and that the fluids of the body transmit the pressure equally and instantly to all parts, so that the mechanical effect is nil. How little protoplasm is affected by fluid pressure is shown by the existence of abundant life in the abysm of the ocean, where at a depth of 2000 metres the pressure of the water equals 200 atmospheres.

The neglect of a simple physical law is the less excusable seeing that Poisseuille in 1835 observed the capillary circulation

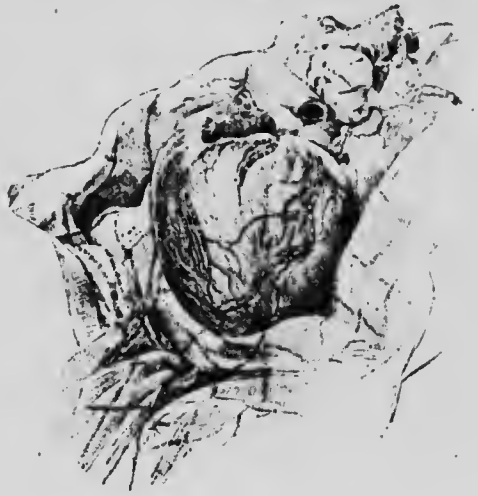


FIG. 11.—Air Bubbles set free in Vessels of Heart after rapid Decompression (*v. Schrötter*).

in frogs enclosed in a strong glass chamber and submitted to 3–9 atm. pressure. The compression had no influence on the circulation. The body of a workman exposed to compressed air supports at +1 atm. an additional 15,000 to 20,000 kilograms. If it were not for the incompressibility of fluids, and the equal and instant distribution of the pressure to all parts of the body, life would be impossible under *any* variations of atmospheric pressure.

The only mechanical compressions which can occur on exposure to compressed air are the compression of the air in the middle ear, producing tension of the membrana tympani, noises in the ear and pain, if the Eustachian tube is blocked up by a cold,

and compression of the gas in the alimentary canal, which leads workmen to tighten their belts, and allows an ampler descent of the diaphragm in inspiration.

Paul Bert, by a remarkable series of experiments (*La Pression Barométrique*, 1878), first proved that the true cause of compressed-air illness is the effervescence of gas in the body fluids, an effervescence which takes place when a man is rapidly returned to the normal atmospheric pressure. The gas that is set free on rapid decompression may obstruct the circulation in various parts and produce one or other of the protean symptoms which caisson workers and divers suffer from. Gas frothing in the heart may instantly kill one man; bubbles in the heart or vessels of the lungs, or in the respiratory centre, produce embarrassment of breathing in another; air embolism of the cerebral vessels may cause aphasia, mono- or hemi-plegia, of the spinal vessels paraplegia; bubbles in the joints and aponeureses, or possibly in the posterior nerve-roots and spinal cord, may cause the severe pains or bends from which caisson-workers so frequently suffer, and a bubble set free in the labyrinth of the ear explains the cases of auditory vertigo. Lastly, bubbles may frequently form in unimportant places, such as the fat and glands, and produce no symptoms.

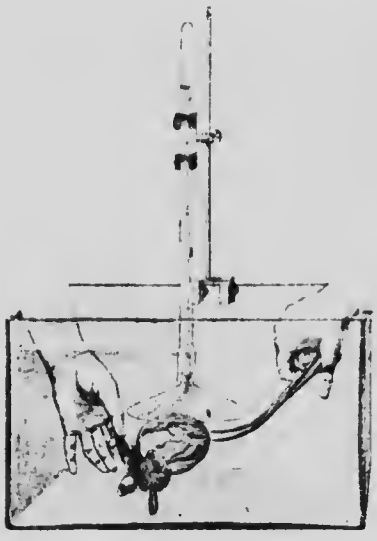


FIG. 12.—Method of obtaining the Gas set free in the Heart on Decompression (*v. Schrotter*).[1]

Bert also found that a high partial pressure of oxygen acts as a general protoplasmic poison. It lessens the respiratory

[1] This method was employed by C. Ham and the writer, and has shown us that the bodies of rats, decompressed after exposure to 10 atm. of air, yield, when cut up, about the theoretical amount of nitrogen gas—*i.e.* the amount calculated as dissolved, supposing 67 per cent. of their weight is water. Most of the gas is free in the abdominal cavity and bowels.

exchange, depresses the body temperature, and provokes convulsions. These effects we will now consider in further detail.

The Metabolism in Compressed Air

The rate at which fuel burns is increased by a greater supply of oxygen, and it has commonly been held that the same holds good for protoplasm. This, however, is not the case. It has been proved that oxygen tensions between 11 per cent. and 94 per cent. of an atm. have no influence on the rate of metabolism. Within these limits the cell rules its own rate of metabolism (Pflüger). Artificially increased pulmonary ventilation does not increase; and the withdrawal of even half the blood does not diminish the oxygen use.

Durig has recently put to the most exact test the effect of breathing different percentages of oxygen (11–94 per cent.), and confirmed the conclusion of previous workers that not the slightest effect on metabolism can be detected. **Oxygen inhalation, therefore, cannot be used as a therapeutic agent to increase metabolism.** Its only value is to supply sufficient O_2 when by reason of anæmia, CO poisoning, nitrite poisoning, &c., the tissues are not adequately supplied. Oxygen cannot do much good to cases of obstructed air-way, for CO_2 has to be got out of, as much as oxygen into, the blood. It can do no good if the circulation is too feeble to keep up the normal rate of supply to the tissues; there can be little doubt, then, that most of the oxygen inhalations given to patients are useless. The tissues are unable to combine with and store up more than the normal amount of oxygen. Fallaise's experiments show this to be the case. He found that asphyxia produced by breathing H_2 occurred only 45″ later than usual after 80 per cent. oxygen had been inhaled by the victim, and this delay was abolished by making the animal breathe air for a minute or two after the inhalation of oxygen. Breathing pure O_2 has little effect on the capillary O_2 tension, as is seen by the following considerations. There is about 14 per cent. Hb in the blood. Each grm. combines with 1·34 c.c. O_2 (Hüfner), and $14 \times 1{\cdot}34 = 18{\cdot}76$ per cent. O_2 when fully saturated. At an alveolar O_2 tension of 110 mm. O_2 the Hb is 81·5 per cent. saturated—that is, the blood contains 15·31 per cent. O_2 combined; it also contains 0·3 per cent. simply absorbed, in all 15·61 per cent. On breathing pure O_2

the alveolar oxygen tension works out as 680 mm. after deducting tension of water vapour and CO_2. The Hb is 96·5 per cent. saturated at this tension—that is, the blood contains 18·4 per cent. O_2 combined, it also contains 1·81 per cent. dissolved, in all 19·9 per cent. The difference between the two conditions is 4·6 per cent. O_2. Now in the capillaries 8 per cent. O_2 is used up, so in the first case 7·6 per cent. O_2 remains over in the venous blood and 11·9 per cent. in the second case. In the first case the relative saturation of the venous blood is $\frac{7{\cdot}6}{18{\cdot}76} = 40{\cdot}4$ per cent., corresponding to 47 mm. O_2 tension; in the second case $\frac{1}{18{\cdot}76}$ 63·4 per cent., corresponding to 434 mm. O_2 tension. Thus while the alveolar O_2 tension rose from 440 to 670 mm., the O_2 tension in the capillaries rose only 264 mm. It takes about 3 atm. O_2 to make a relatively high O_2 tension in tissue (Zuntz and Loewy). The continued action of oxygen above 1 per cent. of an atm. acts as a poison, producing inflammation of the lungs and convulsions. Very high pressures kill by asphyxia.

J. J. R. Macleod and the writer have studied the respiratory exchange in mice and rats placed in compressed air or oxygen. The pressure chamber was fitted with thick glass windows and a pressure gauge. The outgoing current of air was led through sulphuric acid and soda lime absorption tubes, and the output of CO_2 and H_2O obtained by weighing these tubes. From the results obtained we concluded that compressed air above 4–5 atm. lessens the CO_2 output and lowers the body temperature of mice and rats. This effect is not due to the partial pressure of oxygen, for 10 atm. of air is less depressing in its effect than 2 atm. of oxygen. There are other factors to consider, such as the cooling effect of compressed air—it is a better conductor of heat and the resistance of highly compressed air may establish a gradient from the alveolar air to the tidal air. The saturation of the air with moisture which occurs in pressure chambers is said to kill such small mammals as mice and rats, but this does not have no effect on men. Turning to the study of the CO_2 output, we found that no noteworthy change took place in dogs exposed for 7 hours to 8 atm. of air.

Effects on the Lungs

While [illegible] ving the oxy[illegible]n [illegible]nsion of the blood by Haldane's method of [illegible]ntrasting the [illegible]sorp[illegible] of CO by shed b[illegible]d and blood in the living animal, [illegible]orra[illegible]S[illegible]n found "that

Fig. 13.—Section of Lung showing Bronchial Tube and Alveoli. Inflammatory Exudation produced by 3 atm. O_2. (*Bullock and Hill.*)

of animals to a tension of 170–180 per cent. atm. O_2 causes in a short time diminution in the power of the lungs to *actively* absorb oxygen, and that with a continuance of this exposure the arterial oxygen falls till it reaches the level for which mere diffusion of oxygen from the alveolar air might account."

This is because a high partial pressure of oxygen exercises a

marked irritant effect on the lungs, producing at first congestion of the alveolar capillaries, and afterwards hæmorrhagic exudation and consolidation. To the naked eye the lungs present in the early stages a suffused redness. Patches of more intense exudation occur in the apices and edges of the lungs. At a later stage

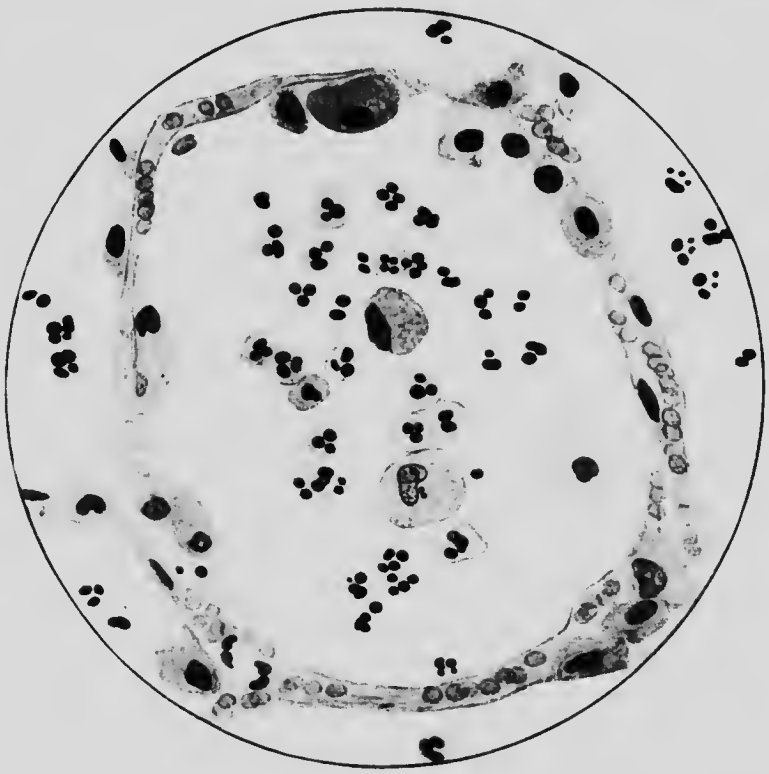


FIG. 14.—Alveolus from the same preparation as Fig. 13 shown under a high power. Note the polymorpho-nuclear leucocytes, detached alveolar cells, and congested capillaries. (*Bullock and Hill.*)

the congestion passes into typical hepatisation, the lungs sink in water and are of a dark purple colour. The pneumonia is patchy if quickly, and universal if slowly developed. Three days after exposure to the oxygen the alveoli and bronchioles show an exudation containing numberless polymorpho-nuclear leucocytes, cocci, and shed epithelial cells.

Lorrain Smith found that 180 per cent. atm. O_2 killed in

about 24 hours, while 300 per cent. O_2 produced inflammation in 5 hours. Our results confirm his.

Lorrain Smith suggests that inflammation of the lungs may be a cause of caisson disease as well as decompression gas embolism. We do not find much in our experiments to confirm this view. The highest pressure hitherto used in caissons is 4·45 atm., and the men never work for shifts longer than a few hours. It seems to require about 24 hours at +7 atm. (= 168 per cent. atm. O_2) to produce marked symptoms of pulmonary congestion. No pneumonia results at this pressure if the partial pressure of oxygen is reduced by the addition of nitrogen.

We observed no sign of lung trouble in a monkey which was exposed on many days to 8 atm. for 4–5 hours at a time.

The Immunity of the Swim-bladder to Oxygen-Poisoning

The epithelium of the swim-bladder of deep-sea fishes, an organ analogous in its development to a lung, seems to be immune to high pressures of oxygen.

The swim-bladder is a long sac usually lying dorsal to the gut, of which it is an outgrowth. It is present in most, but not all fishes. In some cases there is a ductus pneumaticus communicating with the gut. The function of the swim-bladder is to adjust the specific gravity of the body at any depth to that of the water, so that the fish remains suspended without muscular effort. When the fish descends in the water the air in the bladder is compressed, the specific gravity of its body increases and the volume decreases, so that it tends to sink farther; when it rises the converse holds true.

Thus a fish, hooked in deep water and once well started on the upward journey, floats irresistibly to the surface and arrives with a bladder either burst or vastly distended and projecting from its mouth. The fish is apparently made uncomfortable by alterations in the volume of its bladder, and the discomfort causes it to stay at its proper depth. The swim-bladder, placed as it is in the fore part of the body, also helps to preserve the normal position of the body—the head higher than the tail.

The following ingenious experiments demonstrate the function of the swim-bladder. A piece of cork is tied on to the dorsal fin of a gold-fish large enough to float it to the surface of a tall jar of water,

and a piece of lead is tied on to the ventral fin of another just heavy enough to sink it to the bottom. The animals by next day will have adjusted their specific gravity by means of their swim-bladder, and both will be found freely swimming about. The cork and lead are now removed—the fish relieved of the cork irresistibly sinks to the bottom, and the other rises to the surface, and there they stay until a new adjustment of the bladder has been made (Moreau).

Analyses of the gas in the bladder of deep-sea fish show a high percentage of oxygen. In the case of a fish (*Symapho-branchus pinnatus*) caught at a depth of 4500 ft., analysis of the gas yielded 85 per cent. O_2 and 12 per cent. N_2. The pressure at this depth is 150 atm., and thus the tension of the oxygen in the bladder equalled 127 atm., and that of the nitrogen about 18 atm.,[1] while the tension of these gases dissolved in the surrounding sea-water was only $\frac{1}{5}$ atm. and $\frac{4}{5}$ atm. respectively.

Bohr brought up some cod in a bow net from a depth of 45 ft.; the gas in their swim-bladders expanded to such a degree that the fish were forced to swim on their backs. Analysis of the gas showed 52 per cent. O_2. Next morning the fish were again the right way up and of normal size. Analysis gave 10–16 per cent. O_2. From one fish 8 c.c. of gas were drawn off by a trocar, and found to contain 15 per cent. O_2; forty-eight hours later the bladder yielded $7\frac{1}{2}$ c.c. and 79 per cent. O_2; twenty-four hours later 7 c.c. and 84 per cent. O_2. After cutting the intestinal branch of the vagus by making a small opening just behind the gill-slit, Bohr found that the secretion of oxygen ceased. In fish with a closed bladder, without a ductus pneumaticus, there is a vascular area, "the oval," which is permeable to oxygen. The extent of the oval and the dilatation of its blood-vessels are controlled by muscle and the escape of gas from the bag regulated. The epithelium of the bladder is in another place differentiated into a glandular-like structure, "the red body," which has the function of secreting the gas. In the red body are to be seen the débris of red corpuscles. Jaeger suggests that the gland secretes a lysin which, by producing hæmolysis of the corpuscle, sets oxygen free. But Bohr calculates that on this theory the fish would soon have no red corpuscles. It is evident that the epithelium of the swim-bladder is non-susceptible to the poisonous influence of high-tension oxygen, for, while 5 atm. of O_2 rapidly poisons all other forms of protoplasm, we find the *Symapho-branchus pinnatus* with an oxygen tension of 127 atm. in its bladder. Whales, too, must be immune to oxygen-poisoning, if they sound to great depths,

[1] A. Jaeger is of opinion that nitrogen enters the bladder by diffusion, after the fish have been brought to the surface.

for then the air in their lungs must be compressed to many atmospheres. Must they return slowly to the surface to avoid the effervescence of gas in their blood, or do they never seek the depths?

Effect on the Neuro-Muscular System

The writer has exposed nerve-muscle preparations (the frog's gastrocnemius and sartorius) in a small chamber to 50–60 atm. O_2. After one hour the preparations were decompressed and contraction curves recorded, and compared with curves of control preparations. In the case of the gastrocnemius the curves showed remarkably little difference. The muscle was both directly and indirectly excitable; the rate of conduction in the nerve, the latent period and the form and period of the contraction curve, were scarcely altered. The thin sartorius, on the other hand, showed a greatly diminished height of contraction and a prolonged latent period. The frog's heart exposed to the same enormous pressure continued to rhythmically beat for one and even two hours. The size of the contraction only gradually became lessened. After exposure for about an hour and decompression the cardio-inhibitory mechanism was tested. Inhibition by excitation of the sino-auricular junction was readily obtained. Excitation of the vagus, on the contrary, remained without effect. The action of the vagus proved effective before the period of compression. It is probable, then, that cell-stations are paralysed, while nerve, nerve-endings, skeletal, and cardiac muscle are but slowly affected by high-tension oxygen. Paul Bert exposed frogs to 335 per cent. atm. O_2. The animals appeared to be dead in about forty hours. The heart continued to beat and the muscles were perfectly contractile. The central nervous system was alone paralysed and no reflexes could be excited.

Effect on the Central Nervous System

Exposure to high pressures of oxygen produce convulsions (Bert). In 4–5 atm. O_2 the convulsions occur in about 10–20 min.; they resemble in type those produced by strychnine or tetanus. Exposure to higher pressures, 6–20 atm. O_2, produces dyspnœa and coma, and, as a rule, no convulsions occur, while exposure to 50 atm. O_2 instantly throws any animal, vertebrate or inverte-

brate, into convulsions, which resemble those of acute asphyxia. Lorrain Smith observed that a bird could be thrown into convulsions by 3 atm. O_2 after its blood had been 38 per cent. saturated with CO. It is evidently the extra tension and not the quantity of oxygen in the blood which excites. Animals which have been briefly exposed to high oxygen pressures and then rapidly decompressed exhibit reflex hyper-excitability and tetanic convulsions. The spasms may increase in intensity till the whole animal becomes rigidly extended and can be lifted by one leg like a piece of wood. The animals can completely recover from so grave a condition within twenty-four hours, and this is so because the oxygen gas which bubbles off on rapid decompression is rapidly absorbed by the blood and tissues.

The difference is most striking between two rats, one decompressed from air, and the other from oxygen, after five minutes' exposure of each to 20 atm. The rat exposed to air dies after a few convulsive movements, and is swollen out with gas. The heart, the veins, the fat, the liver, &c., are full of gas bubbles (nitrogen). The rat exposed to oxygen, on the other hand, is convulsed, and continues to be convulsed for many minutes if a string is tied round its windpipe, for it lives on the bubbles of oxygen gas set free in its blood. These bubbles are fairly numerous in the veins, but neither block the circulation nor appear evident to the naked eye in the fat and organs. The lungs of the oxygen rat are intensely congested.

Effect on the Blood Gases

The analyses of the arterial blood gases of animals exposed to compressed air show that the amounts of nitrogen simply absorbed increase with the pressure as required by Dalton's law (Bert, Macleod, and Hill). Example:—

Atm.	O_2.	CO_2.	N_2.	N_2 Calculated taking Coefficient of Absorption in Water at 37° as 1·23.
1	18·3	37·1	2·2	1·23
2	19·1	37·7	3	2·46
5	20·6	40·5	6·4	6·15

The nitrogen in blood gas analysis is always slightly too much owing to leakage of air during the manipulations.

The blood can easily be collected if the carotid artery is connected with one of the exit tubes of the pressure chamber, for when the tap on this tube is opened the compressed air forces the blood out of the animal's body.

Effect on the Circulation

This can be studied by placing a recording manometer within the pressure chamber, or by observing the capillary circulation in the web of the frog's foot or bat's wing—the web or wing being spread over the window of the chamber and illuminated with the arc light. Our observations showed us that compressed air has no mechanical effect on the circulation, and negatived all the mechanical congestion theories of caisson-sickness.

The Effects of Decompression

Out of 24 dogs exposed by Bert to 7 9½ atm. and then rapidly decompressed in 1 4 minutes, 21 died from the setting free of gas in the blood and tissues and only 1 escaped without symptoms. The most striking of Bert's results is the following: A dog was put at 9½ atm. The apparatus burst, the dog instantly died. Enormous subcutaneous emphysema was found with gas in stomach, omentum, anterior chamber of eye, spinal cord, cerebro-spinal fluid, &c. The right heart was full of gas, which on analysis yielded 15·2 per cent. CO_2, 82·8 per cent. N_2, and 2·0 per cent. O_2. Similar results have been attained by von Schrötter.

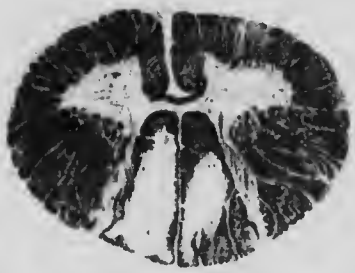


Fig. 15.—Lesion in Spinal Cord of a Diver, produced by Bubbles of Nitrogen, set free after rapid decompression. The man had dived to a depth of about 130 ft. (*T. Schrotter.*)

Having observed the effect of rapid decompression, Bert found that dogs may be safely exposed to +10 atm. if 1 1½ hours be taken for decompression. The animals must not, of course, be

exposed too long, or oxygen-poisoning will result, and the circulatory and respiratory mechanisms will fail to bring about the escape of the dissolved air from the lungs.

J. J. R. Macleod and the writer placed a frog in a small steel chamber which was connected with a cylinder of compressed air and provided with two windows by which an arc light could be passed through the chamber. The web of the frog was stretched on a wire, and fixed so that the small blood-vessels in it could be seen by applying a microscope fitted with an inch objective to the outside of the window. We raised the pressure to 20 atm. and could see no difference in the capillary circulation. After waiting for ten minutes we suddenly decompressed the animal, and then noted first one or two and then a number of air-bubbles scurrying through the capillaries, until finally columns of air filled the vessels and the circulation ceased. On reapplying the pressure the gas-bubbles again went into solution and the blood circulated normally. The dissolved gas in the blood could escape from the body without forming emboli provided we made the decompression gradual. The whole process is exemplified by a bottle of aerated water: if the cork in such a bottle be drawn, the dissolved gas escapes as bubbles of froth; if the cork be again pushed into the bottle, the gas re-enters again into solution and the fluid becomes quiet.

FIG. 16.—Nitrogen Bubbles in Vessels of Spinal Cord of Dog, a Result of rapid Decompression (v. *Schrötter*).

A large pressure chamber provided with observation window, electric light, air-pump, and other facilities provided by Messrs. Siebe & Gorman, the well-known marine engineers, enabled us to thoroughly study the effects of rapid and slow decompression. The chamber was provided with a large tap, by means of which

the pressure could be lowered from 8 atm. to 1 atm. in about 10 to 60 seconds. It was also provided with a pin-point opening through which the period of decompression could be made to occupy one, two, or more hours.

A cat, two rabbits, two rats, and two mice were placed in this chamber and the pressure raised to 8 atm. A ventilation current was maintained. All the animals appeared to be perfectly normal. At the end of an hour rapid decompression was brought about. The chamber filled with mist owing to the cooling of the expanded air. When the mist cleared we saw that the cat and one rabbit were dead, while the other rabbit was in violent tetanic convulsions.

On opening the chamber the rats were found to be dead.

The second rabbit died also and the mice alone survived.

There was emphysema of all the tissues and frothing of the blood in the right heart and lung. In the albino rats we could see extensive retinal hæmorrhages.

A large cat, a rabbit, two white rats and two mice were compressed to 8 atm. in 50 minutes, and kept at this pressure for 1 hour. Decompression occupied 1 hour. None of the animals showed any discomfort.

A Rhesus monkey, a rat, and two mice were compressed to 8 atm. for 4 hours. The animals seemed untroubled by the pressure. Decompression was started at 4.30 P.M. by opening the small tap; the last part of the decompression was hastened, and when at 5.25 the pressure registered 10 lbs. to the sq. inch, the large valve was opened and the pressure quickly brought to zero. On opening the chamber the monkey and the other animals seemed perfectly normal. On removing the monkey from the chamber it struggled to escape, but in the course of a minute or two suddenly became quiet and lay on its side gasping, and with a peculiar cry. It gradually got more and more dyspnoic, and its lips, tongue, and face became markedly cyanotic. Despite energetic artificial respiration it died in about 10 minutes after removal from the chamber.

On opening the right heart a little deep purple frothy blood exuded. Small air columns were to be seen in several of the mesenteric veins.

The other animals in this experiment did not show any decompression symptoms. The cause of the trouble was the acceleration of the last part of the decompression.

The experiment was repeated with another monkey (Rhesus). After being subjected to 8 atm. air for 4 hours 2½ hours were taken to decompress. There was not the slightest sign of decompression symptoms.

This experiment was repeated three or four times a week for a month, the time for decompression being in each case 2 hours.

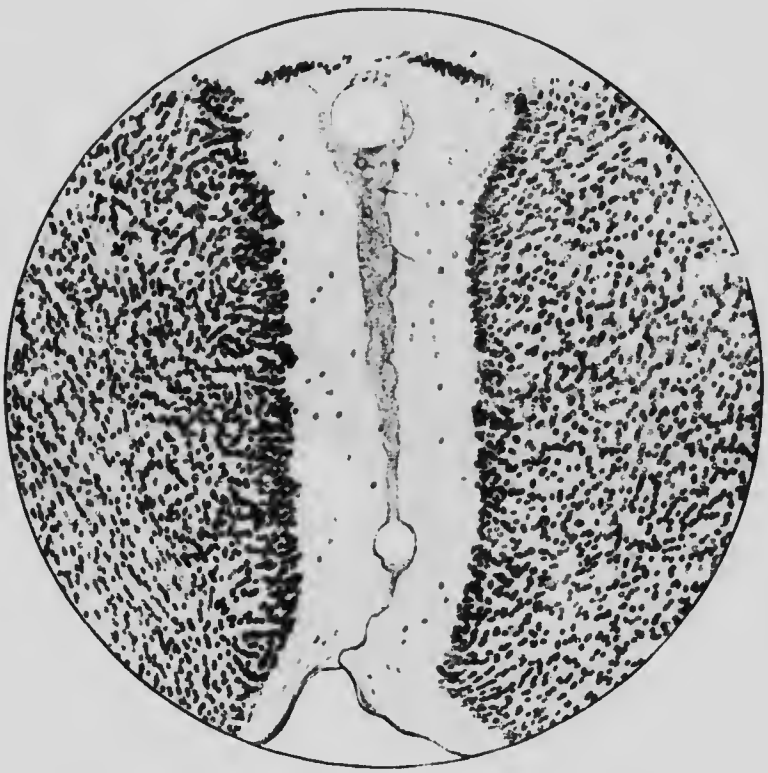


FIG. 17. Nitrogen Bubbles in Vessel of Brain of Mouse, a result of rapid decompression (*Eubanyon, L. Hill, and Macleod*).

There was never the slightest sign of decompression symptoms, and the monkey remained in perfect health and maintained its weight. Towards the end of the period of compression it sometimes seemed to become sleepy. The body temperature remained normal.

The microscopical examination of the organs of animals killed by decompression show that gas-bubbles form small cyst-like

cavities surrounded by compressed and flattened cells. These cavities are especially evident in the liver and central nervous system. The bubbles set free in the blood-vessels run together at less resistant points, and the vessels become occupied here with columns of corpuscles and there with long bubbles of gas. Bubbles are set free in all the connective tissue spaces and especially in adipose tissue. The alimentary canal becomes distended with gas. We have never seen bubbles actually within a muscle, nerve, or other cell; the cells are not torn but compressed by the bubbles. The longer the exposure to compressed air the more completely do the tissue fluids become saturated with dissolved gas.

The Treatment of the Decompression Symptoms

As we have seen in the experiments on the frog and bat, the bubbles of air, which develop in the capillaries, pass back into solution on a rapid reapplication of the pressure.

We have tried this in the case of larger animals.

A rabbit was kept under a pressure of 8 atmospheres of air for hours and was then quickly decompressed. In a minute or so the rabbit developed typical decompression symptoms (*i.e.* fell on side and limbs showed tetanic convulsions). The pressure was now quickly reapplied up to about +5 atm. by emptying a large cylinder of compressed air into the chamber. The symptoms, however, remained unabated and the rabbit soon died. It was evident, therefore, that for the reapplication of pressure to be of any avail, the pressure must be very quickly re-established, and no time be given for the air-bubbles to tear up and damage permanently the nervous tissues, or to produce stasis of the circulation for too long a period.

We therefore repeated the experiment, with the modification that the pressure was more quickly reapplied.

A cat and a rabbit were subjected to an air pressure of 8 atm. for 4 hours. Decompression was effected to zero in about five seconds, and as quickly as the taps could be opened (about five seconds) a large cylinder of compressed air was delivered into the chamber, thus raising the pressure to 75 atm. in about 2 minutes.

In a few seconds the cat became entirely paralysed in the limbs so that it fell helpless on to its side. On recompression, the symptoms gradually disappeared. Some two or three minutes after recompression the cat tried to walk. The pressure was maintained for 15 minutes and then slowly lowered. The cat recovered and on removal seemed *perfectly normal*.

The rabbit was recompressed before it showed any symptoms of decompression, and was quite normal on removal from the chamber.

All our experiments show that for 8 atm. 2 hours is a safe period for decompression. The only case in which it fails is when the animals have developed symptoms of oxygen-poisoning and have become comatose, their body temperature lowered and lungs congested by too long a stay in the compressed air. The circulatory and respiratory organs then fail to rid the body of the gas with which it is saturated.

The *post-mortem* examinations of men stricken with divers' palsy — paraplegia, decubitus, paralysis of sphincters — show extended necrosis in the region of the posterior and lateral columns of the cord, especially in the cervical region. The necrosis is due to ischæmia produced by air embolism. The necrotic tissue consists of the detritus of nervous tissue, and overgrowth of glia tissue. The blood-vessels are distended by the air-bubbles, and the corpuscles driven into clumps which resemble small hæmorrhages (von Schrötter). Recompression has been found to alleviate the minor symptoms, such as "bends" which occur in caisson-workers. Prevention is, however, better than cure, and experimental evidence shows that the grave symptoms can be entirely eliminated by making the period of decompression last one to two hours. The periods of decompression used at present in caisson-works are far too short,[1] and there can be no doubt that the men very frequently must have bubbles of nitrogen set free in their blood. Whether this produces ill effects is largely a matter of chance. Young men with elastic arteries who are deep breathers and of spare habits, are least likely to suffer, for being spare they will dissolve less gas, while their arteries being elastic will be less easily blocked by air-bubbles, and the dissolved air will quickly be expelled by the vigour of their pulmonary ventilation. For deep-sea work the writer has designed a diving-bell into which divers can enter and enclose themselves after completion

[1] Twenty minutes per atm. is a safe rule.

of their work; the bell being then hoisted on deck can be slowly decompressed.

Fatigue brought on by over-work, bad ventilation, alcoholism, oxygen-poisoning from long exposure to high pressure, or any other cause which lowers the vigour of the circulation and respiration, will delay the escape of the dissolved gas from the lungs,

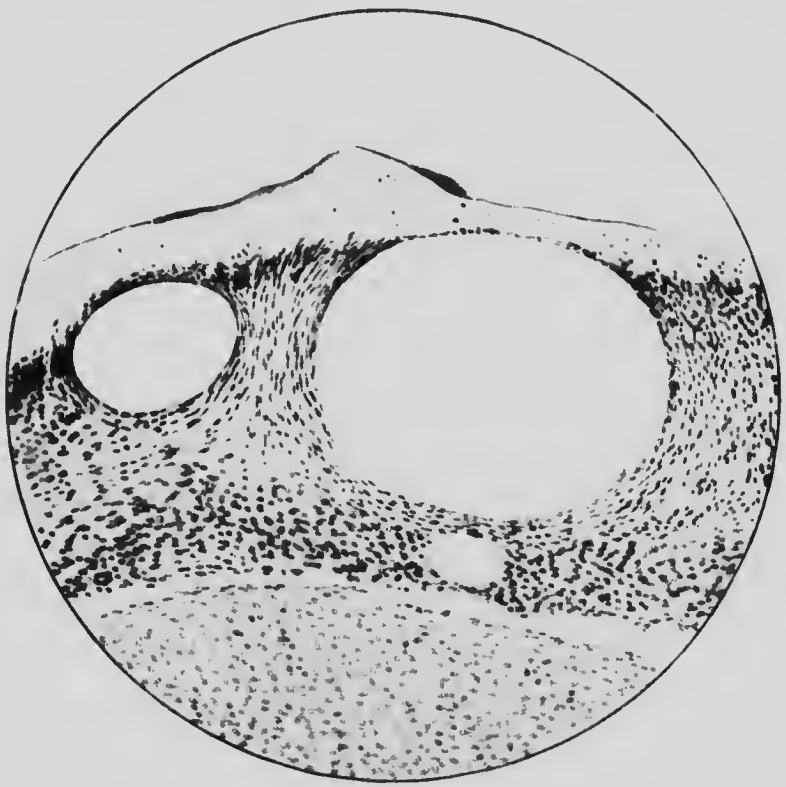


FIG. 18. Nitrogen Bubble in Brain of Mouse, compressing the Cells of the Cortex (*Finlayson, L. Hill, and Macleod*).

and increase the risk of the workers. The replacement of air by oxygen in the air-lock previous to decompression would render the process of decompression safe, and allow its execution in a much shorter time. The O_2 carrying power of the blood renders the escape of much oxygen gas as bubbles impossible. Against the use of this method is the deleterious effects which oxygen produces on the lungs and central nervous system. It would be

unsafe to expose a man for more than a very few minutes to 2 or 3 atm. O_2.

Snell has laid particular stress on the importance of free ventilation in caissons. He says that the sickness increases whenever the CO_2 tension rises to 0·1 per cent. atm. C. Ham and the writer have found that a tension of CO_2 of 3·4 per cent. atm. produces dyspnœa equally when an animal is exposed to compressed air or to ordinary air. Thus 1 per cent. of CO_2 in the air breathed at 4 atm. pressure has the same effect as 4 per cent. at 1 atm. Such high tensions of CO_2 as 1·4 per cent. seemed to have no particular influence on the dangers of decompression. Any agent which produces exhaustion of the work, such as hot moist air, CO arising from flare lights, H_2S emanating from foul soil, will increase the risk of rapid decompression by lowering the vigour of the workmen, and thus the efficiency of the pulmonary ventilation.

The Effect of Water Pressure on Bioplasm

The sea covers 141 million square miles, or three-quarters of the surface of the globe, and everywhere in this vast expanse, from the cold Arctic and hot Red Seas, from the shallows and the depths, the dredge has brought to view evidence of abundant life. Up to 1865, and in spite of the results of soundings by John and James Ross at 1800 m., naturalists considered the abysm of the ocean just as barren of life as the highest mountain peaks. While taking soundings for the Atlantic cable the naturalist Wallich observed star-fish entangled by the sound brought up from a depth of 6500 ft. Upon a piece of the broken Sardinian cable, which was recovered from a depth of 2000 m., Milne Edwards observed Polyps, Pectens, and even an oyster. These animals had been living at a water pressure of 200 atm., and had covered the cable with a crust doubling its thickness.

The Channel, North and Baltic Seas, have a mean depth of about 100 m. The Mediterranean mean, 1300 m.; deepest, 4000 m. The Atlantic mean, 3500 m.; deepest, 5000 m. off Cape Verd. The Pacific mean, 4285 m.; deepest, the fossa of Tusearora off Japan, 8573 m. A column of water 10·33 m. high equals one atmosphere. An animal living in the fossa of Tusearora would therefore be exposed to 857 atm. No direct sunlight pierces to the depths,

and thus a flora does not exist excepting bacteria and fungi, while the fauna lives in darkness, except for a dim phosphorescent glow produced by the radiolaria and other animals which compose it. Water is a bad conductor of heat. So that the temperature in the tropics drops from 80° F. at the surface to 0°–5° F. at a depth of 5000 ft. (Regnard). The deep-sea animals prey upon each other, and upon the débris of dead organisms which falls in a ceaseless shower from the higher strata of the ocean.

At 7000 ft. the dredge of the *Challenger* netted no less than 240 specimens, including 78 species of fish, Tunicates, Crustaceans, Molluscs, Echinoderms, Worms, Coelenterates, and Protozoa. The species were much the same as those in shallow water, but were very much more abundant. The abyss yielded no primeval forms of life, the deep-sea fauna appears no more ancient than any other. The colour of the animals are in many cases startlingly brilliant, and, owing to the darkness of the depths, have not been evolved for the purposes of protection. The eyes are either rudimentary or enormous in size, while the tactile organs are greatly developed in the form of long barbels, fins, or antennæ. The skin, bones, shells are generally soft, and scales absent or little developed. Most of the animals show phosphorescence, and in some —as the deep-sea angler fish—a phosphorescent light is suspended as a bait over the gaping jaws of the fish.

Regnard has studied the effect of enormous pressures of water on all kinds of life. He used a hydraulic pump, and a small steel pressure chamber fitted with thick quartz windows and illuminated with the arc light. He found bacteria and yeasts at 700 atm. produced very little fermentation, so that urine, milk, meat, &c., kept sweet for days. He says that softened but never putrid dead fish have been brought up in the sounds, which are ingeniously contrived to open and shut, and collect samples at the bottom of the sea. Certes has obtained aerobic cultures from all samples of sea-water obtained with due precautions at 500 to 5100 m. The existence of bacteria at any depth is thus assured. Possibly the deep-sea bacteria have become acclimatised to the pressure. Roger found that bacteria—coli and staphylococcus—are not killed by exposure to the enormous pressure of 2903 atm. (3000 kg. to the sq. cm.), while anthrax in its asporogenous but not in its sporogenous form deteriorated in virulence after such exposure.

At 400-600 atm. Regnard found paramecia became swollen and immobile. Their cilia, too, became swollen. After ten minutes' exposure recovery was possible, while death resulted from an hour's exposure. Actinia compressed to 1000 atm. were swollen to double their weight; star-fish, worms, ascidians likewise increased in weight. Crustacea, protected by their carapace, withstood the pressure better, and recovered quickly after five minutes' exposure to 1000 atm. Among vertebrates he experimented on the cyprin after first emptying its swim-bladder. At 200 atm. the cyprin became listless, at 300 atm. it died, and at 400 atm. it was swollen and rigid. The critical depth for this fish was about 300 atm. = 3000 m., and Regnard says surface fish are never hooked below this depth. At about this level the fauna belongs entirely to the abysmal species.

In small transparent eels the heart could be observed beating at a time when the superficial muscles became affected by the imbibition of water. Seeds of cress germinate after exposure to 1000 atm., while the ova of salmon are destroyed by 400 atm.; the chlorophyll bodies of algæ continue to act at 600 atm., as is shown by their oxidising indigo white to blue. These pressures have no action on the unorganised ferments such as pepsin and ptyalin.

The phosphorescence of the insect *Lampyris noctilucus* was inhibited by exposure to 600 atm. and the animal became swollen and rigid. Left for twenty-two days in a bottle with a dead Lampyris, it remained soft and flexible, while the dead Lampyris became dried up. It was finally exposed in a vacuum over calcium chloride. The excess of water was thus evaporated off from its protoplasm and its phosphorescence returned. The muscle fibres of animals exposed to 400 atm. become rigid and increased in weight owing to the imbibition of water. After exposure to 600 atm. the cross striæ became indistinct and the sarcoplasm separated from the sarcolemma by water. The myelin of the nerve-fibres becomes interrupted near the nodes of Ranvier. The blood-corpuscles are destroyed in the superficial vessels, while the protoplasm of mucous and ciliated cells is either thrown into granules or compressed round the nucleus. Pieces of gelatine or agar imbibe water and swell under the high pressure just as the bioplasm. Since the dredge has brought up abundant living forms from all the depths yet explored, it is clear that

the protoplasm of the abysmal species must have become modified so as to stand the enormous water pressure without imbibition of excessive amounts of water. These experiments of Regnard require repeating in the light of recent work on the properties of solutions, and the effect of minute traces of electrolytes on bioplasm.

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CHAPTER X

WATER—ITS RELATION TO METABOLISM AND THE REGULATION OF BODY TEMPERATURE

Water vapour virtually forms one of the components of the atmosphere, and its presence is revealed by condensation as rain or cloud. The quantity may vary from 1 to 32 grm. per c. metre of air. The amount required to saturate the air varies with the temperature. At 32° F. saturated air holds $\frac{1}{160}$th of its weight, at 59° F. $\frac{1}{80}$th, and at 86° F. $\frac{1}{40}$th. For every 27° F. increase in temperature the amount is doubled. The density of vapour is less than air—as 0·623 is to 1·0. Thus a cubic foot of air saturated with water vapour is lighter than a cubic foot of dry air. On this largely depends the motive power of the atmosphere. The water vapour in the atmosphere exerts a most important influence on the heat of the sun, and thus upon bioplasm. According to current hypothesis, the material molecules of hot bodies put into vibration the contiguous ether, and this transmits the vibratory movement as radiant heat. *In vacuo* the propagation of heat by radiation is perfectly analogous to the propagation of light, and the velocity is the same. The quantity of heat received by unit surface in unit time is inversely as the square of the distance of the source of heat. In radiation through matter part of the vibratory movement is transmitted to the material molecules and absorbed. Dry air is almost equally diathermic and transparent for all rays, and absorbs so little that its presence can be neglected for small distances, and the rays received by an object in it can be taken as equal to the inverse square of the distance. Water vapour, like glass, is almost opaque for the least refrangible rays—the infra-red—and transparent for the middle luminous and calorific radiations. Thus on a cloudy day the water vapour both scatters and absorbs the dark heat rays and less heat reaches the earth. On the other hand, clouds after a sunny day prevent the escape of dark heat from the earth and cause a warm night. In a clear night the dark heat radiates into space and the ground cools. The transparency and diathermancy of the air are properties of the greatest importance, since living energy is entirely derived from sunlight and heat. Water vapour and dust serve both to soften

the scorching power of the sun and to prevent the rapid scattering into space of the heat gained by the earth.

Owing to the high specific heat of water, enormous quantities of sun-heat are stored up by the sea, while the temperature of the latter nowhere rises above 85° C. Land, on the other hand, in the tropics may be heated by the sun even up to 140° C.!

The heat contained in any given breadth of sunbeams is most concentrated at the tropics, where at noon the earth's surface lies directly transverse to the path of the sun's rays. Towards the poles and in the winter the sun never rises high in the sky; the rays fall so obliquely that the given breadth of sunbeams is diffused over a much wider area. The air, diathermic to the luminous rays of the sun, and bad conductor of heat as it is, is warmed by contact with the earth or sea, and the heat is conveyed from the lower to the upper strata of the atmosphere by convection currents. The motive power due to convection is enormously increased by evaporation. The air, lightened by heat and the addition of vapour, ascends, and on cooling parts with vapour which condenses as cloud; the condensation sets free the latent heat of vaporisation, and this heat serves to carry the neighbouring air to still loftier altitudes. The process may be repeated over and over again, until finally the cirrus clouds form at a height of five or six miles. The origin of the vast motive power of the winds (the pressure of whirlwinds reaches 100 lbs. to the sq. ft.) is brought home by the consideration that the energy required to raise 1 lb. of water 1° F. is equivalent to the energy of a pound weight falling 783 ft., and that 564 Cal. are set free when 1 grm. of steam at 100° C. condenses to water at 100° C. The water vapour raised in copious abundance over the equatorial and tropical seas is transported to the temperate and colder regions of the earth. The winds arise from the displacement of warm moist air by colder and heavier air, and are modified by the effect of the earth's rotation. The south-west wind comes to us warmed by the sun-heated tracks of the Atlantic, while the north-east sweeps from the ice and snow-bound lands of the high latitudes of Europe. Condensation takes place when warm air becomes cooled to the point of saturation. The cooling is brought about by the mixture of cold and warm currents of air, by warm air blowing upon cold land surfaces, by the sliding of warm masses of air up the slopes of hills, where the air is not only chilled, but expands on reaching higher altitudes regions of lowered barometric pressure and from expansion cools. Such local variations in the earth's structure enormously influence the rainfall.

The violence of tropical rains (the annual rainfall in the Bengal mountains exceeds 650 inches) is explained by the following figures. At 20° F. air takes up 1·3 grains of water per c. ft., at 60° F. 5·77,

at 85° F. 12·78. If air saturated at 85° F. be cooled to 60° F. every c. ft. of air will yield 7 grains of water. Vaporisation is produced by the shooting forth of water molecules which are separated from the free surface by their vibratory action. The rapidity and force of these molecular flights is greater in proportion to the heat of the molecules. The full saturation of a vacuous space is brought about far more quickly than one containing air, for the air particles, by crossing the path of the vapour molecules, retard the penetration of the aqueous molecules among them. When air drifts in conditions of wind it carries the floating vapour with it and accelerates evaporation. The rate of evaporation of the sweat then depends upon the relative humidity of the atmosphere, and upon the temperature, movement, and pressure of the air.

The relative humidity is obtained by dividing the weight of vapour actually existing in the atmosphere by the weight of vapour which would be present if the air were saturated. The dew-point and the actual temperature must first be observed, then the tension of aqueous vapour (a) at the temperature of the dew-point, and (b) at the actual temperature of the atmosphere can be found in tables. Then relative humidity $= \frac{a}{b} \times 100$. The dew-point can be directly found by running iced water through a chamber covered with black glass. The chamber contains a thermometer, and the temperature is read when dew forms on the glass. The atmosphere is, on the average, about 75 per cent. saturated in this country. The relative humidity is greatest near the surface of the earth at night when the temperature approaches the dew-point. It is also great in the morning when the sun has evaporated the dew, and the vapour has not diffused upwards.

In winter the saturation may be 75 per cent. at 2 P.M. and 95 per cent. at midnight, while in summer the saturation at 2 P.M. may be 50 per cent. and at 6 A.M. 75 per cent. In any one day the variations may be much greater. At the seaside the daily variations in saturation are much smaller. Air in forests is 10–20 per cent. moister than air in the open. In California the saturation drops from 100 per cent. at dawn to 22 per cent. at noon. The Föhnwind when it reaches the Riviera lowers the saturation 50 to 60 per cent. in an hour or two. The difference in saturation between out of doors and a stove-heated room in winter may be 50 to 80 per cent.

Water vapour is a far better conductor of heat than dry air, and thus air saturated with vapour at 35° F. is raw and chill, and damp houses and clothing increase the loss of body heat. The beauty of earth and sky, the glories of sunrise and sunset, depend upon the particles of dust and frozen vapour in the atmosphere. The particles

transmit the longer waves of light, and reflect and scatter in all directions the shorter blue and violet ones. The scattered blue rays give the blue colour to the heavens. At high altitudes the blue is found to change towards black, and outside the atmosphere the sun would appear not yellow but blue. The colourlessness of the landscape in intense sunlight as seen by direct vision is very noticeable in contrast to the depth of colour seen in a reflection of the same in water or in a mirror. This is due partly to the white light reflected from all surfaces without penetration—colour being due to reflection after penetration and absorption of certain rays—partly to irradiation. Any bright spot appears larger than it really is, owing to the stimulus overflowing into the region of the retina which borders that directly stimulated. The colours of sunrise and sunset are due to the obliquity of the sun's rays, and their passage through a far greater depth of atmosphere, whereby the more refrangible rays are scattered by dust and vapour particles.

Protoplasm contains almost 80 per cent. of water, and its vital activity is inseparably bound up with this high percentage. The water is in a state of chemical combination so that it can only be separated by very high pressure or by processes of chemical disruption, which, as a rule, are associated with coagulation and death. Nevertheless the spores of bacteria, seeds, rotifers, &c., can be dried, and after being kept inert for years, can be restored to activity by the addition of water. It is a question whether life continues to persist in some particle of moist protoplasm enclosed by an impermeable dry envelope—a cuticle formed by the drying process—or whether water is entirely removed from the chemical combination without destruction of the protoplasmic molecule.

The organs of different animals, taken free from fat, yield an almost constant percentage of water. Thus, in the muscles of the sheep, ox, swine, the percentage is 78 79, in those of the lobster 79, of the snail 78 79. There is also no great difference between the various organs; *e.g.* the blood contains 80 per cent., the liver 77 78 per cent., the spleen 78 79 per cent., the heart 78 79 per cent. Exceptions to this percentage are found only in tissues modified by the storage of food material or for skeletal purposes tissues, in other words, which have no direct share in the activities of life. Thus the umbrella of the jelly-fish is so lightened by a content of 95 per cent. water that it almost floats without muscular effort. The percentage of water in the body of man is about 63.

The water lost per diem under the usual atmospheric conditions of temperature and humidity is :

Under conditions of rest and hunger	1·25	per cent.	of body	weight.
Under conditions of rest and average diet	1·32	,,	,,	,,
Under conditions of rest and rich diet	1·72	,,	,,	,,
Hard work and average diet	2·91	,,	,,	,,

In the height of a hot summer day man may lose water at the rate of 1·87 per cent. of the body weight (calculated for twenty-four hours), and if he works hard, at the rate of 7·71 per cent. (Rubner). A hot day and hard work may increase the rate of water output no less than *six* times.

The total water in a man's body is equivalent to the amount normally excreted in fifty to sixty days.

While frogs can bear drying at a *low* temperature—where their metabolism is at a minimum—until their muscles contain in place of 79 per cent. water only 18–28 per cent., man cannot lose more than about 10 per cent. of his body water and live.

The immediate effect of drying is thirst, a sensation which arises from the lessened percentage of water in the palate and pharynx. The cholera patient thirsts because he loses water from his gut, the diabetic from his kidneys, the labourer from the sweat of his brow, the orator from the local drying of his throat. Thirst results no less from the loss of blood than from the taking of a dose of salts.

Straube found that dogs could be fed on raw meat and live without taking more water than that in the meat. On the other hand, he could not feed a dog on dry meat powder and fat for more than four days. The body of the animal lost 10 per cent. of its water on this diet, the muscles losing most, viz. 20 per cent. There occurred increased destruction of tissue proteid indicated by a rise in the output of N_2 and P_2O_5. The hind limbs of dogs so reduced showed signs of paresis and a tendency to convulsions, which were immediately set aside by the giving of water. The excretion of water from the skin and lungs diminished with the increased dryness of the body.

In the case of a man who reduced his water intake by 27 per cent., the blood temporarily became more concentrated, the solids

of the plasma rising from 8·8 to 11·6 per cent., the red corpuscles from 4·8 5·58 million per c.mm., and the specific gravity of the serum from 1027·4 1033·4. Equilibrium was soon established by the tissue juices passing into the blood, and a repetition of the experiment produced little effect. Attempts have been made to drain the fluid from dropsical tissues into the blood by restricting the water intake, but the results have not been commensurate with the discomfort caused. The problem of dropsy is a complex one, for the permeability of the capillary wall and the osmotic pressure of the tissue cells are altered either by a deficient circulation, bacterial infection, or other abnormal condition of the blood.

In cases of severe and extensive burns and cholera the blood thickens, the urine rises in sp. gr. to 1037 1040, and spasms may occur, possibly from the drying of the neuro-muscular system. The blood in cholera may lose 6·4 per cent. of its water, and if the loss from the tissues were in the same ratio, the total loss from a man weighing 70 kg. would be 1·5 kg., or about 10 per cent. of the body water.

To sum up, then—while a fasting mammal can use up almost the whole of the body fat and 50 per cent. of its proteid before dying, a thirsting one becomes moribund when it has lost little more than 10 per cent. of its body water.

Some of the lower animals, such as frogs, can be dried to a far greater extent without loss of life. When kept in a dry glass chamber fasting frogs may lose 25 to 30 per cent. of their weight in 2 3 days, while the same frogs if kept in water only lose $\frac{1}{2}$–$\frac{3}{4}$ per cent. By gradual drying Durig has diminished the weight of a frog by no less than 39 per cent. without killing it. On the other hand, the *rapid* loss of 15 per cent. of its water killed a frog. In the case of a frog, dried 30 per cent., the blood-corpuscle counts increased from 1 to $2\frac{1}{2}$ millions. The brain and heart lost relatively less water than the other organs.

Frogs take up water through their skin: they do not drink. A thirsting frog with gullet tied increases in weight in a moist chamber no less than one with the gullet open. The absorption through the skin can be easily demonstrated by placing the frog in a bath of dilute ferric chloride after injecting a solution of potassium ferro-cyanide into the dorsal lymph sac. A blue band is soon seen on section of the skin. Reid found that fluid is more easily

transferred through the living skin of the frog from without inwards. The converse holds good for dead skin. A small dose of alcohol increases and chloroform depresses the rate of transference. He found that the transference took place when an isotonic solution (·6 per cent. NaCl) was placed on either side of the skin and at the same pressure. In this case neither our conception of the processes of filtration nor of osmosis is adequate to explain the transference.

In its relation to metabolism, water differs from other foodstuffs in that it does not become stored in the body if taken in excessive amount. An enormous consumption of water only lessens for a few hours the osmotic pressure or solid content of the blood, and by the adjustment of excretion both the blood and the tissues steadfastly retain their normal percentage of water. A melancholic took 7 litres of water a day "to purify his blood," and yet the percentage of hæmoglobin in his blood remained unaltered. The energy spent on excreting water must be very little, since neither the CO_2 output of a fasting cat was found increased by giving it much water, nor was the O_2 intake of a child influenced by an attack of diabetes insipidus during which she drank 8 litres of water a day.[1]

The taking of water with food neither slows digestion nor hinders absorption. Water does not appear to be absorbed by the stomach, and escapes into the intestine at the rate of about one pint in three-quarters of an hour. The nature of the diet influences the amount of water taken, for proteid food occasions diuresis. Thus with a diet of 500 grm. meat and 200 grm. starch, 650 grm. of water were taken, while with a diet of 1500 grm. meat, 1238 grm. of water were taken. Peschel, seeking to accustom himself to a very low proteid diet, found he diminished his water intake from 1300 to 620 c.c.

The taking of large amounts of water provokes diuresis and increases the N_2 output. Exact observation conducted for 24 days on man shows that the water only washes out the urea stored in the tissues, and does not provoke increased destruction of tissue proteid. This influence is the greater, the greater the concentra-

[1] There is possibly an error in this observation, for the warming of this amount of water from 12° C. to body temperature must have required 200 Cal., which was 13 per cent. of the total worth in Calories of the diet taken by the child (von Noorden).

tion difference between blood and tissues brought about by the taking of water. Lessening the water intake may lessen the N_2 output by hindering the digestion and absorption of proteid food. On the other hand, an abnormal concentration of the blood and tissues increases the breaking down of tissue proteid and the N_2 output.

Of the total amount of water which the body takes up partly in the form of drink, partly in food, and partly through the oxidation of the hydrogen in the food, only a very small part is removed in the faeces. The amount given out by the kidneys varies with that taken in the food and drink, and according to the amount transpired from the skin and lungs, and so is a very variable quantity. The amount transpired by the skin and lungs is less influenced by the uptake in the food than by muscular activity and the temperature of the air. Atwater finds that in work experiments two to three, or even four times more water is given out than in rest. This more than compensates the increased uptake in food and drink, and thus less water is excreted in the urine. The sweat in hard work may carry away as much as 12 per cent. of the total nitrogen excreted (285 mg. N_2 per litre, Zuntz and Schumberg).

	Uptake for 24 hrs. per Man.			Output for 24 hrs. per Man.			
	In Food.	In Drink.	Total.	Faeces.	Urine.	Respiration and Sweat.	Total.
4 men resting, average of 49 days	1022	1267	2289	58	1660	935	2653
3 men working, average of 66 days	1658	2045	3703	130	1328	2848	4306

The work done in these experiments was driving a bicycle wheel against friction.

In the rest experiments the amount of water given off by the skin and lungs was approximately the same during the day and night, but was least in the second part of the night when the effect of complete rest was greatest. In the work experiments the output per hour was as follows: —

7 A.M. 1 P.M.	1–7 P.M.	7 P.M. 1 A.M.	1 7 A.M.
183·1 c.c.	188·2 c.c.	57·4 c.c.	44·5 c.c.

Thus the increased output takes place *during* the working period.

Atwater calculates that if the whole of the water, derived from the oxidation of hydrogen in the food metabolised, were given out from the lungs and skin it could not equal more than $\frac{1}{3}$ of the water given out during rest, and $\frac{1}{4}$ of that given out during work.

The water given out as sweat can be calculated if the carbon and water loss from the lungs and oxygen uptake is found by analysis, and the loss of body weight determined. The difference between these two losses is the sweat, supposing neither urine nor faeces are passed and no food taken.

Rubner measured the loss of water from the lungs and found it to be 17 grm. per hr. during rest, 28 grm. on reading aloud, and 34 grm. on singing.

The relative humidity and the movement of the atmosphere have a most important relation to the evaporation of the sweat and the regulation of body temperature.

The means of heat regulation depend on the rate of heat production and heat loss. The former is the chemical and the latter the physical method of regulation. The rate of loss depends, firstly, on conduction and convection, and this depends on the relative temperature and conductivity of the surface of the body, and of the substance with which it is in contact, and in the case of air on the rate of its motion, *i.e.* winds, draughts. The rate of heat loss is proportional to the sq. rt. of the velocity of the wind (Schuckmann). The velocity of the wind round houses in towns is rarely more than 10 per cent. of that in the open. The wind, by cooling the skin, increases the rate of metabolism and promotes the growth of subcutaneous fat—the natural garment of the body. Coddling over fires in hot rooms and avoidance of cold and wind has the opposite effect—enfeebles the heat-regulating mechanism, lowers the rate of metabolism, and lessens the power of resisting the invasion of the tubercle bacillus. Secondly, the rate of loss depends on radiation, and this in its turn upon the specific radiating power of the surface of the body, and upon the difference in temperature between the latter and surrounding objects. Thirdly, on evaporation, which depends on the amount of sweat evaporated, and upon the relative humidity of the air and the rate of its movement. The evaporation of 1 grm. H_2O at body temperature

requires about 0·550 Cal. heat. Fourthly, on the heat lost in the excreta, an amount which is insignificant in comparison.

The heat loss, then, depends on two sets of conditions: (1) the external conditions—the temperature, relative dryness, and movement of the air; (2) the internal conditions of the body, controlled by the vaso-motor and sweat nerves.

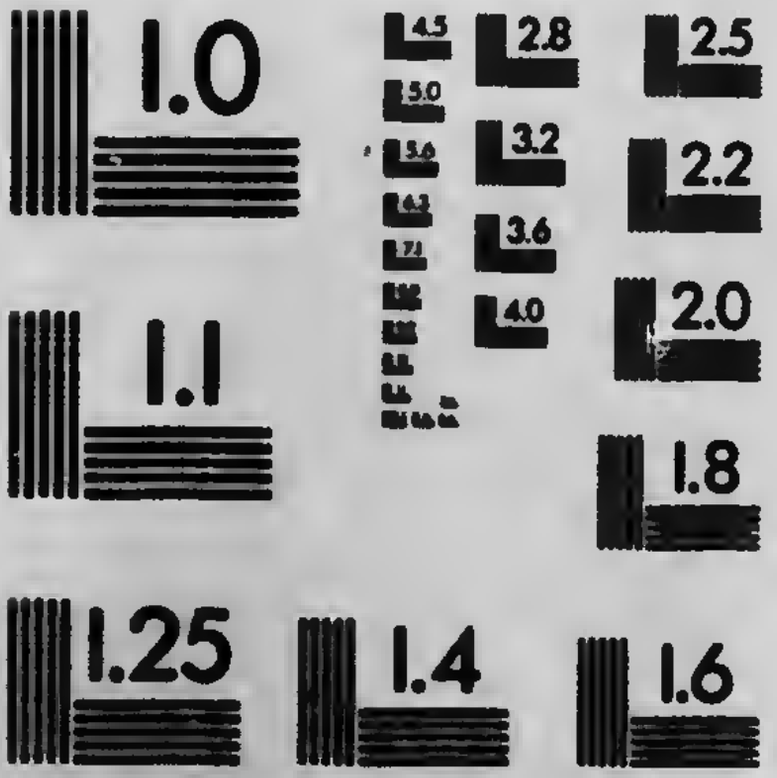
Since all the energy spent in the internal work of the body finally becomes heat, the determination of the total heat produced by the fasting animal, in the state of rest, either directly in a calorimeter or by computation from the amount and kind of matter metabolised, gives us a measure of the total energy of this internal work. It is found to be relatively constant, for the fasting animal adjusts itself to the minimum metabolism necessary for the maintenance of the action of the heart, respiratory muscles, &c., and of the body heat. Thus—

Dog Fasting. (Rubner.)			
Day.	N Excretion.	Fat Metabolism.	CO_2 Output per kg. of Body Weight.
1	4·23	51·74	20·70
2	2·89	45·94	17·83
4	3·65	42·90	17·99
10	2·59	45·55	18·70
11	2·41	41·83	17·86
12	2·53	36·18	16·13

Since the animal is constantly producing heat, it must be able to give off heat at the same average rate, for if not, the heat produced in one day would raise the body to a temperature at which bioplasm is destroyed. Moreover, since the external conditions are subject to frequent and sudden changes, the balance between heat production and heat loss must be capable of prompt adjustment. This adjustment is however, possible only within narrow limits. With the rise of external temperature a point is reached at which the animal is unable to lose heat as fast as it is produced, and with the fall of temperature a point is reached at which the greatest amount of metabolism cannot balance the heat loss. The extent of this thermic range varies for different animals, and in any given animal can alter with changes in its surface, such as in the thickness of its summer and winter coats, in its colour, &c.

MICROCOPY RESOLUTION TEST CHART

(ANSI and ISO TEST CHART No. 2)



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The quantity of heat furnished by an animal in a given time depends on the coefficient of cooling, and this depends not only on the form, but on the surface of the body, as is seen in calorimetric experiments where the body is varnished or covered with oil or glycerine. The varnished surface loses heat so rapidly that the animal dies from cooling. Similarly the relative dryness or moisture of the skin can modify the coefficient of cooling. So, too, the colour, for white rabbits lose only 75 per cent. of the heat lost by black or grey. The loss of heat before and after shaving off the fur of a rabbit is as 100 to 150–160 (Richet, Laulanié).

	CO_2.	H_2O.
Sheep before shearing excreted	719·6 grm.	1939 grm.
,, after ,, ,,	725·1 grm.	434 grm.

Man modifies his heat loss by clothing, shelter, artificial heat, and so extends his adaptation to wider limits of climate.

Clothes lessen the heat loss 47 per cent. (Rubner). D'Arsonval found a 74 kg. man at 18° C.

Lost 79 Cal. per hour clothed.
,, 124 ,, ,, naked.

Clothes are formed of substances of feeble conductivity and entangle air in the mesh. If the conductivity of silver be taken as 493, that of wool or cotton relatively is 0·04, and of air 0·000288. The power of clothes to imbibe sweat modifies their worth, as water is a far better conductor than air. White is a feeble absorbent of radiant heat, and thus is chosen for tropical climates. In the night, on the other hand, white loses less body heat, and is thus warmer than black.

The loss of heat from an animal is not as simple as that from the surface of a metal vessel of water, for the deeper parts of the body where the temperature is constant are separated from the air by layers of fat, skin, fur, or feathers. Thus the peripheral temperature differs notably from that of the central parts. Fat has about half the conductivity of muscle for heat. The diathermancy of white skin is twice that of black skin. Thus his black skin protects the deeper tissues of the negro from the sun's rays (P. Schmidt).

The water excreted per kg. of body weight is about the same in small and large animals. The relative amount of heat lost

by evaporation of water is thus greater in large than in small animals. In the latter the loss by radiation and conduction is relatively greater. Thus loss of heat is estimated to be in

	Kg.	By Radiation and Conduction. Per Cent.	By Water Evaporation. Per Cent.
Man . .	70	77·1	22·9
Dog . .	30	79·5	20·5
Dog . .	4	91·0	9·0
Guinea-pig .	0·55	93·5	6·5

The small animals, owing to their great surface exposure, not only do not need to sweat, but have to live at a fast rate to maintain their body heat. On the other hand, large animals must sweat during exercise to keep the great bulk of their bodies at normal temperature, so lessened in them is the surface exposure in proportion to the body mass.[1]

The rate of metabolism of animals is in proportion to the relative surface exposures of their bodies.

Dogs. (Rubner.)

Body Weight.	Relative Heat Production.	Production per Sq. M. of Surface.
30·66	100	1046
23·71	112	1112
17·70	126	1097
9·51	178	1183
3·10	241	1214

The same proportion has been found to hold good in comparing men with children and dwarfs.

In solids of the same shape the surface is proportional to $\frac{2}{3}$ power of the volume $S = kV^{\frac{2}{3}}$, k being a constant determined for any given form. The weight can be put for volume, and then $S = kW^{\frac{2}{3}}$. For man $k = 12{\cdot}9$, dog $11{\cdot}2$, rabbit $12{\cdot}9$, guinea-pig $8{\cdot}89$, rat $9{\cdot}13$. The formula is only approximately correct, for animals are not of the same geometric figure and vary in specific gravity with the amount of body fat

[1] A dog loses water by opening the mouth and hanging out the tongue.

and contents of the intestines. Whether the relation of heat production to surface holds good in comparing animals of different species is a matter of controversy.

The Effect of Humidity of the Air on Heat Loss

Rubner has studied the effect of altering (1) the humidity of the air with constant temperature; (2) the temperature with constant humidity.

The effect on the dog of altering the relative dryness of the air with constant temperature is seen in the following table.

Temp.	Relative Dryness of Air.	Water Output per Kg. per Diem.
Deg. C.	Per Cent.	
20·2	70·9	16·1
"	28·1	6·2
"	67·2	20·1
"	25	2·5
"	68·4	12·6

The water output thus varies inversely as the humidity of the air,[1] and may be reduced 75 per cent. in wet air without sign of disturbance, so perfectly is the body heat adjusted by other means. The adjustment is brought about by nervous regulation, and its perfection is well illustrated by the following observation. A fasting dog with a daily expenditure of 250 Calories diminished its water output by $53\frac{1}{2}$ grm. when the relative wetness of the air increased 35 per cent. Now the evaporation of this quantity of water requires 32 Calories. Nevertheless as no noteworthy change occurred in heat production—the nitrogen and carbon output remaining the same—it is evident that the heat loss remained undiminished. A compensatory increase occurred in radiation and conduction. Vaso-dilatation took the place of evaporation. The actual difference in heat production which resulted from varying the wetness of the air 35 per cent. was not equal to that produced by altering the

[1] In marching soldiers Zuntz found increased dryness of the air actually lessened the amount of sweat which was produced per 1000 Calories of energy output owing to the more perfect evaporation from the clothes.

external temperature 1 C. The negative results obtained by Rubner do not apply to small animals like mice. J. J. R. Macleod and myself have found that air saturated with moisture at 20 C. increases the CO_2 output of mice. In one case after 18 hours' ventilation with wet air we found the mouse almost dead with a rectal temperature of 22·5° C. It recovered in a current of dry warm air. So long as the temperature of the wet chamber was maintained at 24–25° C. the mouse maintained its temperature by increased combustion. At 20° C. the compensatory mechanism failed, and the increased heat loss over-passed the increased heat production. The weight of the mouse fell in spite of the large amount of food it consumed. Mice are, of course, extremely

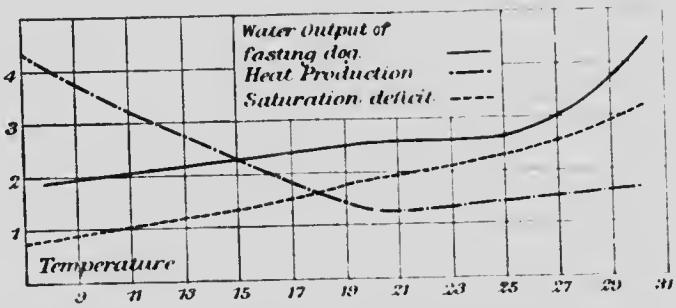

FIG. 19.—The Effect of Raising the External Temperature on the Water Output and Heat Production. Saturation deficit indicates relative saturation of air with moisture. (*Rubner.*)

susceptible to cold, but prolonged exposure to wet cold air must have a similar though far less marked effect on larger animals.

To determine the influence of wet air on radiation and conduction, Rubner constructed an air calorimeter surrounded by a water-jacket which was kept at a constant temperature of about 20° C. The animal was placed in a wire cage isolated from the copper walls of the chamber. The heat given out by the animal expanded the air in the air-jacket, and this affected a recording spirometer. A copper cylinder placed in the water-jacket, and connected with a second spirometer, recorded any change in the temperature of the water, or barometric pressure. The readings of the second spirometer were used to correct those of the first. The temperature of the incoming and outgoing air was also re-

corded. Rubner found that if 100 Calories were lost by radiation and conduction when the air was dry, 107 would be lost in this way at 75 per cent. relative dryness, 116 at 50 per cent., and 125 at 25 per cent. In other words, 1 per cent. increase of humidity raised the loss by radiation and conduction 0·32 per cent. A 25 per cent. increase in humidity equalled in effect a change in external temperature of 2° C. In the case of warmed air (25–30° C.) the evaporation of sweat proved to be a factor of greater moment, a 50 per cent. increase of humidity having the same effect as an increase in temperature of 5° C. A temperature equal or above that of the body cannot be tolerated if the air be saturated with moisture. The favourable conditions of relative dryness of the air for workers are at 18–20° C., 60–40 per cent. relative dryness; at

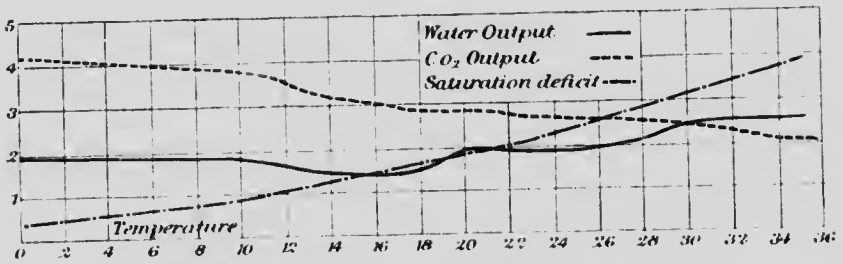

FIG. 20.—Effect of raising External Temperature on Water and CO_2 Output (*Rubner*).

15° C., 30 per cent. relative dryness; and at 25–30° C., as dry as possible; at 25 30° C., 30–40 per cent. relative dryness is distinctly unfavourable (Wolpert).

To study the effect of varying the temperature under conditions of constant humidity, Rubner constructed a chamber which he surrounded with a water-jacket and kept at a constant temperature. The urine and fæces of the animal fell into a vessel of rape-oil, and sinking to the bottom did not influence the cutaneous and respiratory output of water which was measured in the ventilation air. In a small animal such as the guinea-pig the minimum output of water occurred at 15° C. At lower temperature, in spite of the greater relative saturation of the air with moisture, the loss of water increased—a physiological reaction due to the increased respiration and rate of metabolism excited by the cold. In larger animals this reaction is not marked.

The Critical Temperature of an Animal

At a certain temperature, which has been called the "critical temperature," the metabolism of the fasting animal is minimal, for man at 15°, dog 20°, guinea-pig 30–35°. Below this point the production of heat rises or falls with the fall or rise of temperature, and the regulation of the body heat is chiefly brought about by chemical means. Above it the heat production slightly increases, and the regulation depends mostly on physical means. The increased production is to be explained by the greater activity of the vascular, respiratory, and sudorific organs. Above a certain temperature the loss by conduction, convection, and radiation diminish to such an extent that visible sweating appears, and at this stage the heat production becomes further increased. In the fasting dog Rubner found:—

External Temp.	Total Heat Produced.	Loss by Conduction and Radiation.	Loss by Evaporation.
Deg. C.	Cal. per Kg.	Cal.	Cal.
7·6	83·5	71·7	11·8
15·0	63·0	49·0	14·0
20·0	53·5	37·3	16·2
25·0	54·2	37·3	16·9
29·0	56·2	30·0	26·2

The metabolism at the "critical temperature" of the fasting and resting animal is that which is needful for the performance of the various functions of its organs, and the heat production at this temperature indicates the amount of energy which must be consumed in the internal work of the body. The reactions of the body under changes of the temperature of the environment can be compared to that of a cook in regard to her kitchen fire. In spring just enough fuel is used necessary for cooking, in winter more fuel is used, while in summer the windows are opened, and if very hot the walls and floor may be sprayed with water (Armsby). Between 20° and 30° the heat production of the fasting animal remains practically stationary, and the regulation of the body temperature becomes dependent on the increased evaporation of water.

Now the heat production can be greatly and suddenly increased by rich feeding—even to the extent of 44 per cent. (Rubner,

Lanlanié). The food-stuffs vary in this respect, the effectiveness of proteid, carbohydrate, and fat being as 20 : 10 : 7. When the external temperature is high even a moderate increase in diet raises the heat production, and the body temperature must then be regulated by a corresponding increase in the water output.

Thus in guinea-pigs while at 0° C. the consumption of food did not materially affect the total metabolism, at 30° C. it increased it by 39 per cent. At 30° C. the water output of a fasting dog was increased 2 per cent. by feeding 100 grm. meat, 39 per cent. by 200 grm., and 104 per cent. by 302 grm. On the other hand, such a diet had little effect on the dog at 20° or 7° C.

Rubner calculates that the heat lost by evaporation by a moderately stout man on a hot day while out of doors may be at the rate of 3200 Calories per diem. At 37° C. Wolpert observed a loss of 441 grm. of sweat per hr. during rest, and 535 grm. during work, or 3 l. in six hrs.—a great loss for a man with 6–7 l. of blood in his body.

A fat man observed by Wolpert at 37° and 34 per cent. relative dryness of atmosphere could not perform a slight amount of work without rise of body temperature. 30° C. seems to be the limit of heat which fat people can easily endure.

These results show how the danger of heat-stroke is increased by overfeeding in very hot moist days. Under conditions of high temperature and great humidity, the production of heat on the ordinary maintenance ration may be so great as to produce pathological effects, and the consumption of food is then instinctively reduced below the maintenance requirement, with consequent loss of body weight.

A naked man exposed to wind (8 m. per sec.) used 52 grm. O_2 per hr. against 27 grm. when clothed. The effect of the wind was greater than that of a cold bath (Wolpert).

The Effect of Fur and Clothes

A fasting dog after shaving stood 30° C. without any noteworthy increase of water output, for lessened heat production aided by radiation and conduction sufficed. The temperature at which increased sweating came into play was by this means raised 10°. In certain of the workings of Cornish mines the temperature may reach 93° F. owing to the oxidative processes which occur in the soil. The air is also saturated with moisture. The men can work at 80° F., but

treat with caution places where the temperature reaches 85° F. The men work nude, pour with sweat and drink enormously, and work so leisurely that probably an hour or two a day is the real period of active work. A mine inspector who entered such workings in his ordinary flannel clothes quickly reached a body temperature of 103° F. and could hardly walk for breathlessness. The body temperature slowly rises as the regulatory mechanism reaches the end of its tether. This happens at 88° F. in an atmosphere saturated with water vapour (Haldane). Even in air saturated with moisture at 88° F., evaporation of sweat is active owing to the higher temperature of the skin. Thus while at 32° F. 1 c. ft. of air takes up 2·13 grains of water, at 60° F. it takes up 5·77, at 80° F. 10·98, at 90° F. 14·85, and at 99° F. 19·28. The working efficiency of mines [illegible] holes obviously can be enormously increased by the circulation of dry air. By such means at Pendleton Mine work is done at 95½° F. In dry air a man can sit for some minutes while his dinner cooks in an oven beside him, *i.e.* at 100° C.

Zuntz and Schomberg estimate that a resting soldier weighing 70 kg. produces 1·2–1·3 Cal. per min., while marching with a load of 31 kg. he produces 7·73 Cal. per min. The heat thus produced is sufficient to raise the body 1° C. in 8·7 min., and yet the trained soldier, properly clothed and loaded in a suitable manner, shows but a slight rise of temperature.[1] The importance cannot be overrated of supplying light, porous clothing to the soldier, and arranging his load so that neither the respiration is impeded nor the balance of his body disturbed. Hitherto, says Pembrey, he has been "clothed in open defiance of common sense and physiological principles. His tunic generally fits as tightly as possible, is made of thick material and is fastened right up to the neck; his waist is hampered with a tight belt which interferes with abdominal breathing, and other straps supporting valise and haversack still further impede the movements of his limbs and body." His efficiency as a marching mechanism is thus destroyed, his body becomes overheated in hot days and he in danger of sunstroke; his heart and respiration fail to maintain the extra demands put upon them: his muscular movements are not carried out with the maximum economy.

The danger of catching cold from damp clothes or beds is

[1] The normal rectal temperature after exercise on a warm day may be as much as 101° F. The mouth temperature may vary as much as 3°–4° F. from the rectal, owing to cooling of the mouth by neighbouring skin and nose (Pembrey and Haldane).

subject of common insistence. How the damp increases the conductivity is shown by the following observation. An arm clothed in dry flannel and enclosed in a calorimeter lost 4·5 Cal. in an hour; when the flannel was wet the loss rose to 22·7 Cal., an increase of 344 per cent.

The Japanese have especially acquired the liking for very hot baths. But they sit with head and most parts of their limbs out of the water. The writer found a Japanese gentleman stand a bath of 44·5° C. (114° F.) for a few minutes while totally immersed except for his head and neck. His body temperature rose to 39·5° C. (103° F.). A man in a hot bath sweats as much as in hot air, as may be determined by the amount of sodium chloride he adds to the bath (Spitta). Prolonged immersion in a cold bath at 25° C. increases the O_2 use from 21 to 37 grm. per hr. (Zuntz).

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CHAPTER XI

THE METABOLISM OF FAT

In the animal fats are found in all the organs and tissue, and this is so before birth, so fat is a normal tissue component. The percentage amount in each tissue is, however, so variable that there is little profit in giving figures. In the heart freed from connective tissue fat anything up to 16 per cent. of the dry matter may be regarded as normal—1·2–1·3 grm. fat to 1 grm. nitrogen (Rosenfeld); in the muscles up to 4 per cent. There are three chief fat depôts—the intra-muscular, the abdominal, and the subcutaneous connective tissues. The connective tissue cells at the outset have no particular character, but as the globules of fat accumulate and coalesce within them, they enlarge until their protoplasm forms a thin envelope and the nucleus is pushed to one side. In plants fats occur, especially in seeds and fruits; in some cases they are found in the roots. The fat of animals consists of neutral fats with small quantities of fatty acids. The neutral fats are esters of the trihydroxyl alcohol glycerine wit the mono-basic fatty acids. Three hydrogen atoms of the hydroxyl groups of the glycerine are replaced by a radical of fatty acid forming a triglyceride, $C_3H_5O_3R_3$. Animal fat chiefly consists of the esters of stearic, palmitic, and oleic acids. In addition, there are small quantities of esters of some other fatty acids—especially myristic. In milk there also occur glycerides of volatile fatty acids—butyric, caproic, caprylic, &c. In plants other fatty acids are sometimes richly present, such as erucic in colza oil, linolic in linseed-oil, &c.; oxy-fatty acids, and waxes or alcohols of high molecular structure, are also found in many plants, and may occur in small amounts in animal fats. The average elementary composition of animal fat is: C, 76·5; H, 12·0; O, 11·5. In different animals, and even in different parts of the body of the same animal, the fat has a different consistence. The more solid fat contains a greater proportion of stearin and palmitin, the more fluid, a greater proportion of olein. The latter kind of fat exists in cold-blooded animals, so that their fat remains soft at ordinary temperatures. Human fat contains 67–80 per cent. olein. Olein is the solvent of the other fats, and the melting-point of a fat depends on the proportionate composition of the mixture. Stearin, $C_3H_5(C_{18}H_{35}O_2)_3$, melts at 55°–71°.

Palmitin, $C_3H_5(C_{16}H_{31}O_2)_3$ melts at 50·5°–66°. Olein, $C_3H_5(C_{18}H_{33}O_2)_3$ melts at −5°. The neutral fats are colourless or yellowish, and in the pure state are without smell or taste. They are lighter than water and insoluble in it. Soluble in boiling alcohol, they separate out on cooling and generally in the crystalline form. They are soluble in ether, benzol, and chloroform; form emulsions with gum or egg white, and very lasting fine emulsions with soap solutions. They are not volatile, boil at about 300° C., undergoing partial decomposition, and burn with a luminous smoky flame. Neutral fats on being strongly heated yield the powerfully smelling vapours of acrolein.

$$C_3H_5(OH)_3 - 2H_2O = C_3H_4O.$$

On hydration by the lipase of the pancreas, or by similar enzymes obtained from other animal organs, or from plants, or by steam, the fats undergo decomposition as represented by the equation $C_3H_5(OR)_3 + 3H_2O = C_3H_5(OH)_3 + 3HOR$. On boiling fat with a not too concentrated solution of potash, or better, an alcoholic solution of potash, it decomposes with the formation of soaps. Fat on becoming rancid decomposes into fatty acid and glycerine, the fatty acids are subsequently oxidised to lower volatile fatty acids with unpleasant odours. Air and light can bring about this change even in the absence of micro-organisms, which are the usual cause. All commercial fats contain fatty acids.

To determine the existence of fat, the tissue in question is extracted with ether, the ether evaporated off, and some of the residue tested for neutral fat by the acrolein test. Another part of the residue is dissolved in a neutral alcohol-ether mixture which has been rendered faintly alkaline and coloured with phenol-phthalein. If the red colour disappears, fatty acid is present. The fatty acid can be saponified by a hot solution of alcoholic potash. After saponification water and ether can be added, and the neutral fat dissolved in the ether, and some of the soap in the water. The soap-water can be decomposed by mineral acid, and then an oily layer of fatty acid will float out.

In the quantitative estimation of fat the tissue must be dried and ground down to the finest degree, and even then ether will not extract the whole of the fat. Pflüger and Dormeyer digest with pepsin and HCl before extraction, while Rosenfeld boils the substance in alcohol for 30′, extracts with chloroform 6 hours, and then repeats both processes. The residues of the alcohol and chloroform extracts are taken up with ether and finally weighed. This method gives 40 per cent. more than the method of Dormeyer. The boiling alcohol, however, renders nitrogenous matter besides the fat of the

tissue soluble in ether, as is shown by the fact that on saponification the extract yields only about 60–70 per cent. of its weight as insoluble fatty acids, while pure animal fat yields 95 per cent. Glikin extracts with petrol-ether for 48 hours, and removes lecithin by acetone, in which it is insoluble. This method extracts very little nitrogenous matter. The following are chemical processes which are of value in investigations into the nature of fats:—

(1) The acid number, which indicates the amount of free fatty acid. The alcohol-ether extract of the fat is titrated with $\frac{N}{10}$ alcoholic potash, phenol-phthalein being used as indicator.

(2) The saponification number, which indicates how many milligrams of KHO become combined with a fatty acid on treating 1 grm. of the fat with $\frac{N}{2}$ alcoholic potash solution.

(3) The Reichert-Meissl number, which gives the amount of volatile fatty acids. 5 grms. of the fat are saponified and then acidified with H_2SO_4 and distilled. The volatile fatty acids are caught in standard alkali.

(4) The iodine number, which gives the amount of iodine which a weighed amount of fat will take up. It mainly indicates the content of fat in unsaturated fatty acid, *i.e.* olein.

The Digestion of Fat

Fat undergoes no change in the mouth. In the stomach the taking of fat tends to inhibit the secretion of gastric juice (Pawlow). Neutral fats introduced as oil or fat into the stomach of a man scarcely suffer any change, and yield after some hours only 1–2·7 per cent. free fatty acid. On the other hand, finely emulsified fats are split to a very large extent. Thus egg yolk removed from the stomach after 1–4 hrs. was found to be very strongly acid, and split to the extent of 78 per cent. into fatty acid (Volhard). The finer the emulsion the more intimate the mixture and the greater the action of the gastric lipase. In new-born babies this lipase must play an important part, since milk is a fine emulsion of fat, and the pancreatic juice is inactive at this early age. Such assimilation of fat as takes place after the extirpation of the pancreas depends largely on the action of the gastric lipase, and thus the absorption is found to be much greater when milk or finely

emulsified fat is given. Over-acidity of the gastric juice lowers the action of this lipase. The ferment can be extracted from the mucosa by glycerine, and is then very susceptible to the action of alkali. This susceptibility is not observed in the case of gastric juice, so that probably a zymogen exists in one case and the enzyme in the other. As in the case of other ferments, the products of its action are in proportion to the square root of the amount of enzyme present, and of the length of the period of reaction. The bile contains no lipase.

In 1834 Eberle recognised that pancreatic juice had the power of emulsifying fat. Cl. Bernard discovered that it set up an acid reaction in fat, and Berthelot at Bernard's request tested the action of the juice both upon mono-butyrin and on neutral fat. In both cases he obtained fatty acids. Many later workers failed to obtain results owing to the sensitiveness of the ferment, which renders manipulation difficult. It is soluble neither in water nor in glycerine, so either pancreatic juice or minced gland must be used. With either of these it is easy to obtain rapid decomposition of either fluid or solid fats. The ferment works best in weak alkali; it is harmed either by strong alkali or acid. A mixture of bile and pancreatic juice will split 3½ times more fat than the juice alone. Macerated pig's pancreas split no less than 86·4 per cent. of the fat of milk in 24 hours (Fr. Müller). The modern view of fat absorption is that no neutral fat is absorbed, but all split and brought into complete solution. The pancreatic lipase is sufficiently powerful to effect the decomposition.

Pawlow determined that taking of fat excites the secretion of pancreatic juice, and increases the amount of lipase in the juice. Thus the first two hours after the taking of milk a juice relatively rich in lipase is secreted, while if fat-free milk be fed an equal volume of juice is secreted, but the amount of lipase is markedly lessened. No lipase has been obtained either from the mucosa of the intestine or from the chyle. By the action of the gastric and pancreatic lipases the neutral fat is split into glycerine and fatty acids. Some of the latter unite with the alkali of the pancreatic and intestinal juice and form soaps. The fatty acids are entirely insoluble in water, while the sodium soaps are only slightly soluble.

The bile has a dual function as a solvent. Firstly, it

acts as a solvent for lecithin and cholesterin, and so aids in the excretion of these otherwise insoluble bodies. Secondly, it acts as a solvent in the intestine for both free fatty acids and soaps, conferring their entire solubility on the former, and largely increasing the solubility of the latter (Moore). The solvent properties of the bile are chiefly due to the bile salts, but in the case of the fatty acids and soaps the amount dissolved is greatly increased by the simultaneous presence of lecithin. As the supreme function of the bile salts is to render the fatty acids and soaps soluble and fit for absorption, the utility of the reabsorption of these salts—in other words, the circulation of the bile salts—is obvious. Lecithin and cholesterin become precipitated when the bile salts are reabsorbed. Cholesterin possesses a very low solubility even in bile, hence gall-stones are frequently formed of this substance. These important discoveries show why it is the fat-absorbing power of the intestine is most seriously impaired either by absence of the bile or pancreatic juice. In the absence of the juice the unemulsified fats are not broken up, till they reach the action of bacteria in the large intestine, and then it is too late, for the solvent bile salts have been mostly absorbed. On the other hand, in the absence of the bile the fats are split by the juice, but the fatty acids and soaps are not brought into solution.

Absorption

From the intestine at the height of digestion of a fatty meal there are absorbed fatty acids and soaps dissolved in the bile and glycerine. On the other hand, in the thoracic duct 90–95 per cent. of the fat is in the form of neutral fat. Thus somewhere, either in the cells of the intestinal mucosa or in the lymphoid tissue, lymph, the synthesis of neutral fat, must be brought about. Is it effected by an intracellular enzyme, or is it a process which the living cell protoplasm effects, acting as an energy transformer? These are the questions which Moore has recently answered. He collected the chyle by pricking the milk-white lacteals of a dog after a meal rich in fats, taking up the drops in capillary tubes. The tubes were weighed, and then placed in a test-tube of ether. After extraction of the fat

and washing of the tubes with ether, the tubes were dried and reweighed. Thus the weight of the chyle was obtained. The ether was evaporated from the extract, the dry fat dissolved in hot alcohol, and titrated with $\frac{N}{10}$ NaOH, phenol-phthalein being used as an indicator. From this was calculated the amount of fatty acid. The neutral alcoholic solution thus obtained was next evaporated almost to dryness, a measured volume of standard alcoholic potash $\left(\frac{N}{2}\right)$ added, and the mixture boiled for 20–30′ in a flask fitted with a reflux tube. The contents were then neutralised with $\frac{N}{2}$ HCl, and the difference between the amount of acid required to do this, and that necessary to neutralise an equal volume of alcoholic potash, gave the datum for calculating the amount of neutral fat which had been saponified by the alkali. The following is an example of the results obtained:—

Weight of lymph	= 1·8712 grm.
Weight of ether extract	= 0·1450 grm.
Weight of fatty acid	= 0·0042 grm.
Weight of neutral fat	= 0·1326 grm.
Per cent. of neutral fat	= 96·9

These experiments of Moore prove that the synthesis takes place in the intestinal mucosa.

C. A. Ewald submitted soap and glycerine to the action of the fresh minced mucosa, and thought that he obtained evidence of a synthesis of neutral fat. Hamburger confirmed this result, using the mucosa of the large intestine.

Moore has repeated these experiments, and found that the positive results of these authors were due to faulty methods of analysis. After collection of the chyle Moore removed the intestine, cut it open and washed it, and then scraped off the mucosa on a glass plate. He found neither the cell-free extracts nor the detached cells of the mucosa capable of synthesising neutral fats. Weighing a portion of the mucosa, he then extracted it with alcohol (one part) and ether (three parts), and then with ether alone. The solvents were decanted off, mixed, and evaporated to dryness, the dry residue extracted with ether, and the ether extract filtered and evaporated to dryness.

The dry residue was weighed and the fatty acid and fat estimated in it. The results were:—

(1) 84·3 neutral fat 15·7 fatty acid.
(2) 64·6 „ „ 35·4 „ „

They showed that the synthesis is taking place in the mucosa, and that this is at any moment incomplete.

Moore's conclusion is that the synthesis of neutral fat takes place in the living cells of the mucosa, and is not brought about by enzymic action. It is, he says, theoretically highly improbable that the lipase should in the intestine cause almost complete conversion into fatty acid and glycerine, and within the columnar cells cause a complete reversal into neutral fat. Such a reversal is the function not of an enzyme but of the living cell wherein there is a supply of external energy and means for its transformation.

The absorption of fat is most complete when a fat-rich diet is given. The taking of carbohydrate does not interfere with the absorption of fat; but less proteid is absorbed when the other two food-stuffs are given than when proteid is fed alone. Muscular work does not influence the assimilation of fat (Atwater).

No fat-splitting ferment exists in chyle. In the blood, on the other hand, Connstein and Michaelis have discovered that fat is changed into an easily reversible, soluble, filtrable, and dialysable form, by means of which change it is transported into the tissues, for the fat droplets which enter the blood from the thoracic duct cannot wander out of the intact capillaries. Connstein and Michaelis collected the chyle of a fat-fed dog and injected it into the vein of another, thereby raising the fat content of the blood five-fold. They found no trace of fat in the tissue lymph flowing from the thoracic duct of this animal, but the amount of dialysable material in the lymph increased, at the same time the fat introduced into the blood steadily diminished in amount. Thus, at 3.10 P.M. 85 c.c. of chyle were injected containing 6·145 grm. fat.

Blood.		Lymph.	
Normal .	0·171 per cent. fat	Normal . .	0·296 per cent. fat
At 3.12 P.M.	0·732 „ „	At 3.10 3.25 P.M.	0·296 „ „
„ 3.20 „	0·641 „ „	„ 3.25–3.30 „	0·298 „ „
„ 3.40 „	0·600 „ „	„ 3.30–3.35 „	0·294 „ „
„ 4.2 „	0·408 „ „	„ 3.35 3.52 „	0·180 „ „

These authors mixed 157·3 grm. blood containing 0·04 per cent. fat with 31·7 grm. chyle containing 2·607 per cent. fat. The total fat of the mixture was reckoned then at 0·889 grm. Analysis, however, gave 0·225 grm. In other words, 74·2 per cent. of the fat had disappeared.

The essential conditions for this action of the blood are—(1) the presence of the red corpuscles—the serum does not act; (2) the presence of oxygen; (3) a very fine emulsion of the fat (milk is too coarse). The velocity of the reaction at 40° C. is double that at room temperature. Watery extracts of blood which has been dried at 40° C. produce the effect, but not so if from blood dried at 100° C.

This action of the blood on fat is not the same as that of pancreatic lipase, and it cannot correctly be termed a lipolytic action. What the fat changes into is unknown, but the products are insoluble in ether, soluble in water and dialysable. Soaps are probably formed to a certain extent, but not in amount corresponding to that of the fat disappearing.

Hanriot, by studying the action of serum on mono-butyrin, has been led to ascribe a lipolytic action to this fluid. Experimental results obtained with so simple an ester as mono-butyrin cannot be safely applied to the neutral fats. Mono-butyrin is split both by the action of the sodium carbonate, and by that of the proteids of the serum, and there is no evidence that a lipase really exists in the serum.

Since the depôt fat in the tissues can both wander in and wander out, according to the metabolic needs of the body, the tissue cells must possess the power of splitting and dissolving fats as well as the power of synthesis, for there is no reason to think that fat in the droplet form can pass out of the depôts into the blood stream, any more than it can pass in the reverse directions. Extracts of minced liver, intestinal mucosa, muscles, &c., have at any rate the power to split mono-butyrin, and after oil has been injected into the substance of the muscles of an animal, fat drops are found within the sarcolemma, which suggests that the fat has been dissolved, absorbed, and resynthesised (Hester).

To sum up, then—finely emulsified fat is split in the stomach, and other fat in the intestine. The products, soap and fatty acid, dissolved by the bile, are absorbed and built again into neutral fat by the intestinal mucosa. It is probable that

the glycerine of the fat is not used for this synthesis, but for the production of energy, which is required for the synthesis. That the mucosa can supply glycerine is shown by the fact that fatty acids when fed alone can be synthesised into neutral fat. The neutral fat passes by the chyle into the blood, and is there changed into an unknown soluble, filtrable, dialysable modification, and passes in this state through the capillary wall into the tissue. Herein the process is reversed, neutral fat synthesised and laid down as depôt fat.

Many micro-organisms (mostly aerobic) have the power of splitting fat. Bacterium Coli, for example, can split milk fat up to 62·7 per cent. Mucor-like moulds, when growing on cheese or butter, can increase the fatty acid value in the ether extract from 2·7 to 47·7. No glycerine is found, so probably it is used by the moulds as a source of energy. The expressed juice of such moulds has a lipolytic action.

In the seeds of oil fruits the fat acts as a reserve food supply, and on germination is split by a lipase, and rapidly diminishes.

The Metabolism of Fat

The body of an adult man who has lived on an average maintenance diet consists of about 60 per cent. water and 40 per cent. dry matter; about 18 per cent. of the latter is fat. The difference between fat and lean animals is shown by the following:—

	Sheep.		Swine.	
	Lean.	Fat.	Lean.	Fat.
	Per Cent.	Per Cent.	Per Cent.	Per Cent.
Water . . .	61	46·2	58·2	42·9
Dry matter . .	39	53·8	41·8	57·1
	Composition of the dry matter.			
Ash . . .	3·4	2·9	2·8	1·7
Fat	19·9	37·9	24·6	44·0
Proteid and other organic matter	15·7	13·0	14·4	11·4

While the total percentage of water is very markedly lowered in fat animals, the percentage in the actual living tissue, excluding

the fat, is undiminished. The depôt fat is so much dead matter—an oil store—which does not affect the composition of the living bioplasm.

Apart from the ash, the body consists essentially of proteid and fat, for the capacity of the body to store carbohydrate is limited to about 300 grm. of glycogen. The percentage composition of fat—practically the same in man and the domestic animals—is C, 76·5; H, 12; O, 11·5. Thus in metabolism experiments a gain of 100 grm. fat equals about 76·5 grm. C, and a gain of 1 grm. C, as determined by the respiratory exchange, would equal $1 \div 0{\cdot}765 = 1{\cdot}307$ grm. fat, while the respiratory quotient, $\frac{CO_2}{O_2}$, on a diet of pure fat calculated from the above percentage composition, should approximate to ·07.

Adipose tissue itself holds about 30 per cent. H_2O, a fact which must be taken account of in calculating gain in body-weight in metabolism experiments.

In considering the relative fatness of a person 10–15 per cent. under or above the average weights given below must be regarded as normal, 15–30 per cent. above as fat, and 30–50 per cent. as over fat.

The average length and weight of body in the Teutonic race is as follows :—

Year.	Length in Metres.	Weight in Kilograms.
0	0·496	3·2
1	0·696	10·0
5	0·990	16·70
10	1·282	26·12
20	1·711	65·0
25	1·722	68·29
30	1·722	68·90
40	1·713	68·81
60	1·661	65·50

The more slender Latin race at twenty-five years has an average of 1·680 m. and 62·93 kg. The average of woman at the same age is 1·577 m. and 55·08 to 53·28 kg.

The record weights of obese subjects are sufficiently startling; thus a fifteen-month child weighed 26·5 kg., a ten-year-old girl 109·5 kg., a man 304·5 kg., and another 490 kg.

Albert finds that the fat in swine and sheep is subject to very considerable individual variations as to melt-point, refractive index, and iodine number. The difference depends on the food and external temperature. Even in the same individual the fat is not of the same composition in different parts of the body, but varies in the superficial parts exposed to cold.

The following figures show the effect of exposure to cold (Henriquez and Hansen):—

	Iodine Number.	Melt Point.
Pig kept for two months at 30–35° C.	69·4	24·6
Pig kept for two months at 0° C.	72·3	23·3
Pig kept for two months at 0° C., and partly covered with sheep's pelt	67	25·4 (Part under pelt)
	69·1	24·1 (Part exposed)

The panniculus of the new-born infant is a harder fat than that of the adult, owing probably to the sheltering warmth of the uterus.

	Infant.	Adult.
	Per Cent.	Per Cent.
Oleic acid .	65·04	86·21
Palmitic acid .	27·81	7·83
Stearic acid .	3·15	1·93

In the fat of an Esquimaux girl, aged twelve, fed on fish, Rosenfeld found an iodine number of 79, while that of a European of the same age is 61.

The metabolism of fat can be investigated either by the Pettenkofer or Zuntz method. Atwater in America has perfected a most complete calorimeter and respiration chamber, in which a man can live many days working if required on a bicycle which drives a friction wheel, sleeping on a proper bed, and receiving his victuals and handing out his urine and faeces for analysis through a double window. A steady current of air ventilates the chamber, and by analysis of the incoming and outgoing air the respiratory exchange is arrived at. The heat given off is measured by circulating through the double walls of the chamber a constant stream of water which has previously been cooled to a constant

temperature. The temperature of the in and out going water is taken, and the volume of circulating water measured.

By determining the carbon and nitrogen balance between the food fed and the total excreta, any gain or loss of fat and proteid can be calculated. A gain of 66·67 grm. N equals 100 grm. of proteid, and this will contain 52–52·6 grm. of carbon. Any gain of carbon in excess of the amount stored as proteid must be in the form of fat, excepting the very limited amount of glycogen. Every grm. of carbon stored—above that put on as proteid—will represent 1·3 grm. of fat. Similarly if carbon be lost while the nitrogenous balance is preserved, the loss must come from body fat. The experiments require to be conducted for comparatively long periods, because any small gains or losses of carbon are rendered ambiguous owing to the presence of some 300 grm. glycogen in the body.

By the Zuntz method the subject breathes through a meter, and the revolution of the meter causes the continuous collection of a sample of expired air. The total volume breathed is thus obtained, and analysis gives the composition of the expired air, while that of the inspired air can be reckoned if the barometric pressure, temperature, and humidity be known. The O_2 use is taken by Zuntz as the better indicator of metabolism, because of the tendency either for the CO_2 or its precursors to accumulate in the muscles during hard work, the excess being given out during the subsequent period of rest. From the respiratory exchange the respiratory quotient is calculated, and this throws light on the character of the material consumed. When the quotient approaches 1·0, as after a mixed meal, energy is chiefly obtained from carbohydrate, and when the quotient nears 0·7, as before breakfast after a light supper, the body fat is the source of energy. Intermediate values of the quotient give more ambigous results; but if the amount of O_2 consumed and CO_2 produced in the oxidation of any one of the three groups of food-stuffs be known, it is a comparatively simple matter to calculate the proportion in which the other two enter into the reaction. Now the total urinary N gives approximately the measure of the proteid katabolism. Knowing the composition of the nitrogenous urinary products, it is possible to compute approximately the amount of C, H, and O in these, and then to calculate the amount of oxygen required to oxidise the non-nitrogenous residue of the

proteid katabolised. During moderate muscular work the proteid metabolism is scarcely affected, and thus the respiratory quotient will indicate whether the additional matter oxidised is fat, or carbohydrate, or both.

The method of computation is illustrated by the following example :—

In a dog one kg. metre of work increased the O_2 use (per kg. body weight) by 1·6704 c.c.; the CO_2 output by 1·4670 c.c ; the respiratory quotient = 0·878.

Now let x = amount of O_2 consumed in the oxidation of fat, and $1·6704 - x$ the amount consumed in the oxidation of carbohydrate. Since the R. Q. of fat is 0·7069, x c.c. O_2 would yield $0.7069\ x$ c.c. CO_2, and since the R. Q. of carbohydrate is 1·0, the $1·6704 - x$ c.c. of O_2 used to oxidise carbohydrate would yield $1·6704 - x$ c.c. CO_2. Therefore $0·7069\ x + (1·6704 - x) = 1·4670$, and $x = 0·6939$.

Thus the oxygen consumed in the oxidation of fat was 0·6399 c.c., and by carbohydrate $1·6704 - 0·6399 = 0·9765$ c.c., and the CO_2 produced by fat was 0·4905 c.c., and by carbohydrate 0·9765 c.c.

From these data the actual amount of fat and carbohydrate metabolised can be computed, as 1 grm. fat requires 2·8875 grm. or 2·028 litres of oxygen for its oxidation, and produces 1·434 l. CO_2, while 1 grm. carbohydrate requires 1·185 grm. or 0·832 litre O_2 and produces 0·832 l. CO_2.

The Metabolism of Fat during Fasting

As soon as the influence of the food previously taken has passed away, the body settles down to a steady level of fasting metabolism, and the expenditure both of proteid and fat shows only slight variations for many days.

	(Voit). Dog. I.			Dog. II.	
Loss per Kg. of Live Weight.	2nd Day.	5th Day.	8th Day.	6th Day.	10th Day
Proteids .	2·21	1·13	0·96	1·19	1·09
Fats . .	2·62	3·25	3·25	3·43	2·76
Weight, kg. .	32·87	31·67	30·54	31·21	30·05

Both the respiratory exchange and the urinary nitrogen per kg. of live weight remain approximately constant (Lehmann, Zuntz). The proteid metabolism tends to a somewhat uniform percentage of the total, namely, from 10 per cent. to 14 per cent., and only begins to increase in the final stage of starvation when the reserve of depôt fat is almost exhausted, and there is increasing difficulty in transferring an adequate amount of fat from the adipose tissue to the blood. If the body be well nourished and contain much fat this increase in proteid katabolism may be long deferred.

The following example illustrates this:—

Fat Dog.	Proteid per Cent. of Total Metabol.
Day.	
2–4	16·3
10–11	13·1
12	15·5
13	17·1
14	20

In a rabbit, on the other hand, the proteid metabolism was 25 per cent. of the whole by the eighth day, 50 per cent. by the sixteenth day, and 96·4 per cent. by the seventeenth to eighteenth day.

The rise in proteid coincides with the exhaustion of the supply of visible fat.

As the fasting herbivorous animal comes to live on its own fat it is interesting to note how the respiratory quotient changes from a carbohydrate to a fat value.

Guinea-pig (Finkler).

O_2	CO_2	R. Q.	Fasting. Hours.
1202·19	1141·80	0·93	0
1154·53	923·75	0·80	24½
1146·76	811·12	0·71	49

The Metabolism of Fat when Proteids are Fed

When proteids are fed, the proteid metabolism—which equals only 10–14 per cent. of the whole day's energy in the fasting animal—becomes greatly stimulated, and a large proportion of

the proteid food rapidly undergoes cleavage into nitrogenous and non-nitrogenous moieties, and is not used as a tissue-builder. E. Voit reckons the amount of proteid required for nitrogenous equilibrium to be no less than 2½ to 3 times more than that metabolised in the starving animal. Thus if 25·42 per cent. of the total energy expended by the fasting animal be given as proteid food, nitrogenous equilibrium results, and the remaining 58–72 per cent. of the energy is derived from the body fat. Examples: –

Dog Food (Rubner).	N of Food.	N Excreted.	Body Fat Metabolised.	
0	0	4·38	49·33	
115 grm. lean meat	14·11	13·72	25·11	average of several days
0	0	2·80	79·91	
760 „ „	25·16	20·63	30·73	first two days of feeding

These and many similar experiments of Rubner show how material a loss of fat occurs on a proteid ration which is sufficient to prevent any loss of N_2 from the body.

An animal puts itself promptly into equilibrium with its supply of nitrogen, and only a slight and evanescent gain of body proteid can be produced in the adult by the most liberal supply of proteid food. Muscular work plus a sufficient diet of proteid increases the flesh of the body.

The food proteids are cleaved into a nitrogenous and a non-nitrogenous moiety. The nitrogenous moiety is rapidly excreted as urea, while the fate of the non-nitrogenous residue cannot as yet be traced. It may become glycogen or fat. That it is not oxidised simultaneously with the excretion of the nitrogenous moiety is shown by the fact that the heat production of the body does not rise after a meal. Much of the proteid we eat is used wastefully for the energy of the non-nitrogenous moiety. That it is oxidised in due course is shown by the fact that any excess of proteid extra to that required for N equilibrium is substituted for more or less body fat as a source of energy.

The Source of Body Fat—Is it Formed from Proteid?

The physiologists of the earlier part of the nineteenth century held that animal fat was food fat which had escaped oxidation and been deposited in the tissues. Liebig taught the origin of fat from carbohydrate. After much debate Voit introduced the doctrine that proteid is the chief source of fat, and this became generally accepted in 1880 and remained so until Pflüger in 1891, in a trenchant criticism of Voit's experiments, proved that the latter were inadequate. Pettenkofer and Voit's conclusion, said Pflüger, were based upon an erroneous calculation of the elementary composition of the lean meat on which their test dogs were fed. The percentage composition which Voit chose as correct was not based on analysis but on opinion, and is in truth in opposition to the analysis of other workers. Pettenkofer and Voit fed dogs on great quantities of lean meat and found in the excreta all the N but not all the C which they calculated to be in the food. The amount of C retained in the body was, they said, too much to be regarded as stored glycogen.

The proportion of C to N in muscle proteid Voit chose as 1 : 3·4, while Rubner as the result of analyses put it at 1 : 3·28, and even this figure must receive a slight correction for the small per cent. of glycogen present in muscle. The carbon in the urine was estimated by Voit from the amount of N on the assumption of the ratio being 1 : 0·60 while it should be 1 : 0·67. Using these corrections, Pflüger has recalculated Voit's experiment and found no proof of fat formation on a diet of lean meat, thus:—

Meat Fed.	Body Fat.	
	Voit.	Pflüger.
Grm.		
1800	+ 1·4	−35·8
2500	+56·7	+ 3·93
1500	+ 3·4	−29·3
1500	+ 7·3	−23·1
1500	+34·4	+ 3·7
1500	+20·7	−14·1

These were the figures in one typical experiment where the uptake was 2500 grm. of flesh:

		Voit.		Pflüger.	
		1·3682		3·277	
Food		N 85·9 grm. C 313·0 „		N 85 grm. C 278·6 grm.	
		N.	C.	N.	C.
Output	Urine	84·1	50·8	84·1	56·6
	Faeces	1·0	6·7	1·0	6·7
	Respiration	—	213·6		213·6
Total		85·1	271·1	85·1	276·9
Balance			+41·9 C. =56·7 grm. fat.		+3·0 grm. C (an amount within the limits of experimental error).

Since the publication of Pflüger's criticism E. Voit, Cremer, and Gruber have advanced evidence in favour of the proteid origin of fat. Taking the ratio of N to C in the flesh used as 1 : 3·29, E. Voit obtained an output of 30·65 grm. C in three days, which is equivalent to 69 grm. glycogen, *i.e.* much more than the animal could be expected to have stored up in the time. Cats yield 1·5–8·5 grm. glycogen per kg. (Böhm and Hoffmann). The inference drawn by Voit, therefore, was that fat was stored.

Cremer fed a cat for eight days in the respiration chamber, giving it 450 grm. lean meat per diem: the average daily excretion of N was 13 grm. Taking the ratio N to C as 1 : 3·2, this amount of meat gives 41·6 grm. C as the amount taken in the food, while only 34·3 grm. were excreted per diem. Thus 7·3 grm. were retained. In three other experiments 12·6 per cent. to 17·0 per cent. C were retained. Gruber fed two dogs on 1500 grm. lean meat, the N in the faeces and urine were determined and the CO_2 output for five days. The N to C ratio was taken as 1 : 3·28. The total retention of carbon was—

	C.	Calculated as Glycogen (40 grm. C = 90 grm. Glycogen).
Exper. 1	113·9	= 256·3 glycogen.
„ 2	195·9	= 441·0 „

So much glycogen could not have been put on by dogs weighing about 20 kg., and so the carbon was assumed to be put on as fat. There are possible sources of fallacy in these experiments. Firstly,

Pflüger suggests that amido-acids might be retained, such as tyrosin —the degradation products of pancreatic digestion. This might easily result, considering the large amount of proteid fed. Secondly, in the elementary analyses of lean meat by Rubner and Argutinsky, &c., the ether method of extraction was used for determining the amount of fat present. Now, Rosenfeld's method of boiling the tissue with alcohol and then extracting with chloroform may yield 40 per cent. more extract than other methods. On the other hand, this method extracts more than fat. While there is this doubt as to the amount of fat in the meat, the figures quoted above can prove nothing as to the formation of fat from proteid. M. Kumagawa selected two similar dogs of the same litter, and robbed them of their depôt fat to the utmost by twenty-four days' starvation. He then killed and analysed one, while the other he fed with lean horse-flesh to such a degree that it—an 8 kg. dog—consumed 49 kg. in 49 days, a diet equivalent to 133 Cal. per kg. of live weight. The dog increased from 6·08 to 10 kg. In the control dog there was 120 grm. fat, in the other 1087·7 grm. The amount of fat put on then was assumed to be 928 grm. In the meat absorbed there was 1084 grm. fat, and 356 grm. glycogen—in other words, enough to cover the total output of body fat, so there was no evidence of the formation of fat from proteid here.

We must conclude, then, that the metabolic evidence of the formation of fat from proteid is at present lacking. It is impossible to feed an animal on pure proteid alone, such as casein, and it is exceedingly difficult to determine with any degree of accuracy the amount of fat in lean meat. If it were possible to do this, the question as to whether glycogen or fat is stored could be settled by an exact estimation of the oxygen exchange, since the difference in the oxygen content of fat and glycogen is very great. In support of the view that fat can be formed from proteid is the fact that glycogen can be formed from proteid, and that fat can be formed from carbohydrate.

Attempts have been made to prove the origin of fat from proteid in the formation of adipocere, the ripening of cheese, the formation of fat in fly-maggots fed on blood, and the formation of milk, in the fatty degeneration which occurs in phosphorus poisoning, &c.

Adipocere is the fatty substance into which a corpse changes on long immersion in water. It was supposed to result from the actual change of muscle proteid into fat. What happens, in truth, is the putrefactive colliquation of the proteids, and the fat set free from its depôts floats in the water and percolates through the tissues.

Lehmann put in flowing water pieces of muscle, which contained 3·66 grm. per cent. neutral fat, and found after exposure for some weeks 1 grm. per cent. of fat and 6·27 grm. per cent. of free fatty acids. This change was due to the action of bacteria, as shown by Fr. Kraus, who kept pieces of organs obtained bacteria-free in sterilised water at 38·39° C. and found no change in the fat.

Cheese.—Bacteria can undoubtedly break down proteid and set free among other degradation products fatty acid. Thus fresh cheese which yielded 2·16 grm. ether extract, after fourteen days ripening yielded 4·3 grm. Windisch found the ether extract of Camembert cheese increase from 49·78 to 56·75 per cent. in the process of ripening. That fungi have the power of forming fatty acids and fats out of proteid does not prove that the same thing holds good for the cells of the animal body, but it at least renders it more probable.

Fly-maggots.—Hoffmann collected the eggs of blow-flies and analysed a portion for fat. The rest he set upon a weighed quantity of coagulated blood. The fat percentage in the blood he also analysed. He found the developed maggots contained ten times more fat than that in the blood. Pflüger made two objections to this experiment. Firstly, the micro-organisms in the blood decomposed the proteid. Secondly, such small amounts were used in the experiments that errors in analysis (ether extraction of fat) rendered the results quite untrustworthy. Thus while an ethereal extract of blood yielded to one observer 0·18 per cent. fat, a boiling alcohol and chloroform extract yielded in the hands of another no less than 1·4 per cent. O. Frank repeated Hoffmann's experiment, growing the maggots on meat extracted with ether; but the uncertainty of the extraction and the presence of carbohydrate in the meat rendered the positive result he obtained of little value.

The Formation of Milk Fat

The fat appears in the cells of the mammary glands in the form of minute droplets, as may be seen in osmic preparations. These droplets run together and approach the free edge of the cell. Heidenhain supposed that the front edge of the cells broke down and thus set free the fat; this is denied by Benda, who finds no sign of a torn cell wall in his preparations of active gland. The cell actively produces fat—it is not a product of regressive metamorphosis or fatty degeneration (Bezzozero and Benda).

Voit regarded the milk fat as a decomposition product of body and food proteid, Soxhlet and others as a product of food and body fat, while still others ascribed its origin partly to carbohydrate. Voit, feeding a bitch on lean meat, came to the conclusion that it put on more fat and separated more butter in the milk than was in the food.[1] From metabolic researches both on the bitch and cow he concluded that the proteid in the food sufficed to produce all the fat in the milk. He supposed that the mammary cell substance decomposes into milk and is re-formed, that proteid is essential for rebuilding the cell substance, and that carbohydrate and fat act only as proteid sparers. While the earlier investigators found no effect on the milk on feeding fat, but bettered the quality by giving more proteid, the more recent workers have undoubtedly influenced the milk by increasing the fat in the food.

Thus Stohmann observed the favourable effect of feeding oil to a goat, and the lessening of the yield of butter on removing fat from the diet. The melting-point of the butter has been found affected by the feeding of cotton-oil, palm-oil, and cocoa butter. Rosenfeld, by constantly feeding bitches with mutton fat, caused this fat to accumulate in the fat depôts, and the animals produced a butter with the iodine number of mutton fat. Henriquez and Hansen found feeding cows with linseed-oil raised the iodine number in milk fat from 30 up to 59·7 and even to 70·4, showing the increased proportion of olein. Stellwaag found that cows fed on a mash of maize gave a butter too low in melting-point to be marketable. Oil-cake fed to cows produces an oily milk. Baumert and Falke fed sesame and almond oil, and found the milk fat to consist of a mixture of butter fats with these foreign fats. Winter-

[1] The possible errors in Voit's researches have been considered above.

nitz injected iodifin, a stable compound of iodine and fat, and found it secreted in the milk, an observation which has been confirmed on woman.

Caspari likewise fed a bitch on iodised fat and found 23 per cent. of this appeared in the milk. If much rice or sugar were also fed no less than 32 per cent. of the iodised fat passed into the milk. The greater part of the remainder became stored in the fat depôts. He then fed with lard, and found 8 per cent. of the iodised fat stored in the depôts passed into the milk. From this observation he concluded that the milk fat comes from both the food and the depôt fat. It has been objected to this conclusion that when iodised casein is fed, a measurable amount of iodised fat appears in the milk. This does not prove that the casein is converted into fat. Probably the body or food fat robs the casein of its iodine, when the latter undergoes decomposition during or after absorption.

There can be no doubt, then, that milk fat can come from food or body fat. It can also come from carbohydrate food. Jordan and Jenter placed a strong Jersey cow in the height of lactation on hay and seed food, from which the fat had been largely extracted. In ninety-five days they estimated the cow assimilated only 5·7 lbs. of fat, yielded 62·9 lbs. of butter, and increased 47 lbs. in weight. It excreted urea equivalent to the decomposition of 33·3 lbs. of proteid. The non-nitrogenous moiety of this amount of proteid could at most have produced only 17 lbs. fat. Granting the fact, which is unproven, that this amount of fat could have been derived from proteid, it is clear that the rest must have come out of carbohydrate food, seeing that body fat was not lost but gained.

The formation of sebum has been supposed to be an example of fatty decomposition of the cell proteid, but it is now recognised that it is a secretion which stands in close relation to the depôt fat (Plato). By feeding geese with sesame oil this fat was found secreted by the rump gland.

Pathological Evidence as to the Formation of Fat from Proteid

Histological examination has led to the widely accepted theory of fatty degeneration of the cell proteids (Virchow), but chemical analysis of the tissue does not bear this out. The fat previously existent in the cells comes prominently into view in the degenerated

cells owing to the wasting of the proteid. The necrotic changes lead to the fatty contents running together into visible droplets. In degenerated nerves the fat droplets are striking objects, but the nerves are poorer, not richer in fat. A patch of yellow softening of the brain in the region of the cuneus yielded 6·17 per cent. fat, while the normal cuneus gave 8·81 per cent. (Rosenfeld). In old pus cells in which microscopically the fat droplets appeared most numerous analysis showed only 1·94 per cent. fat.

In sections of the normal heart and kidney no histologist sees evidence of fat in the cells, and yet chemical analysis may give 15–23 per cent. in the kidney, and as much as 15–16 per cent. in the heart. This shows that microscopical evidences of fatty degeneration are utterly untrustworthy. The pre-existent fat may not only come more prominently into view in cells suffering from mal-nutrition, but the depôt fat may be attracted and lodged within such cells, owing to the effort of the dying protoplasm to maintain its energy.

Thus phosphorus poisoning has been quoted over and over again as a typical example of fatty degeneration of proteid, but Rosenfeld has collected much evidence to show that the fattiness is brought about by the transference of depôt fat to the poisoned organs. He robbed dogs to the utmost of their depôt fat by fasting, next filled up the depôts with mutton fat by feeding, then freed the liver from fat by a second and shorter period of fasting, and finally gave phosphorus. The liver of the control dogs yielded 7·9 per cent. of the dry substance as fat with the normal iodine number for dog's fat (100·99), while the poisoned dog's liver yielded 41·44 per cent. fat, with the iodine number of mutton fat (57·4). The mutton fat had been transported from the depôts to the liver.

When Rosenfeld had fasted animals to such an extent that the depôt fat had disappeared to the utmost extent, he found phosphorus produced no increase of fat in the liver. Thus—

Phosphorus administered to	Fat in the Liver per Cent. of the Dry Substance.
A dog fasted to the utmost was	6·14
A dog fasted, but containing a little visible fat .	17·1
A dog fasted, but somewhat fatter . . .	36·98

The deposit of fat in the liver cells begins in the peripheral part of the lobules, where the connection with the blood stream is closest. The same results have been obtained on starved fowls, and on rabbits in which the diphtheria toxin was used as the poisoning agent. Kraus and Sommer have determined that the total fat in white mice is not increased by phosphorus poisoning but decreased, and even to the extent of one-half.

In opposition to these results is the fact that phosphorus produces abundant signs of proteid decomposition, viz. the presence of lactic acid, tyrosin, leucin in the urine, &c. These substances all contain less nitrogen than proteid—lactic acid 0, leucin 10·67, tyrosin 7·77; but we have no evidence of the excretion of the whole of the non-nitrogenous residue of the decomposed proteid, and can formulate no reasonable hypothesis of its fate except that of its becoming fat. Thus Bauer executed a metabolism research on a dog which had fasted twelve days, and to which he administered small doses of phosphorus. The N_2 output doubled while the CO_2 output and O_2 intake sank to one-half, which suggests that much of the non-nitrogenous moiety of the decomposed proteid remained in the body. The dried liver substance of this dog yielded 30 per cent. of fat, while the normal is about 10·4 per cent.; the dried muscles 42·4 per cent., while the normal is about 16·7 per cent. This observation and those of Rosenfeld and his school appear at present, therefore, to be in direct antagonism.

Arsenic, antimony, phloridzin, alcohol, and many other poisons, such as CO and bacterial toxins, cause infiltration of the tissues with fat. 4 c.c. per kilo of alcohol fed to fasting dogs produces a fatty li

Rosenfeld obt the same results with phloridzin as with phosphorus.

Mutton-fatted dogs gave mutton fat in their livers on giving 2 grm. of phloridzin per kilo. No fat accumulated in the livers of dogs starved to the utmost. In fasting animals the proteid is decomposed by phloridzin, and the non-nitrogenous moiety excreted as sugar, so that the fat cannot be derived from proteid in this case.

Fatty infiltration of the organs is produced by keeping animals in an over-heated atmosphere—the livers of geese are thus prepared for *paté de foie gras*. The same thing coupled with rickets has been observed in high stud foals whose dams have

been kept in hot stalls, over-richly fed, and given deficient exercise. This condition was at once remedied by giving proper exercise to the mares. In this and every other pathological case Rosenfeld argues that the fat is brought from the depôts, that the tissues undergo fat infiltrati not fatty degeneration. In the conditions of intoxication and mal-nutrition the cells increase their energy at first by oxidation of glycogen—hence the total disappearance of glycogen in phosphorus, chloroform, phloridzin, arsenic poisoning—finally they fall back upon fat and take up increasing quantities of this from the depôts through the blood. The probability of this view is established by the fact that feeding glycogen-producing foods lowers the output of fat in the liver. Thus in phloridzin or alcohol poisoning the fat infiltration can be prevented by feeding glycogen builders, and dogs which on a pure fat diet put on 25 per cent. of dry liver substance as fat have this percentage lowered to half or less by feeding glycogen builders at the same time. The restoration of a fatty liver after poisoning by phosphorus, &c., is signalised by the return of glycogen. In the aseptic autolysis of organs the microscope reveals apparent signs of fatty degeneration of the cells: but here again chemical analysis shows that the percentage of fat is not increased.

In the case of salmon it has been suggested that the fat of the generative organs is formed at the expense of the muscle proteids. The salmon, on entering fresh-water rivers for the purpose of breeding, cease to feed, and may fast from five to fifteen months. They enter the river with maximally developed muscles and minimally developed generative organs. During the ascent of the river the ovaries increase to $\frac{1}{4}$ of the body weight. In the muscles of the back, which atrophy as the organs of generation grow, Miescher found enough proteid and fat available to build respectively the proteid and fat of the ovary. There was no evidence of transformation of proteid into fat. The muscle fibres do not degenerate but dwindle in size, and again become restored when the fish return to the sea.

There is no evidence that the developing chick can build fat out of the proteids of the egg. In the new-laid egg there is about 10 per cent. fat, after seven days' incubation 9 per cent., and after twenty-one days 6 per cent. Until the chick can feed itself on fat and carbohydrate, the fat contents of its body decreases, consumed as it is to supply the energy of growth.

Nerve degeneration is the only authenticated example of a proteid-containing substance yielding fat on decomposition. But in this case the fat does not come from the proteid, but from the lecithin moiety of protogon.

The Weigert stain depends on the existence of protogon, the Marchi stain on the setting free of oleic acid by the decomposition of lecithin which follows the disruption of the protogon molecules. The lecithin breaks down into phosphoric acid, fatty acids, and cholin. Both the phosphorus and total fat content of the degenerating nerve decrease, and the cholin passes into the blood, and may, it is said, be detected therein; but this is a disputed matter (Halliburton and Mott).

Origin of Fat out of Carbohydrate

Laws and Gilbert, feeding swine on meal, found one pig (compared with another of the same litter and weight) gained 55·5 lbs. of fat, while the food contained only 13·7 lbs. Reckoning that the whole of this food fat was put on, that the whole of the nitrogen in the food was in the form of proteid, and that the whole of the non-nitrogenous moiety of this proteid went to form fat, the total comes out as insufficient to cover the fat put on. Therefore, fat must have been formed from carbohydrate. Example:—

	lbs.
Fat put on	71·2
Fat in food	12·4
Fat produced	58·8
Proteid consumed	64
Proteid put on	6·5
Available for fat . . .	57·5
Carbon in fat put on . . .	15·3
Carbon in available proteid (deducting the carbon excreted as urea) .	27·4
Difference	17·9

Swine are especially suitable for these researches, as they eat a large amount of digestible food, pass a relatively small amount of excreta, and put on fat rapidly.

Tscherwinsky chose two ten-week-old piglings out of one litter of almost equal weight. One was killed and its fat and proteid content estimated. The other was fed four months with barley. The amount of barley eaten was weighed and the barley analysed. The undigested fat and the nitrogen in the excreta were determined. From these data the fat and proteid assimilated in the four months were calculated. The pig increased to 24 kg., and the fat and proteid in its body were estimated.

	Kg. Proteid.	Kg. Fat.
No. 2 held	2·52	9·25
No. 1 held	0·96	0·69
No. 2 had put on . .	1·56	8·56
In the food assimilated there was	7·49	0·66
Difference . . .	5·93	7·90

Of the 7·9 kg. fat only a small part could possibly have come from the 5·93 kg. proteid. At least 5 kg. must have come from the carbohydrate.

Meissl and Strohmer fed a one-year-old pig weighing 140 kg. for seven days with 2 kg. of rice per day. The rice, urine, and fæces were analysed, and on the third and sixth days the pig was brought into the respiration chamber and the CO_2 output determined. 289 grm. C and 6 grm. N were retained daily. Now 6 grm. N = 38 grm. proteid, containing 20 grm. C. Therefore 269 grm. C were put on as fat. In the food there was 5·3 grm. fat and 104 grm. proteid. Of the latter 38 grm. were put on. The remaining 66 grm. proteid and 5·3 grm. fat were insufficient to supply the 269 grm. C retained.

The formation of fat from carbohydrate has also been proven on sheep, dogs, geese, &c., by Rubner and others. It is a process of reduction. The excess of oxygen in the carbohydrate molecule appears to unite with the carbon of another carbohydrate molecule, oxidising the latter to CO_2 and H_2O. "The process is an intra-molecular change analogous to a fermentation producing CO_2 without the intervention of O_2 from outside." The respiratory quotient is therefore increased, and becomes more than 1 when much carbohydrate is transformed into fat.

The end stage of the reaction is represented by Hanriot in the equation :—

$$13(C_6H_{12}O_6) \quad C_3H_5 \begin{cases} C_{18}H_{34}O_2 \\ C_{18}H_{32}O_2 \\ C_{16}H_{30}O_2 \end{cases}$$

(Olco-stearo palmitin)

$$+ 23CO_2 + 26H_2O.$$

Bleibtreu calculates that 270·06 grm. glucose can form 100 grm. fat, 54·61 grm. water, and 115·45 grm. CO_2. Rubner calculates that 6 per cent. of the available energy is lost in the chemical work of transmutation.[1]

Respiratory quotients greater than 1 have been observed by Regnault and Reiset and others. Hanriot obtained an R. Q. = 1·28 in man fed on dextrose. Pembrey has observed an average respiratory quotient of 1·21 in a marmot during the stage of fattening. In rats after a rich meal of carbohydrate he and Spriggs have obtained R. Q. of 1·17. These high quotients are due to increased output of CO_2, not to decreased intake of O_2. Laulanié has observed R. Q. above unity in dogs fed on much bread or sugar. As to the possible seat of this transformation, it is worth noting that Leathes finds that liver pulp, when suspended in Ringer's solution and aerated, forms 10–40 per cent. more fat than it originally contained, and the amount formed is increased by the addition of glycogen.

The fat formed from carbohydrate seems to contain more stearin and palmitin and less olein.

In geese fed on potatoes Rosenfeld found the fat put on of higher melting-point and poorer in olein with an iodine number 63, while the iodine number of normal goose fat was 79. He found the same kind of hard fat put on in dogs, rabbits, ducks, and carp when fed on carbohydrates.

The iodine number of the body fat of various carnivorous animals approaches that of the fat on which they feed. Rosenfeld fed a dog on mutton fat, and then for a month on food in which no fat was given. At the end of this time the body fat had still

[1] "The conversion of the resorbed nutrients of the food into the ingredients of tissue involves profound chemical changes, and we can hardly suppose these take place without some evolution of heat." Johansson, Tigerstedt, &c., compute that 15 per cent. of the total energy of resorbed food is thus spent.

all the characteristics of mutton fat. Rabbits fed on barley have fat like barley oil. Green-food eaters have a hard fat poor in olein, seed-eaters a soft fat. Horses fed on oats have a soft fat, on hay a hard fat. Hard fat is characteristic of fat derived from carbohydrate food. If there is some fat and much carbohydrate in the food the body fat takes on the character of the fat.

In the formation of carbohydrate into fat a higher compound is built, and energy must therefore be expended, and thus food fat is laid on in preference.

The Origin of Body Fat out of Food Fat

Hoffmann fasted a dog 30 days till the body had lost 39·5 per cent. of its weight, and was almost fat-free; then fed it for five days with bacon fat, giving 370·8 grm. fat and 49·4 grm. proteid daily. The dog put on 1353 grm. fat, of which not more than 131 grm. could have arisen from the proteid assimilated.

Henriquez and Hansen fed two pigs for nine months with barley meal to which they added linseed or cocoa-nut oil. Samples of subcutaneous fat were excised from the back at intervals of time, and showed abundant deposit of these oils.

Munk fasted a dog for 19 days to a loss of 52 per cent. body weight. In the next 14 days he gave altogether 3200 grm. meat and 2850 grm. mutton fatty acids. The body weight increased 17 per cent., and he separated from the dog's body no less than 1100 grm. fat with scissors and scalpel. This fat melted at 40°, while normal dog's fat melts at 20° C. Feeding rape-seed oil to fasted dogs he obtained a fat fluid at body temperature containing 17 per cent. more oleic acid than normal, and some erucic acid, which is a constituent of rape-seed oil. Erucic acid was also detected in the chyle in a man with a fistula of the thoracic duct to whom this oil was given.

Quantitative Relations of Feeding Fats

When Rubner gave more fat to a previously fasted animal than was sufficient to cover the amount of body fat metabolised during the fast, he found 82 to 92 per cent. of the excess was stored up in the body. Pettenkofer and Voit calculated that 87·86 per cent.

was deposited when fat was added to a ration already more than sufficient for maintenance.

On the other hand, in the case of peanut oil added to a maintenance ration Kellner found only 35 to 61 per cent. of the fat stored. The loss of energy here implied suggests that the fat underwent molecular changes after assimilation and was converted in part into animal fat.

To sum up, then, experiments show that food fat is stored up in the depôts as body fat, and that excess of one kind of food fat modifies the characters of the depôt fat to that of the food fat. How far the organism strives to adjust the food fat to a normal standard peculiar to the individual is undetermined.

Fat as a Source of Energy

The well-being of an adult animal is not conditional upon the maintenance of any fixed relation between the fats, carbohydrates, and proteids in the food supply, apart from the minimum proteid ration which is absolutely necessary to cover body loss. The organism has the power to use all three classes indifferently for the manifestation of its energy. A fasting animal uses up its body proteid and fat. Proteid fed in excess of the amount metabolised during starvation serves as a source of biotic energy and takes the place of body fat. The portion of the proteid supply which thus serves as a source of energy may be replaced indifferently by fat or carbohydrate. Thus in a dog brought into equilibrium on a ration of 1500 grm. meat, the feeding of only 100–200 grm. fat or carbohydrate makes it possible to dispense with no less than two-thirds of the proteid.

The taking of proteid stimulates the metabolic activity of the cells, so that cleavage of the fed proteid rapidly takes place, and the excretion of nitrogen keeps pace with the intake. The non-nitrogenous foods flatten out the curve of nitrogen excretion which follows the intake of proteid, and by their simultaneous absorption lessen the rate of its cleavage.

The minimum amount of nitrogen on a diet of proteid and fat lies between 130 per cent. and 160 per cent. of the amount of nitrogen excreted during fasting. With a very large supply of carbohydrate, on the other hand, the N intake may be reduced even below the output during fasting. Thus Siven maintained himself

on a diet containing 4·52 grm. N, less than one-third of that in an average man's diet. Many physiologists, especially Chauveau and his school, have supposed that isodynamic amounts of fat and carbohydrate (amounts which the same number of Calories when burnt with O_2 in a bomb calorimeter) are not of equal worth as a source of muscular energy. They teach that sugar is the sole source of this energy, and that fat must be converted into sugar before it can be utilised. In the bomb calorimeter 1 grm. fat is isodynamic with 2·26 grm. dextrose; but after conversion into sugar it is only equal to 1·6 grm. In place of 9·5 Cal. it equals after conversion 6·07 Cal.

Now we have no means of determining what part of the energy of the body comes from proteid, fat, or carbohydrate in the body. There is no proof that the body makes any distinction between these food-stuffs. The whole supply of energy is used as a whole for the bodily needs. In any given experiment we can determine the amount of proteid fat, and carbohydrate metabolised, and the amount of work done, but we cannot tell what part of each of these stuffs is metabolised in performance of the work.

Atwater has settled the question by placing a man in his respiration calorimeter on a diet not quite sufficient for maintenance, so that the food-stuffs were used with the greatest economy. The same amount of easily digestible proteid was given each day, the same amount of external work done, and as the conditions of life and temperature were uniform, the same amount of energy was produced. Fat was fed in addition to the proteid in one case and carbohydrate in the other. In each case the same isodynamic value of the food-stuff was given. If under these conditions the loss of body substance is greater in one case than in the other, then it is clear that one food-stuff has a lower value than the other, as a source of muscular energy.

The following is the average of four experiments on an athletic student, lasting fifteen days altogether :—

Diet.	Energy of Food.	Energy of External Muscular Work.	Energy of Total Material oxidised in Body calculated from Excreta.	Energy of Body Substance oxidised.
Carbohydrate and proteid .	4532	558	5167	635
Fat and proteid . .	4524	554	5238	712

The conclusion from these experiments is that the worth fat is to that of carbohydrate as 95·5 : 100, while if Chauveau's view were true it would be as 61 : 100. The difference found by Atwater is very small, and may be an individual peculiarity. Rubner also concluded that fat and carbohydrate replaced each other when given in isodynamic amounts.

In the fasting animal the R. Q. may sink below that of an animal fed on fat. This must be due to the conversion of some proteid or fat into sugar. The same thing may happen in cases of diabetes, and in hybernating animals during their winter sleep (Pembrey).

To what an extent the depôt fat can be used as a source of muscular energy is shown by the following observation of Atwater.

The subject pedalled the friction bicycle 16 hours out of the 24.

The whole energy expended = 9981 Cal.

The energy of the food taken = 5138 Cal.

The energy taken out of the body substance = 1813 Cal.

The energy derived from tissue proteid only = 478 Cal.

As the man was in perfect training his muscles were not overdone, and thus the proteid metabolism was scarcely increased. This experiment strikingly shows how surplus body fat can be taken off by hard muscular work.

The Work of Digesting and Absorbing Fat

The taking of food increases the metabolism of the fasting animal owing to the activity of the digestive organs—mastication, peristalsis, glandular secretion, &c.; the processes of solution, hydration, fermentation, and cleavage; the warming of ingesta; the cleavage and synthetic processes of absorption. The injection of sugar or egg-white into the circulation does not provoke any such increased expenditure of energy (Zuntz, v. Mehring).

The average metabolism of a fasting man resting in bed was 2022 Cal. When over-fed his metabolism became 2517 Cal. (Johansson, Tigerstedt, &c.).

Fat fed to fasting dogs in 2–3 hrs. increases the O_2 use 12 per cent. The maximum is reached in 5–9 hrs., and the effect is over in 12 hrs. A large ration of fat may increase the expenditure of energy 19 per cent. The R. Q. sinks almost to

that of the oxidation of fat. Carbohydrate raises the metabolism double as much as fat, and proteid when fed in large amounts may increase it 90 per cent., as so much rapidly undergoes chemical cleavage.

The Putting on of Fat

Flesh cannot be put on by over-feeding proteid, unless the diet be accompanied by bodily effort, with the exception of convalescents and children. In such cases N will be retained even on an insufficient diet, and much more so on a rich one. The deposit of fat, on the other hand, is directly dependent on the amount of food assimilated. Fat can be put on (1) by increased food, (2) by lessened expenditure of energy, (3) by these two causes acting together, and this is the common case.

A man requires a daily diet equivalent to about

30 Cal. per kg. of body weight when resting in bed.
40–45 Cal. per kg. of body weight when doing moderate work.
45–50 Cal. per kg. of body weight when doing strenuous work.
(1 grm. proteid = 4·1 Cal. 1 grm. fat = 9·3 Cal. 1 grm. Carb. = 4·1 Cal.)

About 1·3–1·5 grm. proteid per kg. must be in the food—*e.g.* a man of 70 kg. requires 90–115 grm. proteid = 370–470 Cal. Chittenden says only half the amount of proteid given in ordinary diet-tables is necessary. He has kept soldiers in full vigour and perfect health on this amount, increasing the carbohydrates proportionately. The lean require relatively more proteid and the fat less, because the proportionate weight of living active cells is greater in the former. The tall lean man also requires more Calories than the short fat man, because in the former the surface exposure in proportion to body mass is much greater.

Fattening by Over-eating

In the matter of fattening the whole question is one of the balance of intake and output of energy. The popular idea that potatoes are more fattening than bread, bread-crumb than crust, beer than wine, is baseless; the only thing of moment is the potential energy of the food.

Suppose a man of 70 kg. is living on 40 Cal. per kg. per diem, or 2800 Cal. made up thus:—

120 grm.	proteid	= 492
150 ,,	fat	1395
30 ,,	alcohol	210
170 ,,	carbohydrate	700
		2797

an extra 300 Calories can very easily slip into his diet by some slight change of food or mode of cooking. Thus frying or cooking meat as ragouts may double the Caloric worth of the meat by adding fat. Taking an equivalent weight of bread in place of potatoes doubles the Caloric worth of the latter.

105 grm.	(3·7 oz. approx.)	bread	= 300 Calories.
370 ,,	(13 ,,	) potatoes	
75 ,,	(2·6 ,,	) cheese	
36 ,,	(1·3 ,,	) butter	
300 ,,	(10·6 ,,	) lean raw beef	
200 ,,	(7·0 ,,	) eggs	
83 ,,	(3·0 ,,	) sausage	
500 c.c.	(17·5 ,,	) milk	

An extra 300 Calories put on as fat would equal about 32 grm. This gives more than 11½ kg. of fat in the year, or 16½ kg. of adipose tissue (the latter contains water) (v. Noorden).

Alcohol is not a fat-builder but a fat-sparer. It is easily burnt and takes the place of fat or carbohydrate. 1 grm. alcohol = 7 Cal. = 0·7 grm. fat or 1·7 grm. carbohydrate. 50 grm. is contained in a bottle of wine, or about three pints of beer. Alcohol thus often takes a very large part in the causation of obesity.

Lessening of water in the day's ration has been extolled as a cure for obesity, firstly by drying the body (this can be only a slight temporary effect), and secondly by lessening the appetite.

Fattening by Lessened Expenditure

This may be easily brought about by change in habit, illness, or injury. Suppose, for example, a business man of 70 kg. and of regular habits lives in a flat fifteen metres up, and ascends four times a day. Suppose he changes his flat to the ground floor. The

energy spent in climbing = 15 × 4 × 70 = 4200 kg. Now only 30 per cent. of the muscular energy appears as external work. Therefore the total climbing energy = 1400 kg. But 425 kg. = 1 Cal. Therefore 14,000 kg. = 32·9 Cal., and this is equivalent to 3·54 grm. fat. In the year this equals 1·3 kg. fat, or 1·87 kg. adipose tissue (v. Noorden). Whether the man puts on fat or not depends on whether his appetite lessens in consequence of the lessened expenditure. With increasing age and growing wealth there is growing tendency to take more alcohol and richer food, to use cabs instead of walking, to shrink from exposure to cold and live in over-heated rooms.

Women with child are especially liable to get fat, as they both eat and rest more.

The following figures illustrate the effect of rest and restlessness on metabolism.

A fasting man during sleep excreted on the average 7·243 grm. CO_2 per kg. per diem, while during a restless sleep he secreted 8·136 grm.

A dog lying down excreted 174·3 c.c. O_2 per min.

„ standing up „ 245·6 c.c. „

A man fasting and lying in bed in the ordinary way excreted 24·94 grm. CO_2 per hour; while with complete muscular relaxation he excreted 20·72 per hour.

Zuntz found the metabolism of a horse in the respiration chamber distinctly increased by the presence of a few flies.

In 70 per cent. of the cases of obesity there is an hereditary tendency. Certain races, such as Hungarians, Lapps, Jews, and Dutchmen, are especially characterised by a full habit of body.

In certain, but rare, cases obesity is due to an anomaly of protoplasmic metabolism, consisting in a lessening of the energy of decomposition, a greater economy in working the bodily machine. Individuals differ greatly in this respect. There are pigs that put on flesh and pigs that put on fat; and so it is with men. In two pigs of almost equal weight, observed by Weiske and Wild, A used 40·1 Cal. per kilo, and B only 34·0. A put on 20·9 per cent. of the absorbed carbohydrate as fat, and B 41 per cent., while A increased its proteid 146 per cent., and B only 120·4 per cent.

Von Noorden quotes the case of a man weighing 102 kg., who daily walked 8 km., was on his feet all day, and twice

mounted a hill 165 m. high. Placed on a strict diet equivalent to 2000 Cal. for three months, he lost only 1 kg. in three months. On the other hand, a man who worked 7 hrs. at a desk and took easy exercise on the level for 3 hrs. a day lost 7 kg. in 2 months on the same diet. He also gives the astonishing case of a woman, who was of spare habit up to 55 years, and became after this fatter and fatter. She kept herself on the following diet, equal to 900–1000 Cal.:—

8 A.M., cup of black coffee.
10 „ 1 egg and 1 Albert biscuit.
12 noon, 7 oz. apple and ½ pint white wine.
2 P.M., cup of black coffee.
4 „ 2 oranges and 2 Albert biscuits.
6 „ ¼ l. milk.
8 „ 1 egg, 7 oz. apple, ½ pint white wine.
10 „ glass of seltzer water.

And in spite of it put on ½ kg. in six weeks. She showed no sign of œdema, and her strength seemed unimpaired. In such a case—if the woman did not lie—the lamp of life must burn at a lower level—in other words, the internal work of the body must be run more economically than in most of us.

The net available energy of the absorbed food equals the gross available energy minus the work of digestion and assimilation. It is possible that this is done more economically in some than in others. Likewise in some the ratio of external work may be greater in proportion to the heat produced by muscular work. In the ordinary man not more than 20 per cent. of the total energy appears as external work, while in the highly trained an efficiency of 30 per cent. may be attained. Pregnant animals when starved waste more slowly than others. It is said that their metabolism runs at a lower level in the interest of the young they carry; but this requires exact confirmation.

The Effects of Obesity

The extent and rapidity of the bodily movements are lessened. A man of 120 kg. mounting stairs does double the work of one of 60 kg. The temperament becomes phlegmatic. Fatigue is quickly induced. Fat in the belly and mediastinum may limit the

respiratory excursion, and by lessening the suction force of the respiratory pump impede the return of venous blood. The efficiency of the heart is diminished by the weight of fat on its substance.

The tendency to obesity can be met by a diet of lean meat, green vegetables, and fruit. By taking potatoes or coarse brown bread with much waste in place of white bread. By avoiding all food cooked with fat or flour. By lessening appetite, by drinking between and not at meal times.

The following diet of von Noorden, given to a man of average height, will with systematic muscular exercise in many cases lessen fat by 10 lbs. in the first month, and 5 lbs. in the next two months.

100 grm. ($3\frac{1}{2}$ oz. approx.)	lean meat	= 130 Cal.
1 l. ($1\frac{3}{4}$ pint ,,)	milk	= 600 ,,
1 l. ($1\frac{3}{4}$ pint ,,)	butter milk	= 450 ,,
200 grm. (7 oz. ,,)	potatoes	= 160 ,,
100 grm. ($3\frac{1}{2}$ oz. ,,)	black bread	= 180 ,,
100 grm. ($3\frac{1}{2}$ oz. ,,)	raw fruit	= 40 ,,
Coffee *ad lib.*		...
		1560 Cal. *i.e.* $\frac{3}{5}$ of a normal diet.

Mountain climbing is the most efficient means of relieving a robust man of surplus fat. Climbing should be regulated so as never to disturb deep and regular breathing. Quick superficial respiration overburdens the heart. The influence of climbing is shown by the fact that progressing one metre on the level uses up 0·1196 c.c. O_2 per kg. of body weight; climbing one metre uses up 1·4425 c.c. O_2 per. kg. of body weight.

Massage has no effect on metabolism, and cold baths have little effect unless combined with friction to produce vaso-dilatation and loss of heat.

It has been an article of faith that castration lessens the rate of oxidation and increases fattening, but this Lüetige has failed to confirm.

The feeding of th roid gland increases the O_2 use and CO_2 output, and in many cases the N_2 metabolism. In some it lessens fat, in others not. It may cause glycosuria, nervous symptoms, and palpitation of the heart, and is not a treatment to be recommended. Less food and more exercise, and especially the latter, is the one and only remedy for fat people.

Want of a proper amount of depôt fat destroys the grace of the figure, and deprives the blood-vessels and viscera of the elastic cushions with which they are naturally supported. A tendency for congestion of the blood in the lower parts and a falling down of the abdominal viscera results therefrom.

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CHAPTER XII

THE METABOLISM OF THE CARBOHYDRATES

Carbohydrates constitute the greater proportion of our dietary. The approximate composition of the diet of a man of average weight (70–75 kg.) doing a moderate amount of muscular work is, according to Ranke, proteid 100 grm., fat 100 grm., carbohydrate 240 grm., and when muscular work is undertaken the amount of carbohydrate is usually much in excess of this, viz. 400–700 grm.

The greater proportion of this carbohydrate is taken in the form of starches (polysaccharides), some as cellulose, and the remainder as sugar. During digestion all carbohydrates are resolved, by the various amylolytic and inversive ferments, into simple sugars (monosaccharides), and it is as such that they are absorbed into the portal blood. A study of their metabolism starts from this point—*i.e.* from the moment at which they enter the circulation as simple sugar—it then considers what rôle the sugar plays in the production of energy and in the nutrition of the tissues, and it ends with an account of what becomes of the used-up sugar—*i.e.* in what form or forms it is excreted from the system.[1]

We see, then, that a very large amount of carbohydrate is taken each day in the food. If, however, an examination of the various excreta—urine and fæces—be made, no trace of sugar under normal conditions will be found in them. The ingested sugar must, therefore, become converted in its passage through the organism into something not sugar, and since it is plainly impossible that any large amount, at least, can be built up into the tissues—else would the organism very soon assume enormous dimensions—we are driven to the conclusion that it is oxidised, and thus converted into carbon dioxide and water. An examination of the expired air shortly after a carbohydrate-rich meal

[1] Space in this article will not permit of an account of the chemistry of the carbohydrates nor of the chemical processes which they undergo during their digestion. The necessary information on these points will be found in any of the elementary text-books on Physiological Chemistry.

has been taken will plainly demonstrate that this supposition is correct, although the increased excretion of carbonic acid may not be marked for some time after the meal. As we shall see later, much of the carbohydrate which is not immediately required becomes laid aside in the tissues in a modified form to be afterwards called upon, when required by the organism, to assist in carrying out the processes of metabolism.

Indeed, very soon after its absorption into the blood—it being by this path and not by the lymphatics that carbohydrates are absorbed into the organism—the carbohydrate undergoes a change. When absorbed into the blood from the gastro-intestinal tract the carbohydrate is in the form of a reducing sugar, and as such it may be detected in large amount in the *portal* blood. If, on the other hand, the blood of the *systemic* circulation be examined, only a very small amount of reducing sugar will be detectable in it, and this is true even after a very liberal carbohydrate diet has been taken. As the result of numerous observations, it has been shown that under normal conditions the systemic blood contains from 0·05–0·2 per cent. of reducing sugar. It has also been found that any increase over this amount of sugar in the blood (which condition is called hyperglycæmia) is at once followed by the appearance of the excess of sugar in the urine; in other words, a condition of *glycosuria* is established. With a normal percentage in the blood the kidney allows none of the sugar to pass into the urine, but whenever this percentage is exceeded immediate leakage of the excess through the kidney filter occurs.

Allowing 5000 c.c. for the amount of blood in a full-grown man, we see that, at most, only about 10 grm. of the absorbed sugar can be accounted for in the blood. Nor can we account for the sugar in a free state in the tissues (*e.g.* muscles), for if these be examined in a perfectly fresh state only the minutest traces of sugar will be found in them.

In contrast to the systemic blood, the amount of sugar in the portal blood will be found to show great variation, according to whether absorption of food from the intestine be in progress or not. During fasting, the percentage will be seen to be the same as in the systemic blood, but during absorption to be much higher than this (0·2–0·4 per cent.).

If we consider what the cause of this difference in their sugar content between the two forms of blood can be, we are at once

led to look to some function of the liver, for all the blood must pass through this organ on its way from the intestines to the tissues. We must assume that this organ is endowed with some function whereby it retains the excess of sugar in the portal blood and prevents its overflowing into the systemic circulation, and thus creating a condition of hyperglycæmia, and consequently of glycosuria. If, however, the liver be examined for sugar in a perfectly fresh state, and even after a meal rich in carbohydrates, it will be found to contain only a very small amount, so that the excess of sugar which this organ seems to remove from the portal blood cannot be retained in it as sugar.

It must be as something else. That the liver does contain some substance closely related to sugar was shown in 1855 by Claude Bernard. He found that when the liver was perfused through its blood-vessels with water, until the washings were sugar and proteid free, and then was allowed to stand over night at room temperature, a large amount of sugar reappeared in it. From this result Bernard concluded that by some *post-mortem* process sugar had been formed in the liver out of some substance not sugar.

Claude Bernard[1] in 1857 discovered this substance to be *glycogen*, and dating from that discovery great strides have been made in the subject of carbohydrate metabolism.

At most, however, the liver contains only 10 per cent. of glycogen, and, if we allow 1500 grm. for the weight of the liver of a full-grown man, we can account in this way only for 150 grm. of the sugar absorbed from the intestine; and indeed, not for so much as this, for the liver still contains a considerable amount of glycogen after fasting, unless this be of long duration (see p. 324). There must, therefore, be in the organism other places besides the liver in which the excess of sugar is laid by in some modified form. The muscles contain sometimes as much as 1 per cent. of glycogen, so that, collectively, they could probably hold as much of this substance as the liver does. In these two depôts (liver and muscles) there might, therefore, be laid aside as glycogen about 300 grm. of sugar. But our diet often contains much more carbohydrate than this, so that we must conclude that a considerable amount of carbohydrate becomes converted into, or at least incorporated with, tissues which are not carbohydrate in nature—*i.e.* with fat or proteid.

With regard to fat, there is no doubt that carbohydrates can be ultimately converted into it (p. 285) in the organism: but this transformation is probably too slow to make it of much account as an immediate means of disposal for the excess of carbohydrate. Pavy, it should be mentioned, believes this conversion into fat — as well as into proteid—to be an ever-occurring process, and if his views, which we will discuss later, be accepted, little difficulty can exist in explaining what becomes of the excess of ingested sugar.

Most workers agree that a certain amount of the absorbed sugar is converted into, or rather becomes incorporated with, the tissue proteids, and in this way is laid aside in the organism; but just how much is thus disposed of cannot be stated.

As the presence of a carbohydrate group in the proteid molecule is now fully established, at least for most proteids, and since the existence of this group has a very important bearing on the whole question of carbohydrate metabolism, it will be advisable, before going further, briefly to review the bio-chemical work which has been done on this subject.

The first exhaustive work in this direction was done by Schmiedeberg (2). He found in cartilage proteid a complex polysaccharide—chondroitin-sulphuric acid. By hydrolysis of this substance he obtained chondrosin and acetic acid, and by further hydrolysis of chondrosin he thought that glycuronic acid and glucosamin were formed. Glycuronic acid is, chemically, quite closely related to dextrose, differing from it only in that the end CHOH group is replaced by a COOH group. Glucosamin has recently been shown by Emil Fischer to be α-amido-glucose.

CHOH	CHOH
|	$(CHOH)_3$
$(CHOH)_4$	$CH(NH)_2$
|	|
CHO	CHO
(*Glucose*)	(*Glucosamin*)

The presence of glucosamin in cartilage, if true, is of very great interest from a biological point of view, for it is also found as a constituent of the chitin found in the carapace of Arthropods (where it is also in union with acetic acid) and in the so-called cellulose of fungi. There is also reason to believe that it is present in the insoluble residue of tubercle bacilli.

Orgler and Neuberg (5), in a recent publication, have, however, cast doubt on the presence of glucosamin in chondrosin,[1] for they have succeeded in isolating from the decomposition products of chondrosin sulphate, a copper salt of tetraoxyaminocaproic acid $(C_6H_7O_2(OH)_4NH_2)_2Cu$, with which, in chondrosin, they show it to be impossible that glucosamin can be combined. They conclude this from a comparison of the formulæ of chondroitin-sulphuric acid and tetraoxyaminocaproic acid, also from their inability to detect glucosamin or any related body in the decomposition products of chondrosin.[2]

In *mucin* and *mucoid* it has for many years been known that reducing substances are present. In the fluid from an ovarian cyst, Scherer, in 1852, discovered a proteid, which he named metalbumin, and which by boiling with weak acid yielded a reducing substance. It has since been established that Scherer's metalbumin is mucin. The most exhaustive work on the carbohydrates in mucin, &c., is by Friedrich Muller and his pupils (2). The method used by these workers consisted in hydrolysing the mucin with H_2SO_4. To the resulting solution (with or without previous separation of proteids) benzoyl chloride and caustic alkali were added, whereby a precipitate of the benzoyl ester of the sugar was obtained. By a determination of the melting-point and crystal form of this compound and by its elementary analysis, the exact nature of the carbohydrate was ascertained. In this way, it was found that sputum mucin, stomach mucin, ovo-mucin, and pseudo-mucin contained glucosamin. So far, no other carbohydrate than this has been discovered in mucin, and it is probable that here, as in chitin, the glucosamin is combined with acetic acid.

In the *nucleo-proteids* obtained from the pancreas, thymus, thyroid, spleen, muscles, &c., there has been found, by Hammersten and others, to be constantly present a pentose group which can be dissociated from the nuclein molecule by the action of acids. The exact variety of this pentose in the nucleo-proteid of the pancreas and liver has been shown to be *l*-xylose. Certain nucleo-proteids, *e.g.* of the thymus gland and of yeast, also contain hexoses, for, as Kossel has shown, they yield lævulinic acid amongst their decomposition products, and this can only come from hexoses.

It is with regard to *true proteids* that the most interesting results have been obtained. Schutzenberger was the first to draw attention to the presence of a reducing sugar in *egg albumin*. To liberate

[1] $Ba(OH)_2$ was employed as decomposing agent.

[2] Glycuronic acid also cannot be present.

this sugar, he boiled the mucin free albumin with strong sulphuric acid. He further stated that the carbohydrate existed in the proteid molecule as a polysaccharide containing nitrogen in its molecule, and which he named "*amide cellulosique*."

The more recent methods which have been used to study this carbohydrate group resolve themselves into two classes: in the one of these acid is used to decompose the proteid, and in the other alkali. For the detection of a carbohydrate group in proteid certain colour reactions may also be employed.

By acting on egg albumin with 10 per cent. caustic potash and neutralising the resulting solution with acetic acid, Pavy was able to obtain, by precipitation with alcohol, a gummy like mass which itself had no reducing properties, but which when boiled with mineral acids yielded a reducing sugar thought, on account of the melting-point of its osazone, to be dextrose. The gummy-like substance has been further investigated by Fraenkel (2) (using $Ba(OH)_2$ instead of KOH), who found it to contain nitrogen and named it *Albumin*. This worker was able to obtain it also by acting on egg albumin with gastric juice. By the hydrolysis of albumin a reducing monose was obtained. Langstein (2, 3) prepared a large amount of albumin by the prolonged digestion of egg albumin with gastric juice in the presence of sulphuric acid, and by hydrolysing it he obtained as much as 86 per cent. glucosamin. He thinks it probable that in albumin acetic acid may be present, as in chitin: he was, however, unable to demonstrate its presence.

By the action of 3 per cent. hydrochloric acid directly on egg albumin and separation of the reducing substance by means of benzoyl chloride (Müller's method), Seeman also succeeded in demonstrating the presence of glucosamin, and Langstein, by the same method, has been able to obtain as much as 10 per cent. of this monose from crystallised egg albumin.

There can be no doubt, then, that, like mucins (and chondro-proteids?), the most important carbohydrate group in egg albumin is one which yields glucosamin on hydrolysis.

Langstein has also, by the benzoyl chloride method, separated glucosamin from ex-globulin and cong-albumin in egg white; and in several other proteids, *e.g.* fibrin, serum proteids, vegetable albumin, &c., various workers, by the use of phenyl hydrazine, have detected carbohydrate groups.

No worker has, however, been successful in demonstrating any carbohydrate group in *casein*, and on this account Pavy has suggested that the lactose of milk really represents dissociated proteid carbohydrate.

By various colour reactions,[1] Langstein has shown that pure *serum albumin* also contains a carbohydrate group, and from one preparation of it this worker was able to separate small quantities of glucosamin. There still remains some doubt, however, as to whether this may not have been derived from some adherent mucin of which it is very difficult to remove all traces from the albumin.

With pure *serum globulin*, on the other hand, much more satisfactory results have been obtained. H. A. K. Morner and Langstein ([2, 3]) have independently shown that both the eu- and pseudo-globulin fractions of this can yield monosaccharides when hydrolysed with acid, and by a careful study of the osazones, the products of oxidation and the benzoyl compounds of these, and their power of fermenting with yeast, they have identified these monosaccharides as dextrose, glucosamin, and possibly lævulose. By decomposing the globulin with baryta, a polyose practically identical with albumin has been obtained by Langstein. By decomposing this polyose with acids glucosamin was obtained. The percentage of nitrogen in this albumin was found to be somewhat variable, and, since dextrose has been found along with glucosamin in the products of acid hydrolysis,

[1] There are certain colour reactions by which the presence of carbohydrate groups in proteids may be detected. The more important of these are as follows:—

1. *The Reaction of Molisch.*—To one cubic centimetre of a one per cent. suspension of proteid in water are added two drops of an alcoholic solution of α-naphthol (15%); three cubic centimetres of concentrated sulphuric acid are then run down the side of the test-tube so as to form a layer underneath the proteid solution. If the proteid contain a carbohydrate group, a purple ring forms where the two fluids are in contact. (The green diffuse colour which also appears in the acid is no part of the reaction.)

This reaction is so exceedingly delicate (·0001 grm. dextrose or pentose gives it—that great caution must be taken that the proteid under examination is entirely free of adherent carbohydrates, or of proteids such as mucin, which contain carbohydrate groups. It is indeed too delicate a test on which to base absolute conclusions. This reaction is usually described as due to the liberation of furfurol by the action of the acid.

2. *The Orcin-ferric Chloride Reaction of Bial.* ([5])—Into four cubic centimetres of fuming hydrochloric acid in a small test-tube is dropped orcin till no more will dissolve; two cubic centimetres of the proteid solution are then added and one drop of a dilute solution of ferric chloride. The mixture is boiled for about two minutes. If hexoses be present, bluish-green flakes soon separate out, and if these be dissolved by shaking with amyl alcohol the resulting solution will be found to give, when examined in the spectroscope, an absorption band at the beginning of the green absorbing the orange. Pentose and glycuronic acid give similar bluish-green precipitates, but the orange of the spectrum is not absorbed.

Glucosamin and lævulose do not give this reaction. It is therefore an important one for indicating what carbohydrate groups ought to be sought for by the more elaborate methods

Langstein suggests that, besides albumin, globulin may contain some N-free polyose yielding on hydrolysis dextrose; or it may be that the dextrose is adherent as such to the albumin molecule. It should be pointed out, however, that only 2 per cent. of sugar has, so far, been detected in globulin. Simon has recently described in the liver an albumose which contains sugar.

There is too little matter on hand to enable us to say how the carbohydrate group is attached to the proteid molecule. By the action of acids on proteid, the product which remains behind after the carbohydrate group has been split off, although it gives the biuret reaction and is precipitable by alcohol, is more probably of the nature of a protease or peptone than native proteid.

After we have studied the nature and mechanism of phloridzin diabetes, we shall see that there is reason to believe that a loose chemical compound of a colloidal nature exists between serum globulin and dextrose (see p. 363).

Soon after absorption into the blood, therefore, the sugar in great part becomes converted into glycogen, and possibly also into proteid. The biological evidence of this latter transformation we will consider later; for the present, a careful study of the **derivation and fate of glycogen in the organism** will occupy our attention.

Although, as we have already seen, the liver is not the only organ in which glycogen is found, it is evident that a careful study of the so-called glycogenic function of the liver must be of very great importance, and for many reasons:—the percentage of glycogen is much higher in this than in any other organ or tissue; the anatomical position of the organ, and the fact that the percentage of sugar in the systemic blood is nearly constant whereas that of the portal blood varies according to the activity of digestion, suggest the glycogenic function of the liver as the one which mainly regulates the supply of sugar in the systemic blood: moreover, under certain conditions, we can approximately calculate, from the amount found in this organ, how much glycogen there is in the entire body.[1]

[1] It should, however, be noted that different workers do not agree on the quantitative relationship between the hepatic glycogen and that deposited elsewhere in the body. Ott, for example, states that there is an equal amount in the liver and in the other tissues; whereas Salkowski and Frent found more in the liver. Kulz found in starved hens a large excess in the body compared with the liver.

With regard to the method of investigation of this glycogenic function, we must, in the first place, see what carbohydrates and other food-stuffs cause glycogen to be deposited. To do this it is necessary that we render the organ as nearly as possible free of glycogen, and then, if we find that feeding with a certain food-stuff should cause glycogen to be deposited, we can state that this food-stuff is a glycogen former. A positive result of this nature cannot, however, without further investigation, be taken to show that the food-stuff in question has *itself*, in whole or in part, become transformed into glycogen; for it is always possible that the glycogen may have been derived from the tissues of the animal, the food-stuff having favoured such a process by replacing these tissues in their usual break-down, *i.e.* by becoming oxidised in place of them. As we shall see later, proteid may form glycogen, and it is therefore always possible that the glycogen apparently derived from some ingested non-proteid substance may really be derived from the tissue proteids. In other words, we must carefully distinguish between *true* and *pseudo*-glycogen formers.

Having ascertained the possible sources of glycogen, we must next study what becomes of this substance in the general economy of the animal, and what the conditions are which, apart from food, influence its amount.[1]

The questions we will at present consider are therefore the following:—

How is glycogen distributed quantitatively in the body?

How may the liver and other tissues be rendered glycogen-free?

What food-stuffs lead to glycogen formation?

What becomes of the glycogen in the organism?

How is the glycogenic function controlled?

[1] These questions depend for their satisfactory solution on reliable and accurate methods for the estimation of glycogen. It is beyond the scope of this article to describe these. A critical survey of all the more usual methods has recently been given by Pflüger, who also gives in detail a new method of great accuracy. (A detailed account of this method will be found in "Practical Physiology," 2nd ed., London, 1905.)

The Distribution of Glycogen in the Body

The presence of glycogen in tissues can be ascertained not only by its isolation by chemical methods, but also by its micro-chemical colour reaction, which depends on the deep brown or violet colour which it gives with a watery solution of iodine. By bringing microscopic sections of tissues in contact with such a solution any glycogen which these may contain is at once stained. In this way Barfurth (6) has examined a large number of tissues and organs.

In the *liver*, the amount of glycogen depends very largely on the taking of food. This is very evident in the liver of gastropods. After starvation for about three weeks all the hepatic glycogen disappears in these animals, and it begins to reappear in from nine to ten hours after food is taken. It has been noticed that the glycogen is at first deposited in the connective-tissue cells of the liver and afterwards in the epithelial cells, just as in starvation it disappears first from the epithelial cells and lastly from those of the connective-tissue.

This glycogen is contained entirely in the extra-nuclear portion of the cell; none has ever, in any animal, been seen in the nucleus. By its deposition the cells increase markedly in bulk and the liver increases in weight. In the liver of many animals (*e.g.* rabbit) the glycogen is deposited mostly towards the centre of the lobule—around the intra-lobular branches of the hepatic vein—the periphery of the lobule containing much less glycogen; in other animals, however, this distribution is not so evident. The different lobes of the liver seem to contain the same amount of glycogen; in other words, the glycogen is evenly distributed throughout the liver. The liver of a dog has been found to contain as much as 18·69 per cent. of glycogen (Schöndorff).[1]

In the *muscles* of well-fed resting animals, glycogen is present both in the sarco-substance and in the interfibrillary material

[1] Bernard and Barfurth state that the liver of embryos contains no glycogen until the middle of fœtal life, the other tissues being, however, more or less rich in it. In the observations on which this statement is based, the glycogen was merely extracted with boiling water, and no precautions were taken that the mother animal before slaughter had been normally fed. Pflüger, by his new method (6), has shown that glycogen is always present in the liver of fœtal calves, guinea-pigs, and lambs, at least during the first half of gestation.

(sarcoplasm); but in poorly fed animals it can be detected only in the interfibrillary material. In fœtal life, it appears in the muscles as soon as their histological structure in differentiated. During starvation, the glycogen in the muscles remains for some time after all of it in the liver has disappeared.

The amount of glycogen varies considerably in the different muscles of the same individual, as the following table of observations on three dogs shows:[1]—

TABLE I

Muscle.	Per Cent. of Glycogen.		
	1	2	3
Biceps brachii	0·17	0·25	—
Quadriceps femoris . . .	0·53	0·32	—
Muscles of back	—	—	0·135
Adductors of leg	—	—	0·077

The percentage of glycogen in the muscles of the dog has been found to rise, under certain conditions, to 3·72 per cent. (Schöndorff[7]).

For an estimation of the total amount of glycogen in the muscles of an animal, it is therefore not allowable to determine the percentage in a small piece of any muscle and calculate from this the amount in all the muscles; even by multiplying by two the amount of glycogen found in one-half of the body, only an approximate result of the total amount in the body is obtained, since, as A. Cramer has shown, corresponding muscles on the opposite halves of the body do not contain the same amount of this substance. A difference of 27·7 per cent. was found in one investigation in which the glycogen content of the two hind limbs was compared. Heart muscle contains more glycogen than skeletal muscle.

In none of the other tissues or organs is the percentage of glycogen so high as in the liver and muscles, but traces at least of it are to be found almost everywhere in the body. Even in such tissues as *cartilage* and *bone* has glycogen been found; in the blood it is found in the *leucocytes*, but there is none in the plasma; it is present in the *epithelial cells* lining the gastro-

[1] Cramer. Brucke-Kulz method (6).

intestinal tract, lining the ducts of glands, in the brain, in ganglion cells (of gastropods at least), in the ovaries and testes and placenta.

A more detailed account of its distribution is unnecessary here. An important point to keep in mind is that in the embryo it is much more evenly distributed over the body than in the adult, where it becomes located mainly in the liver and muscles.

With regard to *the seat of glycogen formation*, we would at first sight assume—from a consideration of its distribution in the tissues—that this is in the hepatic cells. It is most plentiful here, and feeding starved animals with carbohydrates causes glycogen to be deposited first of all in the liver and only later in the muscles. We would thus explain the presence of glycogen in the muscles by its being carried there by the blood. Against such an hypothesis, however, stands the fact that no glycogen has ever been detected in the blood plasma. There is also much evidence that other cells than those of the liver can form glycogen; yeast cells form it; it is present in the tissues of pr[illegible] animals which possess no liver; it is formed in the tissue[illegible] liverless frogs when solutions of dextrose are injected subcutaneously (E. Külz); it is present in the tissues of the developing chick before any liver cells have become differentiated, whereas it is absent in the unhatched egg. It is probable, therefore, that glycogen is formed in the muscles as well as in the liver; indeed, Nasmyn has stated that the colour reaction of muscle glycogen towards iodine is somewhat different from that of liver glycogen, and Bernard has noticed that in paralysed muscles a form of glycogen accumulates which gives a blue instead of a port-wine colour with iodine.

Attempts have been made to decide this important question —*i.e.* whether tissues other than the liver can form glycogen by perfusing the muscles of one of the hind limbs of a dog with blood containing dextrose, and comparing the glycogen contents of the perfused muscles with those of the other hind limb. Constant results, on which any definite conclusion could be based, have not, however, been obtained.

It is therefore probable, although absolute proof of the fact is wanting, that glycogen can be independently formed in the liver and other tissues in which it is found.

How the Tissues may be rendered free of Glycogen

By *starvation* alone it is impossible to render the liver glycogen-free or the blood free of sugar. Moreover, if several animals of the same kind be starved for the same length of time, the amount of glycogen which remains in their tissues will be found to vary considerably; for example, in seven hens starved for six days the total amount of glycogen in the body at the end of starvation was found by Edward Külz (6) to be: 0·7010 grm.; 0·5433 grm.; 0·0425 grm.; 0·3332 grm.; 1·3939 grm.; 1·0788 grm.; 1·7607 grm. Pflüger also records a dog which was starved for twenty-eight days, and the liver of which contained at the end of this period 22·5 grm. glycogen. V. Mering (6) states that in one dog after eighteen days, and in another after twenty-one days of complete starvation, 0·48 grm. glycogen was obtained from the liver. Indeed, as starvation proceeds, the amount of glycogen may, after attaining a minimum, increase slightly in amount, as, for example, when all the available fat has been used up and the tissue proteids begin to break down, as occurs in starvation a few days before the death of the animal.

In several animals of the same kind, therefore, starved for the same length of time and to all outward appearance identical, the amount of glycogen may be very variable, and the same is true when the animals are fed with exactly the same amount and kind of food; thus, the glycogen deposited in the tissues of similarly fed dogs may vary from 7·59 grm. to 37·87 grm. per kg. body weight (Schöndorff). On this account, great care must be taken in drawing conclusions from the amount of glycogen found in the body after death regarding the power of any food-stuff in influencing glycogen formation.

Starvation alone cannot be depended on to entirely deprive the tissues of their glycogen. If, however, it be combined with certain other agencies, which also tend to cause the disappearance of glycogen in the body, much more satisfactory results can be obtained. The most important of these agencies is *muscular work*. This causes the liver glycogen to disappear in a few hours, although it takes longer to make the muscles glycogen-free. A method for clearing the organism of glycogen, which includes starvation and muscular work, is that described by Bendix (8), in which dogs

after being starved for several days—or fed on flesh alone—are made to do excessive muscular work, immediately after which they are killed and the tissues examined for glycogen.

Certain poisons—such as arsenic and phosphorus, which act directly on the liver cells, and strychnine, which acts on the spinal cord centres, producing general convulsions (*i.e.* excessive muscular contractions)—cause a considerable depression in the glycogen deposits.[1] In the experimental diabetes caused by phlorhizin or by extirpation of the pancreas the glycogen deposits, especially those of the liver, become very much diminished in amount. We shall have occasion to consider these points later on.

What Food-stuffs lead to Glycogen Formation?

This may be ascertained by seeing whether, by feeding an animal rendered as nearly as possible glycogen-free, an accumulation of glycogen is induced. This is the *direct method*. Its results can be controlled by the *indirect method*. The rationale of the latter is as follows. Since there can be no doubt that dextrose forms glycogen—the absolute proof of this will be considered later—any food-stuffs which during their metabolism yield dextrose must also be glycogen formers. We can ascertain whether a food-stuff yields dextrose during its metabolism by rendering an animal incapable of oxidising carbohydrate—in other words, by rendering it diabetic—and then seeing whether the food-stuff in question causes an increase in the sugar excretion by the urine. If, for example, we find that feeding a diabetic patient with proteid

[1] A very thorough method, depending on the use of strychnine, is that of Frentzel (3). This worker showed that all traces of glycogen can be made to disappear in rabbits by inducing strychnine convulsions for five hours after having starved the animals for from one to two days. Previous to the starvation the rabbits should be fed for some time on milk. During the action of the strychnine, artificial respiration is usually necessary, but care must of course be taken that too large a dose of the drug is not given; it should be such that the convulsions are invoked only when the skin is irritated. After five hours the strychnine is antidoted by chloral, so that the animals fall into a deep sleep. If the animals be killed at any time up to twelve hours no glycogen will be found in their liver or other tissues. After this time, however, glycogen begins to appear and steadily increases in amount. The source of this glycogen may be tissue proteid. To study the influence of any food-stuff on the formation of glycogen in the liver, the food-stuff in question should be given along with the chloral and the animal killed in from ten to twelve hours.

causes a distinct increase in the sugar excretion, then we can state that proteid is capable of producing carbohydrate in the organism. This indirect method has within recent years been very thoroughly investigated, and we shall discuss it in considerable detail later. *Meanwhile we will proceed with a consideration of the results obtained by the direct method.*

It is evident that this direct method of ascertaining whether or not a substance is convertible into glycogen in the organism can be only an approximate one. We can ascribe a glycogen-producing influence to a food-stuff only when in a large number of experiments, an amount of glycogen is found to be deposited which is constantly higher than that found during starvation: and we must further show that, meanwhile, insufficient tissue proteid has been decomposed to account for the glycogen. This latter we can do by estimating how much glycogen could have been derived during the observation from proteid, and seeing whether this amount is sufficient to account for the excess of glycogen deposited. The amount of glycogen liberated from proteid can be calculated from the nitrogen excretion, for each grm. of nitrogen excreted in the urine will equal 2·500 grm. of carbon liberated in the tissues, which is equivalent to 5·700 grm. of glycogen (*i.e.* 3·295 grm. carbon liberated in the tissues *minus* 0·795 grm. excreted as urea, &c.). A method including this factor was adopted by Ott[8] in a series of experiments on the glycogen-forming power of the various carbohydrates.

The food-stuffs which we would naturally expect to be marked glycogen formers are the **carbohydrates.** Let us, therefore, consider these first of all in this connection.

For *dextrose*, the proof of its conversion into glycogen in the body is, as might be expected, an easy matter. Dr. F. W. Pavy [9] found that, whereas in eleven healthy dogs fed only on flesh the average relation of the weight of the liver to that of the whole of the body was 1 to 30, in five dogs fed on vegetable food it was 1 to 15. On animal food also, the average percentage of crude glycogen in the liver was 7·19, whereas on vegetable food it was 17·23. With such results, little doubt can exist that glycogen is derived directly from dextrose. It is, however, always possible, as indicated above, that its source might be the tissue proteids, and that the large accumulation of glycogen in the liver could be accounted for by the proteid not being required by the

organism—there being, on account of the liberal ingestion of sugar, sufficient carbohydrate to supply all the fuel necessary for the tissues—and hence being deposited as glycogen. A definite answer to this question has been furnished by Ott by the use of the method described above. This worker experimented on hens which had been starved for five days, and in which the glycogen in the entire body (according to Külz's analyses) could not have been more than 2·130 grm. On the sixth day 50 grm. of chemically pure glucose was given, and after about eight hours the hens were killed. In a typical experiment the total glycogen in the body was found to be 10·37 grm. so that 8·22 grm. glycogen had been deposited. By an estimation of the nitrogen excretion it was calculated that 3·397 grm. glycogen might have been derived from proteid, leaving 4·825 grm. undoubtedly derived from the dextrose (6).

Of the remaining monosaccharides, *lævulose* is an active glycogen former and probably also *galactose*,[1] although there are no observations on this sugar recorded by either of the above methods.

Of the *di-saccharides*, distinctly positive results have been obtained by the above methods with cane-sugar and maltose, but with lactose the results (on rabbits and hens at least) have been entirely negative; no glycogen formation, undoubtedly independent of proteid break-down, has been noticed by feeding with this sugar.

Of the *polysaccharides*, starch and dextrin are marked glycogen formers.

From an analysis of the foregoing results, we see that all carbohydrates which on digestion yield dextrose or lævulose are active glycogen formers. In the gastro-intestinal tract of all animals there are ferments capable of converting cane-sugar, maltose, and starches into one or other or both of these monosaccharides, and it is as such that the carbohydrate is absorbed into the blood. Lactose, however, in hens and rabbits at least, is not a glycogen former. On hydrolysis this di-saccharide yields dextrose and galactose, and dextrose we have found to be the most active of all glycogen formers. Why then does lactose not

[1] Fritz Voit has shown galactose to be completely oxidised in the organism—subcutaneous injections not appearing in the urine, as would have occurred had it not been oxidised by the tissues and therefore to be undoubtedly a glycogen former.

form glycogen? The only explanation that can be offered of this apparent anomaly is, that in the animals which have been experimented on, there exists no digestive ferment capable of hydrolysing the lactose. Such, indeed, has been shown by Ernst Weinland ([6]) to be the case. Whereas, a watery extract of the intestinal mucosa of all animals which take milk in their food (*e.g.* the young of all mammalia, and omnivorous animals all through life) can invert lactose,[1] no such ferment is present in watery extracts of the intestinal mucosa of herbivorous animals (hens and rabbits).[2]

The subcutaneous injection of cane-sugar or of lactose does not increase glycogen formation, nor can these sugars be oxidised in the organism—*i.e.* they pass unchanged into the urine when subcutaneously injected. From this we may conclude that neither of these sugars is directly transformable into glycogen, and that there is no inverting ferment in the blood capable of producing dextrose and lævulose from them. With maltose, however, different results are obtained. Intravenous injection, in moderate doses at least, is not followed by the appearance of maltose in the urine, for there is present, in blood plasma, a ferment capable of inverting maltose, and the dextrose thus formed is converted into glycogen. Of course, in the normal digestion of starch, &c., most of the maltose will have been already inverted by the maltase contained in the Succus entericus before it is absorbed, but there is reason to believe that some passes as such into the portal blood (Pavy [10]).

The only carbohydrates which the hepatic cells can directly convert into glycogen, therefore, are dextrose and lævulose, and possibly, galactose. These are also the sugars which are fermentable by yeast. The glycogen produced in each case is, chemically, the same, and is undoubtedly produced by a synthetic process, several of the monosaccharide molecules fusing together with the loss of a corresponding number of water molecules; thus:

$$\underset{(Monosacch.)}{n(C_6H_{12}O_6)} = \underset{(Polysacch.)}{(C_6H_{10}O_5)n} + nH_2O$$

[1] This action is due to the extracts containing a hydrolytic ferment called *lactase*, which acts specifically on lactose.

[2] Several workers have reported glycogen formation in dogs after feeding with lactose, but, as Pflüger points out, none of the reported cases is absolutely convincing.

So far all is plain, but a difficulty arises when we try to explain why both lævulose and dextrose should produce an identical glycogen, for dextrose is an aldose (*i.e.* it contains the group $-CHO-$), whereas lævulose is a ketose (contains the group $-CO-$), and to convert the one sugar into the other is, in the laboratory at least, by no means a simple process. There are three possible ways by which this conversion in the body could be explained: (1) that the lævulose is first of all converted into dextrose; (2) that both dextrose and lævulose are converted into some common derivative, which may possibly be a compound of sugar with proteid, and which for want of a better name may be called *active dextrose* (M. Cremer); (3) or that the dextrose and lævulose do not form any common compound until glycogen is produced, *i.e.* that each passes through a special synthesis. Against the first possibility stands the fact, as we shall see later, that in pancreatic diabetes lævulose when given in the food can form glycogen in the liver, whereas dextrose itself cannot. The two forms of sugar must, therefore, be transformed into glycogen by different processes, but whether these be entirely different, or only partially so—*e.g.* only until active dextrose is formed—remains an open question.

With regard to the *glycogen-forming powers of bodies closely allied to the hexoses*—such as hexatomic alcohols (mannit, dulcit, &c.), and the sugars which do not ferment with yeast, such as the pentoses (which contain only five carbon atoms)—there is no unequivocal evidence, obtainable by the direct method at least, that this is of any account.

An elaborate series of experiments, conducted by E. Külz (6) on rabbits and pigeons, has been considered, by most writers, to point to a number of these compounds as being glycogen producers, but Pflüger has conclusively shown that the results, on which these important conclusions are based, are inaccurate and misleading, and that, from observations *by the direct method at least, we cannot positively state that glycogen is formed from other than the fermentable sugars.*

The two most obvious objections to Külz's results are:—*firstly*, that from very small variations in the amount of glycogen—variations which might well be due to experimental error—he drew fundamental conclusions; and *secondly*, that he used, as standards for comparison, the average glycogen content of animals (pigeons, hens,

and rabbits) which had been starved for a certain time without regard to the period of year. For his standard estimations, Külz gives no dates, though he gives dates for the feeding experiments; now, it is well known that the amount of glycogen in the body varies considerably with the season of year, especially in such animals as the rabbit.

Max Cremer, by methods entirely analogous with those of Külz, and indeed using Külz's averages for starving animals as his controls (without, however, rendering his glycogen ash-free, as Külz did, or estimating the glycogen in the whole body) tried to show that pentoses are glycogen formers. In a table, No. 6, Pflüger shows that in every case in which Cramer claims a positive result, the glycogen derived from tissue proteid meanwhile broken down in the organism, could be held accountable for the recorded increase of liver glycogen, so that, if glycogen formers at all, pentoses can only be indirect ones.

A very thorough investigation of this question has been made by Frentzel (5). To clear out all the glycogen from the organism he used the method described in a footnote on p. 325. He found that *xylose*, a typical pentose, when fed to glycogen-free rabbits did not cause glycogen to be formed.[1] This might mean, however, not that xylose is incapable of being converted into glycogen, but that, as a result of the action of the strychnine, the organism had lost the power of producing glycogen. That such an explanation does not hold, was shown by the fact that when dextrose was administered to animals made glycogen-free by the same method, a considerable deposition of glycogen ensued. Xylose is, therefore, neither a direct nor a pseudo-glycogen former; it can neither produce glycogen itself nor can it become oxidised in place of other substances, such as proteids, which, if so saved, might conceivably become converted into glycogen.[2]

In another portion of this article it will be shown that, theoretically considered, a transformation of *glycerine* into dextrose is quite a simple process. When tested by biological

[1] The xylose was proved to be all absorbed, for the intestine was found to be free of it after death, and, moreover, it was found present in the urine.

[2] With *l-arabinose*, another pentose, Salkowski (6), by comparing the glycogen contents of rabbits fed with it with that of rabbits starved for six days, thought that slight glycogen formation had occurred; but, since his results are open to the same criticism as are those of Külz, his statement cannot for the present be accepted.

experiment, however, the evidence is not convincing enough to allow of a positive assertion that such a transformation takes place in the animal body; although there is some evidence to show that it possibly may. Seegen has also shown that if glycerine be added to a mush of liver there occurs a formation of glycogen. It has, however, not been shown whether the glycogen in this case is derived from the glycerine or from some of the liver constituents.

We come now to the most interesting question of all, viz. *can* **proteid** *form glycogen in the animal body?* As this question has recently been the theme of much research, it will be necessary for us to discuss it in considerable detail. It has already been pointed out that, besides the direct method (*i.e.* the power of glycogen formation), there are indirect methods (*i.e.* the behaviour of proteids when fed to diabetic animals) by which the question may be answered, and, since by the direct method only doubtful results have, so far, been obtained, we will pass over it quickly, and thus leave more space for the discussion of the results obtained by the indirect method.

Claude Bernard stated that abundant glycogen was stored in the liver when a diet of flesh was taken, and he argued from this that proteid could be transformed in the animal body into glycogen. The fact that flesh may contain as much as 1 per cent. of glycogen —a fact not sufficiently considered by Bernard—renders this observation valueless. Numerous workers have repeated Bernard's experiments, with the precaution to render the flesh as far as possible glycogen-free. A full review of these researches is given by Pflüger (6), who points out that none of them—on account of errors similar to those indicated above—is of much value in deciding this important question. Most of the earlier workers started out on the assumption that after a few days' starvation the liver was glycogen-free, and they argued that any glycogen which might be found in it, after this period, must have come from whatever food had meanwhile been given.

The inaccuracy of this premise we have already discussed.

Few of these earlier workers, moreover, worked with a proteid which was carbohydrate-free.

It is true that in several of the researches, removal of the glycogen in flesh was attempted by extraction with water, but, as Nerking has shown, such treatment only removes a small proportion

of the glycogen. In others, egg white was used, but, as we have seen, there is a considerable amount of carbohydrate in this form of proteid.

By far the most exhaustive researches on the derivation of glycogen from proteid, as tested by the direct method, are those recorded by E. Külz (6). This worker experimented on pigeons and hens. In pigeons he found that after two to four days' starvation the average amount of glycogen in the liver and muscles was 0·946 grm. per kilo body weight, the maximal amount being 1·259 grm., and that after 4–8 days' starvation the liver was glycogen-free (Brücke-Külz method); but that the muscles still contained glycogen to the extent of 0·716 per kilo body weight, the maximal amount being 1·414 grm. These averages were obtained by observations on thirty-three birds. In two cases proteid—in the form of flesh powder which had been extracted till no glycogen could be detected in it—was fed to pigeons which had been starved for three and seven days respectively. An average of 2·03 grm. glycogen per kilo body weight was found after the pigeons had been fed for fifteen and twenty-five days on the proteid. We have seen, however, that after from four to eight days' starvation there may still be 1·414 grm. glycogen per kilo body weight, so that the positive increase of glycogen in the above experiments would only amount to 0·616 grm. (2·03–1·414), which amount might well be accounted for by traces of glycogen in the flesh powder, too small indeed to give any chemical reaction, yet sufficient *in toto* to yield a considerable amount of glycogen—seeing that during the experiment 1058 grm. water- and ash-free flesh powder, representing about 5 kg. fresh flesh, were administered. Much of the flesh used, too, undoubtedly underwent slight putrefaction before all the glycogen had been extracted, and by this process much of the glycogen had doubtless become converted into dextrin, which would not be detected by the Brücke-Külz method.

The experiments on hens were conducted on the same plan. After from six to seven days' starvation the average amount of glycogen in the liver and muscles per kilo body weight was found to be 1·656 grm., the maximal amount being 1·605 grm. After feeding hens previously starved for three days with flesh powder for periods varying from eight to forty-three days, it was found that on an average 1·4 grm. glycogen per kilo body weight had been deposited in the liver and muscles. This is almost the same as the maximal amount found for starving hens, viz. 1·605.

From such results as these Pflüger remarks that we can conclude that "notwithstanding excessive flesh feeding, no deposit of glycogen had occurred."

With fibrin, serum, and egg albumin and casein similar result

were obtained by Kulz, there being, in spite of these observations, an undoubted glycogen formation.

A repetition of the work of Kulz is required, and, in doing so, the precautions will require to be more than Kulz is employed, but also that the same time of the year as the experiments to which periods are administered for, and even [illegible], at different periods of the year the amount of glycogen present in the body weight varies considerably. Kulz did not take this precaution. Great care must also be taken that the proteid administered is free of carbohydrates.

Even from the more recently recorded observations on the influence of proteid food on glycogen formation little that is definite can be stated. We will content ourselves with only two of the observations. Seegen (7) compared the amount of glycogen extracted by Pflüger's method in frogs which had received several subcutaneous injections of ten per cent. solution of pure casein (given at one time, i.e. doses) with the amount in frogs which had been starved and found that no increase of glycogen was caused by the injections — that is to say, that carbohydrate-free proteid causes no deposition of glycogen. This conclusion for frogs on casein has been confirmed by Blumenthal and Wohlgemuth (8), who also found that similar experiments with egg albumin did cause glycogen to be deposited, since, as we have seen (p. 317), this proteid contains a considerable amount of carbohydrate in its molecule.

Of course, as Bendix points out, it is scarcely allowable, as a general principle, to assume that physiological processes are the same in frogs as in warm-blooded animals; but still it must be remembered that, in the case of glycogen formation, the translation of results from the one group of animals to the other may not be so far-fetched, for both groups of animals live on proteids, fats, and carbohydrates, and cold-blooded animals are energetic glycogen formers.

Bendix (8) made several observations on dogs which he states that he tried to render glycogen-free by starvation for two days followed by four hours' work on a tread-mill. He found that by feeding such dogs with various proteids (egg albumin, casein, gelatin) for from two to five days glycogen deposition occurred. Pflüger, in reviewing these results of Bendix, sees a positive glycogen formation only after gluco-proteid had been administered;

with casein and gelatin, on the other hand, he denies that the results show any undoubted glycogen deposition.

To sum up, then, we may state that, although glycogen formation does probably occur by feeding with proteids which contain a carbohydrate group, there is no unequivocal evidence, so far, that this is the case for such proteids as casein, which contain no such group. The whole question urgently requires reinvestigation, especially since, by the indirect method, as we shall see later, the evidence undoubtedly points to sugar formation from all proteids.

Although, as we have seen, there is some doubt as to whether ingested proteids can form glycogen, it has been recently shown by Hirsch and Rolly ([13]) that the *proteids of the animal's own tissues* certainly can form it. Adopting all the precautions set forth by Pflüger, and by the employment of his method for the estimation of glycogen, these workers, after rendering animals glycogen-free by the use of strychnine, inoculated them with an attenuated culture of the bacillus Coli Communis. Fever followed. After some time the animals were killed, and their tissues were found to contain a considerable amount of glycogen. As is well known, fever induces an active break-down of the proteid tissues, and it was doubtless from the decomposition products of this that the glycogen had been formed.

As explained above (p. 325), evidence of this tissue-proteid source for glycogen is also obtained when rabbits rendered glycogen-free by strychnine are allowed to sleep under chloral for more than twelve hours.[1]

What becomes of Glycogen in the Organism?

Concerning *the fate in the organism of the glycogen stored in the liver*, there are two so-called theories, the one by Bernard ([1]), the other by Pavy ([10]). Bernard's theory ascribes to glycogen a function in animal life analogous with that of starch in plant life, viz.

[1] In a series of investigations carried out on the same plan as that detailed above for proteids, E. Külz has found urea to increase the glycogen contents of the liver in hens and rabbits. Other workers (Rohmann, Nebelthau, &c.) have recorded similar results with ammonium carbonate, glycin, asparagin, &c. In most of these researches, the liver glycogen alone was estimated, which renders the results of little value, and in those in which the glycogen in the entire body was determined, the differences were so slight that nothing definite can be stated from them.

that of a reserve carbohydrate. The sugar in the portal blood which is in excess of that in the systemic blood (*i.e.* above 0·2 per cent.) is converted into glycogen in the liver, and whenever the amount of sugar in the systemic blood tends to fall below its normal percentage, the liver glycogen is, according to this theory, reconverted into sugar, which is liberated into the blood of the hepatic vein, and so keeps up the average percentage of sugar in the systemic blood. At first, Bernard thought that the glycogen was derived from proteids and not from carbohydrates.

Pavy admits that glycogen is derived from excess of sugar in the portal blood, but thinks that this glycogen never again, under normal conditions, becomes transformed into sugar, but is built up into non-carbohydrate substances, viz. fat and proteid, which then undergo metabolism along their own lines.

We will discuss Bernard's theory first. By this theory, it is easy to explain why the percentage of sugar in the systemic blood should be practically constant, although there are considerable variations in the sugar content of the portal blood. The amount of sugar in the body depends mainly on two factors: firstly, on the supply of sugar from the intestine, and secondly, on the rate of oxidation of sugar in the tissues. The second factor is a fairly constant one—varying, of course, somewhat with the activity of the muscles—but the first factor varies enormously according to whether digestion be in progress or not. The balance of action of these two factors would therefore tend to cause, during the absorption of food, an overplus of sugar in the systemic blood, and during starvation, a deficit. We have, however, seen that whenever the percentage of sugar in the systemic blood rises distinctly above 0·2 sugar appears in the urine — a condition of glycosuria is established; but glycosuria practically never occurs under normal conditions; and, on the other hand, the systemic blood always contains between 0·05 and 0·2 per cent. of sugar, even during starvation. To explain this, Bernard offers the above theory.

The most important facts which Bernard brought forward in support of his theory are as follows:—

Firstly, the percentage of sugar in the liver is higher than in any other organ or tissue.

Secondly, after death, the glycogen in the liver gradually dis-

appears and sugar takes its place. This is the result of the action of a ferment in the hepatic cells, which goes on acting after death just as it does during life.

Thirdly, the percentage of sugar in the blood of the hepatic vein is greater than that of the blood in the portal vein when no absorption of food from the intestine is in progress.

Fourthly, the blood going to the muscles (arterial) contains more sugar than the blood coming from the muscles (venous).

If, to these facts, it be added that during muscular work glycogen disappears first of all from the liver and only after some time from the muscles (see p. 324), and that the difference in sugar contents between venous and arterial blood increases during muscular work,[1] then Bernard's theory would seem to be established, for it is inconceivable that when the muscles require more glycogen—as they do when they become active—the liver should proceed to convert its glycogen into some non-carbohydrate material, such as fat, instead of transferring it as sugar to the muscles.

Now, let us see on what grounds Pavy objects to Bernard's theory. In doing this we will take up Bernard's proofs in the order given above.

Firstly, the percentage of sugar in the liver is no higher than that of other organs and tissues if the estimation be made immediately after the death of the animal and precautions be taken—by freezing the organ—to stop at once the action of the glycolytic ferment contained in the blood included in the blood-vessels of the liver (see, however, p. 324).

Secondly, the accumulation of sugar and the disappearance of glycogen which occurs in the liver after death is purely a *post-mortem* phenomenon, there being in the liver cells during life no glycolytic ferment.

Thirdly, the higher percentage of sugar in the blood of the hepatic vein over that in the portal vein is due to the stimulation of sensory nerves, which necessarily follows from the method used to collect the blood for analysis. This method, as practised by Seegen, consisted in passing a gum elastic catheter down the superior vena cava to opposite the entrance of the hepatic veins in animals which were not anæsthetised. That the irritation

[1] There is, however, some doubt regarding the accuracy of this result; see Pavy, "The Physiology of the Carbohydrates" (10).

of sensory nerves does cause increased production of sugar from the liver is an undoubted fact.

Fourthly, as a result of a large number of carefully conducted analyses, Pavy concludes that the amount of sugar in venous and arterial blood is practically the same. The average of his analyses for arterial blood is 0·941 grm. and for venous blood 0·938 grm. per 1000 grm. blood.

The third and fourth "proofs," we see, depend on the quantitative analysis of the blood for sugar, a process which is, technically, very difficult and is subject to a considerable experimental error. Moreover, even if between meals the liver should add dextrose to the blood of the hepatic vein, the percentage increase of sugar thus created could be only very slight, on account of the enormous amount of blood which passes through the organ. In his criticism of Bernard's second proof, Pavy takes it as certain that the hepatic cells contain no active diastatic ferment during life. That this is incorrect has been definitely shown by Pick.[1]

The balance of evidence stands in favour of Bernard's theory.

The transformation of the glycogen of the liver into sugar proceeds very rapidly immediately after death, and then slows down, so that even after several days, there still remains some unchanged glycogen. Thus Pavy states that the living normal liver contains from 0·1 to 0·4 per cent of sugar: a few minutes after death this rises to from 1·2 to 1·5 per cent., and in about twenty-four hours it amounts to from 2 to 3·5 per cent.

Such a result would at first sight seem to point to some vital process—which, gradually dying, causes the falling off in the production of sugar. In speaking of the cause of the transformation as a "vital process," we mean a living state of the protoplasm of the cells; some workers hold to this view.[2] Other

[1] In support of his theory Pavy gives histological evidence of the conversion of glycogen into fat, not only in the liver but also in the intestine. He considers the intestinal epithelium the first barrier against the passage of sugar into the blood; what escapes this retention filter (*sic*) being caught up by the liver.

[2] It should, however, be pointed out that Pavy himself does not believe in the "vital process" theory, but thinks this *post-mortem* sugar production to be entirely due to some ferment—contained in the blood—which comes to act on the liver glycogen after death.

workers (and they include the majority), ascribe the hydrolysis of glycogen to a ferment produced by the hepatic cells. In support of this latter view stand the following observations: ferments can act in weak chloroform water or in a 0·2 per cent. solution of sodium fluoride, whereas in these antiseptics all vital action is stopped; now pieces of liver when minced and placed in either of these solutions go on actively transforming glycogen into sugar. A piece of liver, washed free of all traces of sugar and blood serum through its blood-vessels, hardened in alcohol, and then triturated with glycerine in a mortar yields an extract with marked glycogenetic powers.

There can be no reasonable doubt that the process depends on a ferment. Even if we do state that it is due to the so-called vital activity of the hepatic cells, we can mean nothing more than that it is a ferment process occurring in the living protoplasm of the hepatic cells, instead of at the glycogen depôts themselves: that a zymase, or intracellular ferment, acts and not a extracellular ferment.[1]

A similar ferment, *i.e.* one producing dextrose from glycogen, is contained in the blood. This may possibly owe its origin to the hepatic cells, having escaped from these into the blood.

The presence of this ferment actually present in the liver cells is a strong argument in favour of Bernard's theory.

Accepting Bernard's explanation regarding the fate of glycogen in the organism as the more probable one, we must next consider where, and by what agency, the dextrose, thrown into the blood by the hydrolysis of glycogen, is used up. We must, in other words, investigate the cause of *glycolysis*.

It cannot be in the blood itself that this process takes

[1] This is Dastre's conception of the process (⁶). He considers that there exists in the liver cells an endoenzyme capable of converting glycogen into maltose, just as there exists in yeast an endoenzyme which inverts cane-sugar. This endoenzyme, during life, is firmly held in the hepatic cells, but can be separated from them by various methods, *e.g.* by acting on them with chloroform water or 0·2 per cent. of sodium fluoride in physiological saline. F. Pick (¹⁴) (Hofmeister's *Beitr.*, iii. 163, 1902) confirms this view of Dastre, and shows further that by boiling an extract of liver, made by extracting an alcoholic precipitate of liver with 0·8 per cent. NaCl solution containing 0·2 per cent. sodium fluoride, the diastatic action is lost, and, further, that the diastatic action of such an extract is stronger than that of blood, showing that the ferment is not derived from this source, but is a product of the hepatic cells. This important observation is in direct antagonism to Pavy's view.

place, for, after drawn blood has stood for about an hour in the incubator, there is only a small reduction in its sugar contents, a glycolysis far too feeble to account for the rapid and extensive sugar destruction which undoubtedly occurs in the body. The same is true for blood kept in a doubly ligatured blood-vessel (living test-tube); its percentage of sugar remains constant.

Having obtained some evidence that it is in the muscles that the dextrose is used up—for on examining the blood coming from a muscle *in situ*, especially an active one, there is, according to most authorities, distinctly less dextrose than in the arterial blood going to it—we would expect an extract of muscle, or its expressed tissue juice, to possess a distinct glycolytic power, which, however, is not the case.

As we shall see later, when the pancreas is completely excised, dextrose ceases to be oxidised in the organism so that it accumulates in the blood and overflows into the urine, diabetes being the result. This would lead us to expect that the pancreas must normally furnish something to the organism which can produce glycolysis. When, however, we try to demonstrate the presence of this substance in pancreatic extract, or in the expressed juice of the pancreas, it is impossible to obtain any positive result. In all these cases a slight decomposition of dextrose may ensue, which, if not the result of bacterial growth, is probably due to the action of the oxidase or oxidising ferments universally present in such extracts.

A satisfactory explanation of these seemingly contradictory results has recently been furnished by Cohnheim[15], who has found that if the expressed tissue juice of a muscle be mixed with the expressed tissue juice of the pancreas, a mixture is obtained which has great glycolytic power. Evidently, then, muscle produces a ferment, itself incapable of decomposing dextrose, but which, when acted upon by an internal ferment derived from the pancreas, becomes activated so that it quickly decomposes the dextrose.

This mechanism is analogous with that which under certain conditions exists in the intestine in connection with the action of the proteolytic ferments of the pancreatic juice; until this secretion reaches the intestine it cannot digest proteid—it contains only the precursor of trypsin, viz. trypsinogen—but when it mixes with

the succus entericus, a body which this latter secretion contains—enterokinase—renders the proteolytic ferment active, that is to say, converts it into trypsin. It is likewise comparable with hæmolysis by hæmolytic serum, where two bodies, a complement and an amboceptor, are necessary for the process—and with all analogous processes. It satisfactorily explains why excision of the pancreas should inhibit all glycolysis of dextrose in the organism, and how it is that muscular venous blood should contain less dextrose than its arterial.

This work of Cohnheim has in general been confirmed by subsequent workers ([16]). Not only the muscle but other organs, such as the liver, possess the ferments which the pancreatic zymase activates. When abundance of oxygen is present, the end products of this glycolysis are carbon dioxide gas and water, whereas, when it occurs anaerobically, at first alcohol, and later lactic acid are formed, and later still oxy-butyric acid. Each of these stages is really produced by a different ferment, the alcohol-producing ferment acting first, then the lactic acid ferment, and lastly the butyric acid ferment. It has been suggested that it may be to a suppression of the alcoholic and lactic acid ferments and an excessive action of the oxy-butyric acid ferment, that the presence of the latter acid in Diabetes mellitus is due (Stoklasa [16]).

The conditions which, apart from food and muscular exercise, influence the amount of glycogen in the liver.

Of these the most important are the various forms of **experimental diabetes**.

The first form of experimental diabetes to be discovered was **puncture diabetes.**[1] If the floor of the fourth ventricle of the brain be punctured, sugar shortly afterwards appears in the urine. In rabbits, in which this form of experimental diabetes has been very extensively studied, the point of puncture is bounded above by a line joining the roots of origin of the nervi acustici, and below, by one joining that of the vagi. The puncture is made, by means of a strong steel pencil, in the median line of the occipital bone in front of the occipital protuberance. The puncture is continued till the Pars basilaris is reached, when the instrument is withdrawn. The cerebellum is injured by the puncture but not seriously (Bernard [1]).

[1] *Piqûre.*

In from one to two hours after the puncture, or even sooner, sugar appears in the urine. In the rabbit, the glycosuria disappears after five or six hours, seldom lasting twenty-four. In the dog, on the other hand, it may last as long as seven days. If the animal be killed and the liver examined after the sugar has disappeared from the urine no glycogen, or only a trace of it, will be found. On the other hand, if the animal be starved for several days previous to the puncture—and the store of glycogen be thereby greatly reduced—no glycosuria will follow the puncture.

To illustrate these statements, the following table of results, obtained by Dolley and the author, may be of interest.

TABLE II

Number.	Weight of Rabbit.	Time of Puncture.	Time of Urine Test.	Per Cent. Sugar.	Remarks.
1	1·5 kg.	11.30 A.M.	4 P.M.	2·22	
2	,,	11.45 ,,	1 ,,	1·33	
3	,,	9.45 ,,	3.30 ,,	none	Rabbit previously starved
4	1·8 kg.	10.55 ,,	4 ,,	large amount	
5	1·9 kg.	9.45 ,,	1 ,,	8·33	
6	2·25 kg.	12.50 P.M.	3.45 ,,	3·7	

The percentage of sugar in the blood during the presence of the glycosuria is considerably above the normal. All these facts point to the glycosuria being due to the too rapid transformation of glycogen into sugar. If we accept Bernard's theory, we can state that hyperglycogenesis has occurred; if Pavy's, we must assume that, instead of being transformed into fat and proteid, the glycogen has become hydrolysed into dextrose: in both cases hyperglycæmia would be caused.

It is interesting to note here that tumours in various parts of the central nervous system in man have frequently been found, *post-mortem*, where glycosuria had been one of the symptoms before death.

The puncture of the medulla acts by producing irritation of the diabetic centre, and not, as might be imagined, by its destruction. The short duration of the glycosuria, as well as the fact that if the animal be kept under anæsthesia no glycosuria occurs on

puncture, is evidence of this. Moreover, the centre can be excited by the stimulation of *afferent* nerve fibres. It is a true reflex centre. The afferent fibres run in most of the sensory nerves, and have been exhaustively studied in the vagus nerve of the rabbit, especially by C. Eckhard ([17]). This worker found that by merely cutting one vagus in the neck, a transitory glycosuria lasting a few hours was produced. After the urine had become sugar-free, the central end of the cut nerve was stimulated electrically, off and on, for three-quarters of an hour, whereby the urine became glycosuric. The wound was then closed and the rabbit placed back in its cage. Next morning, the urine had become sugar-free. The nerve was again stimulated and sugar again appeared in the urine, and so on for several days. This experiment was successful only on well-fed animals. The glycosuria which appeared on merely cutting the nerve had doubtless been due to irritation. Cutting the sciatic nerve and irritation of the cardiac depressor nerve in the rabbit similarly tends to induce glycosuria.

Several clinical cases are reported where severe neuralgias (of facial and sciatic nerves) and the pressure of tumours on nerves (vagus) existed in patients exhibiting glycosuria.

The following table of observations by Dolley and the author illustrates this form of experimental glycosuria.

TABLE III

No.	Animal.	Nerve Stimulated.	Length of Time after Nerve Stimulated before Sugar found in Urine.	Per Cent. Sugar (Pavy's Method).
1	Dog	Vagus	23 minutes	trace
2	„	„	45 „	1·6
3	„	„	45 „	1·53
4	„	„	10 „	1·72
5	Rabbit	„	30 „	large amount
6	„	Cardiac depressor	15 „	trace
7	„	„	23 „	1·5
8	„	„	48 „	0·46

So far, then, we see that there must be a centre in the medulla, stimulation of which causes an increased production of sugar in the organism, with a consequent hyperglycæmia and glycosuria;

and, furthermore that this centre can be excited through several sensory nerves. It remains for us to ascertain by what nerve path the *efferent impulses* from this centre are transmitted, and if it be the hepatic glycogen alone which is transformed into sugar when the centre is stimulated, or whether glycogen stored elsewhere in the organism may not also contribute.

If the splanchnic nerves be cut just after their entry into the abdomen, or the upper three thoracic nerve roots torn out, or the spinal cord cut across above the first thoracic root, and the diabetic centre be then punctured, no diabetes will be found to result (Marc Laffont,[6] Eckhard[17]). On the other hand, if the spinal cord be irritated opposite the brachial swelling (Pavy[6]), or if the posterior columns alone be cut (Schiff[18]), or if the lower cervical and upper thoracic sympathetic ganglia be cut, a more or less marked glycosuria will result independent of puncturing the fourth ventricle. The cutting, in these experiments, doubtless acts as an irritation.

All these experiments are usually taken to show that the efferent path of the impulses from the glycosuric centre is by the spinal cord as far as the upper thoracic region, then by the upper thoracic spinal roots and rami communicantes into the lower cervical and upper thoracic sympathetic ganglia, and then by the splanchnic nerves to the liver. It is also concluded that it can be the hepatic glycogen alone which is influenced by the glycosuric centre, since, were the glycogen in the muscles, &c., also under its control, glycosuria after puncture should not be inhibited by cutting the splanchnics or upper thoracic nerve roots.

As stated above, irritation of the cervical spinal cord, or of the upper thoracic and lower cervical sympathetic ganglia, is followed by glycosuria; if, however, the splanchnic nerve be irritated (electrically stimulated), *no* glycosuria is induced. This remarkable result would seem to indicate that in passing through the sympathetic ganglia, the glycosuria-producing impulses had undergone some change; stimulation of the pre-ganglionic nerve fibre producing glycosuria, which, however, is not produced by stimulating the post-ganglionic.

As is well known, Langley has shown that nicotin paralyses the synapses of pre-ganglionic fibres in the sympathetic ganglia; that it institutes a block so that impulses can no longer pass through the ganglia. Wishing, therefore, to see whether the glycosuria-pro-

ducing impulses—resulting from puncture—could be blocked by nicotin, we injected the drug subcutaneously and then punctured the medulla, with the results depicted in the following table.

TABLE IV

No.	Dose of Nicotin per kg. Body Weight Injected.	Time of Injection.	Time of Puncture.	Time of Urine Test.	Per Cent. Sugar.	Remarks.
1	8 mg. (intravenous)	9.10 A.M.	immed. / after	4 P.M.	0·625%	*Osazone crystals fermented
2	9 „ (subcutaneous)	11 A.M.	10.30 A.M.	3 P.M.	0·5	Convulsions
3	7 „ „	12.15 P.M.	immed. / after	3.30 P.M.	none	Carrot-fed
4	7 „ „	1 P.M.	„	3.30 P.M.	none	—
5	7 „ „	11.15 A.M.	„	12.30 P.M. 1.30 P.M.	trace none	Convulsions
6	8 „ „	8.36 A.M.	after / 4 mins.	10.50 A.M.	trace	Convulsions Carrot-fed
7	7 „ „	8.32 A.M.	after / 4 mins.	10.40 A.M.	none	Convulsions Carrot-fed

It will be seen that the nicotin had undoubtedly prevented the usual effect of puncture, possibly by its having instituted a block in the sympathetic ganglia.

All these experiments, however, may not have such a simple interpretation, for in all of them in which it was found that glycosuria did not appear on puncture, there must have been coincidentally established, by the experimental procedure, a condition of extreme splanchnic vasodilatation, and a consequent fall of blood pressure, which alone, as we have shown, is sufficient to cause the glycosuria produced by vagus stimulation in dogs to disappear, or, at least, to become very much less marked.

For example, if the central end of the vagi be stimulated until the urine becomes strongly saccharine, and the dog be then gradually bled from its femoral artery until the blood pressure has fallen to almost one-third its normal level, it will be found that the sugar disappears from the urine or diminishes markedly in amount; or, conversely, if the dog be first of all bled until its blood pressure has considerably fallen and the central end of the vagi then stimulated, no glycosuria will be induced. The glycosuria we have found likewise to disappear when the blood pressure has been caused to fall from other causes than bleeding, such as by opening the thorax.

As to *the exact nature of the glycosuria-producing impulses* we know very little. They may merely be vaso-motor and cause dilatation of the hepatic vessels whereby an increased sugar production is induced (Bernard); or it may be that there are in the splanchnics true secretory fibres concerned in the control of the ferment or zymase production in the hepatic cells.

We have already seen that stimulation of the central ends either of the vagus or cardiac depressor causes glycosuria. In the case of the vagus, a rise, and in the case of the cardiac depressor, a fall of abdominal blood pressure will result, that is, opposite changes in blood pressure and still the same effect on sugar production, which would seem to show that there may be secretory fibres quite independent of those controlling blood pressure. On the other hand, it is a well-established fact that atropin paralyses all true secretory nerve terminations, whereas it does not have any distinct influence on the glycosuria produced either by puncture of the fourth ventricle or by stimulation of the central ends of sensory nerves (Dolley and Macleod)

Pflüger (6) offers an ingenious speculation regarding the rôle in the animal economy of the reflex control of sugar production in the liver. Sugar is the most available food-stuff for muscular contraction, so that when a muscle contracts it uses up some sugar; at the same time, however, by compression of the muscle spindles afferent nervous impulses are set up which are carried up to the diabetic centre, and so lead to the liberation of more sugar from the liver. The heart is the most active muscle in the body, and consequently requires most sugar; its afferent fibres to the diabetic centre—carried in the vagus—are therefore the most active of all.

In connection with puncture diabetes ought to be mentioned certain experimental conditions—such as the inhalation of carbon monoxide, asphyxia, extensive hemorrhage itself, the administration of morphia, curare, strychnine, acids, &c., which are not infrequently followed by glycosuria. What the exact cause of the glycosuria in these cases may be—whether it is by some irritation of the so-called diabetic centre, either directly or through afferent nerves, or whether it is some direct action of these substances on the hepatic cells leading to a too rapid transformation of glycogen

into sugar—cannot at present be considered. In many of these cases, the glycosuria is only slight, and in few of them has the disappearance of hepatic glycogen, so characteristic of piqûre, been described.[1]

Perhaps the most interesting of all these forms of glycosuria is that produced by the intravenous injection of solutions of sodium salts. For example of $\frac{m}{6}$ 100 c.c. of $\frac{m}{6}$ NaCl[2] every fifteen minutes into a rabbit. The glycosuria thus induced can be cut short by injecting solutions of calcium salts (*vide* [40]). Solutions of calcium salts themselves do not cause glycosuria. The action of the sodium is probably on the diabetic centre, and not, as has been supposed, on the renal cells, rendering them more pervious to dextrose.

It is probable, however, that where a mild glycosuria exists, the sugar may be made to disappear from the urine by causing the excretion of urine to diminish.[3] Thus, it has been stated by Walter that atropin diminishes urine excretion, and that, if it be administered to animals rendered mildly glycosuric by drugs, it causes a disappearance of sugar in the urine. It may be that it is to depressed excretion of urine that is due the disappearance of sugar from the urine which follows a considerable fall in blood-pressure in dogs rendered glycosuric by vagal stimulation.

Amongst the most distressing symptoms of Diabetes mellitus in man are great thirst and polyuria. J. P. Sawyer has shown that, in many cases of this disease careful treatment of the stomach (*e.g.* by lavage) greatly diminishes the thirst, that the polyuria almost disappears, and that the sugar excreted by the kidneys becomes very much less in amount. It is just possible, in these cases, that the glycosuria lessens in amount as a consequence of the diminished excretion of urine; and that, less carbohydrate being drained out of the blood, much of it, which would otherwise have been excreted, becomes utilised in metabolism, and thus spares the excessive proteid break-down so characteristic of this disease.

[1] *Cf.* Bock and Hoffman, however ([41]).

[2] $\frac{m}{6}$ means a sixth normal solution, *i.e.* the molecular weight in grm. divided by six dissolved in 1000 c.c. water.

[3] Conversly, by administering diuretics to glycosuric animals, the amount of sugar eliminated is increased.

Pancreatic Diabetes.—On 22nd May 1889 von Mering and Minkowski[1] made the announcement that total extirpation of the pancreas in dogs was followed by severe diabetes, which persisted until the death of the animal. Previous to this, pathologists had noticed that in severe forms of Diabetes mellitus in man morbid changes in the pancreas were not uncommon.

In a later publication, in 1893, Minkowski[2] gives in detail a description of the operation for the excision of the gland, and an account of his investigation on dogs and other animals thus rendered diabetic. Briefly, the most important of these results are as follows.

Total extirpation of the pancreas is followed by diabetes in dogs, cats, pigs, and frogs; if any trace of pancreatic tissue be left, however, no glycosuria, or only a mild form of it, results.[2] Extirpation of any other gland or organ than the pancreas does not produce diabetes, although the operative interference may cause temporary glycosuria.

In rabbits, total extirpation of the gland is technically impossible on account of the glandular tissue being spread out diffusely between the layers of the mesentery. In herbivorous birds glycosuria only occasionally follows pancreatic extirpation, whereas in carnivorous birds it always occurs.

The most important observations have been made on *dogs*. A few hours after the extirpation, in these animals, sugar appears in the urine. In twenty-four hours this usually attains a percentage of about one, and it markedly increases during the second day (3–4 per cent.), to attain on the third day a maximum percentage of from 8–10, after which, if no food be given, a gradual fall occurs. If, instead of merely ascertaining the percentage, the total sugar excretion of twenty-four hours be estimated, it will be found that during starvation, after the preliminary rise described above, a constant level is attained, and, if the amount of dextrose excretion be compared with that of nitrogen—in other words, if the *ratio* of dextrose to nitrogen D : N be determined—a constant

[1] Dominicis almost simultaneously made the same discovery.

[2] In this connection it is interesting to note that where the whole gland has been successfully removed the abdominal wound, despite all surgical care, suppurates, and if it heals at all, does so only by second intention, whereas, if some of the gland be left, and no diabetes, or only a mild form of it be thus established, primary union is more likely to follow. The diabetic state so lessens the resistance of the tissues that infection readily occurs.

value will be obtained. This ratio was found by Minkowski to be between 2·61 and 2·94 in observations on three starved depancreated dogs of different sizes. Practically the same ratio is found when flesh alone or any other form of proteid food (plasmon, casein, &c.) is given, the average ratio for a large number of observations being 3 : 1, and varying between 2·62 and 3·05 : 1.

Where traces of pancreatic tissue are left, as was the case in an experiment conducted by Cobb and the author, the glycosuria is not so marked and almost disappears if no food be given. As the results of this observation show some interesting points, which we will have occasion to refer to later, we give them here in tabular form.

TABLE V

DATE. (Pancreas removed on 13th February at 11 A.M.)	DIET.	NITROGEN IN URINE. Per Cent.	NITROGEN IN URINE. Total Amount in Grm. per Diem.	SUGAR IN URINE. Estimated by Reduction in per Cent. (Pavy's Method).	SUGAR IN URINE. Estimated by Polariscope in per Cent.	SUGAR IN URINE. Total Amount (by Reduction) in Grm. per Diem.	D : N RATIO.
February							
14–15	none	2·42	13·08	4·81	4·87	25·97	1·99
15–16	,,	2·18	10·19	5·10	5·01	20·91	2·06
16–17	,,	2·67	8·00	5·88	5·69	17·64	2·20
17–18	,,	1·91	7·12	4·07	3·92	15·14	2·13
18–20	,,	1·43	7·035	2·82	2·70	13·86	1·97
20–21	,,	2·00	6·17	1·39	—	4·28	0·69
21–23	,,	1·53	3·83	0·18	—	1·38	0·31
23–24	,,	1·02	3·25	0·21	—	0·77	0·24
24–25	(200 grm. flesh)	1·50	1·45	1·22	0·88	3·61	0·81
25–26	,,	3·10	5·02	3·76	—	6·09	1·21
26–27	,,	2·32	—	1·14	—	—	—
27–28	,,	2·20	5·57	2·41	—	6·10	1·10
28–1 Mar.	,,	2·26	5·49	2·19	1·68	5·32	0·97
March							
1–2	,,	2·12	4·93	2·43	2·67	5·66	1·15
2–3	,,	4·02	6·15	2·63	—	4·02	0·65
3–4	,,	2·62	1·83	0·46	—	0·32	0·18
4–6	,,	1·35	1·21	0·00	—	0·00	—

When, however, carbohydrates are included in the diet the sugar excretion is raised, but not that of the nitrogen—indeed by their proteid-sparing influence these food-stuffs may cause the

excretion of nitrogen to fall—and the *D : N* ratio may become 7 or even higher.

All the other symptoms of severe diabetes are also produced by the extirpation. We have noticed that the desire for water is especially marked where some pancreatic tissue has been left, and the D : N ratio is therefore less than 3.

The preliminary rise in the sugar excretion is no doubt due to the elimination from the organism of stores of sugar or sugar-yielding substance derived from the previous diet. Thus it was noticed by Minkowski that the height of the sugar excretion about the third day after the operation was greater in dogs which had been well fed previous to the operation than in those which had been poorly fed. At first sight it may be difficult to imagine how, if this explanation of the preliminary rise be correct, the maximal excretion should not exist on the first day after the excision of the gland; if, however, it be remembered that it is from the withdrawal of an internal secretion—which normally destroys sugars in the organism—that the diabetes results (see p. 339), the apparent anomaly disappears, for an excess of this secretion might be present in the blood when the gland is removed, so that several days might elapse before it was all used up. In support of this explanation, may be quoted an observation of Minkowski's, that in one dog it was not until three days after the excision of the gland that any sugar at all appeared in the urine, and the gland on excision was found to be hyperæmic and apparently very active. We also found very little sugar in the urine the day after the removal (incomplete) of the gland in one dog, with a very large excretion next day.

With regard to the *source of the sugar* in pancreatic diabetes, there can be no doubt that at first it comes from stored-up carbohydrate. If, a few days after the operation, the animal be killed by bleeding and an examination of the liver for glycogen immediately made, only traces will be found, and this is so not only when, previous to death, no food had been given, but also after the liberal ingestion of food. For example, we could find no trace of glycogen (Pflüger's method) or of dextrose in the liver of the above-mentioned depancreated dog, which had been fed with 200 grm. of flesh each day for several days before death. Minkowski has noted the same thing for dextrose; *i.e.* it does not cause glycogen to be deposited. The

only substance, in fact, which does induce glycogen formation in depancreated dogs is *lævulose*. This interesting point we will discuss later. *The sugar must, therefore, first of all be derived from glycogen.*

As we see, however, this soon disappears from the organism after removal of the pancreas, and cannot be the only source of the sugar. In a recently published experiment (by Lüthje[20]), a dog of 5·8 kg., after removal of the pancreas, excreted during twenty-five days a total of 1176 grm. dextrose; during all this time a carbohydrate-free diet was given. Assuming that on an average a dog's body contains 40 grm. glycogen per kilo body weight—an average determined by Pflüger—then 232 grm. glycogen (5·8 × 40) might be held accountable for the sugar; but this could yield only 257 grm. dextrose, leaving 919 grm. to be otherwise accounted for.

Where does it come from, then, when all the available glycogen has been used up? The possible sources are proteid and fat. The fact that the D:N ratio is the same during starvation and proteid feeding would seem to point to the derivation of both sugar and nitrogen from a common source, *i.e.* from proteid. We have already seen how, chemically considered, such a derivation is possible. The extreme emaciation of the dogs which supervenes, and the presence of β-oxybutyric acid, &c., in the urine, is usually considered to support this view.

To explain the constant ratio of *D:N*, if proteid be the source of the sugar, we must assume further that all the sugar liberated in the tissues from proteid or a constant fraction of it reappears as such in the urine. Supposing that all the carbon of proteid were converted into sugar, then 100 grm. proteid could yield 113 grm. grape-sugar, and a D:N ratio of about 7 would be obtained. We have seen, however, that it is only 3. Does this mean that all the carbon of proteid is not converted into sugar, or that all is thus converted, but that some of it is afterwards destroyed? To answer this question, let us see what proportion of dextrose when fed to depancreated dogs reappears in the urine: for if all, or nearly all, the administered sugar reappears, then we may assume that the same will hold true for the dextrose split off from proteid, and that the sugar which is excreted in the urine on a carbohydrate-free diet represents all

of what has been liberated from proteid in the organism. Minkowski, to answer this question, fed depancreated dogs with different amounts of dextrose, and estimated how much of this reappeared in the urine. He concluded from his results that when moderate amounts of dextrose are ingested it all reappears in the urine.[1]

It should, however, be pointed out that in the dogs to which dextrose was given the nitrogenous excretion was usually distinctly below its previous level, which would tend to indicate, either a proteid-sparing action of the dextrose—presumably, therefore, its partial oxidation—or, that the absorbed dextrose in such cases never really enters the tissue cells, but, by still further raising the percentage of sugar in the blood, tends to prevent diffusion of the sugar, which the cells themselves produce from proteid, into the blood—to tend, as it were, to overcrowd the cells with sugar produced by their own metabolism—and hence to lessen the activity of their proteid break-down. An explanation along this line is offered by M. Cremer. By some such process all the administered dextrose would reappear in the urine without its coming into actual contact with the tissue cells.

It is generally concluded by other workers, however, that a small proportion of the dextrose-destroying power of the organism still remains in depancreated dogs, *i.e.* that all the administered dextrose does not reappear in the urine, although the greater part of it does. This being so, it would appear probable that sugar of proteid origin would behave similarly. Why, then, is a D : N ratio of about 7 not obtained? The only answer possible is that all the carbon of proteid is not available to form sugar (as is assumed in the above calculation), but only a portion of it.

How do other carbohydrates behave when given with the food?

Starch is very imperfectly digested in the absence of the pancreatic juice, so that a large proportion of it reappears unchanged in the fæces, and, of what does not thus reappear, much becomes destroyed in the intestines by putrefactive

[1] When large amounts were given, intestinal disturbance (diarrhœa, &c.) occurred, and when small amounts were given it was difficult to measure the increase on account of the normal variations in the sugar excretion in depancreated dogs.

bacteria—for the half-digested starch lies several days in the intestines—leaving only a small proportion to be absorbed as dextrose and pass into the urine. Dextrose is the only sugar which thus appears.

With *maltose* also, only dextrose appears in the urine.

The most interesting results in this direction are with *lævulose*, for, as Külz has shown, this sugar is often oxidisable in the tissues of patients suffering from Diabetes mellitus, whereas dextrose and dextrose-yielding carbohydrates are not. If large quantities of lævulose be given to depancreated dogs on constant diet—and therefore with a constant D:N ratio—a rise in the *dextrose* excretion will be noticed, and a *small amount* of lævulose itself will appear in the urine. If only small amounts of lævulose be given no lævulose will appear in the urine, and the increase in dextrose excretion will be very slight, since a large part of the lævulose becomes utilised in metabolism. It is only when excess is given that some of the lævulose leaks as such through the kidney filter or becomes converted into dextrose, which, as we have seen above, cannot be destroyed, and is likewise excreted.

The dextrose, however, might possibly be derived from proteid, the lævulose having induced a more active break-down of this. That it is not so derived, but comes from the lævulose itself, is proved by the fact that the *D:N* ratio has in some cases been seen to rise to 11 or 13·5, a figure far above what could be obtained were proteids the source of the sugar.

How then is this transformation of lævulose into dextrose brought about? Lævulose, it will be remembered, is, in its chemical structure, quite different from dextrose; it is a ketose, whereas dextrose is an aldose.

An examination of the glycogen contents of the liver and muscles furnishes us with an answer to this question. After feeding lævulose to depancreated dogs, a high percentage of glycogen (8·14 in the liver and 0·81 in the muscles) has been found, and in its chemical reactions, this glycogen is indistinguishable from normal glycogen. From this result we must assume that, in depancreated dogs at least, a direct transformation of lævulose into glycogen occurs. This transformation is not effected by the lævulose being first of all changed into dextrose; for we have found dextrose to form no glycogen under such

conditions. The glycogen thus formed produces dextrose, the trace of lævulose which coincidently appears in the urine representing some which has leaked through the kidney filter before being transformed into glycogen.

With *inulin*, a polysaccharide yielding on hydrolysis lævulose only, somewhat similar results are obtained; and with *cane-sugar*, a disaccharide composed of dextrose and lævulose, the dextrose excretion is raised to an amount corresponding to somewhat more than half of the administered cane-sugar—all the dextrose and a part of the lævulose reappearing as dextrose in the urine.

Lactose, which on hydrolysis yields dextrose and galactose, causes also a considerable increase in the dextrose excretion, but it is difficult—on account of this sugar very readily undergoing fermentation in the intestine—to determine quantitatively just exactly how much does thus appear. So far there seems little doubt that not only does all the dextrose of the lactose reappear but also a certain amount which must come from galactose.

We must conclude, therefore, *that the sugar in the urine comes from the dextrose given in the food and from the proteid both of the food and of the tissues.*

In about four weeks after the extirpation of the gland the animal dies. As the condition proceeds, and whether food be given or not, the animal becomes extremely emaciated, and, when at last it is too weak to move about, the excretion of sugar begins to fall and may indeed disappear for a few days before death. The excretion of nitrogen may also fall, although not to so marked a degree as does that of dextrose. Can this disappearance of dextrose indicate that the organism has reacquired the power of oxidising dextrose? According to Minkowski, if dextrose be added to the food during this period it will almost all reappear in the urine, so that a reacquirement by the organism of its lost glycolytic powers cannot be the cause of the disappearance of the sugar.

On the other hand, Luthje and Cobb and the writer have found that the sugar may disappear from the urine much earlier than Minkowski states, provided the dog be starved. In our experiment it had practically disappeared in nine days after the

extirpation,[1] but immediately reappeared on feeding with flesh, to greatly diminish again just before death, even although flesh was still given (*vide* table). Minkowski seems to have considered that starvation and flesh feeding are analogous conditions; this, however, does not seem to be the case, sugar being more readily produced from flesh than from tissue proteid. This question requires reinvestigation.

About the same time as the sugar excretion begins to fall and the emaciation of the animal becomes extreme, β-oxy-butyric acid, aceto-acetic acid, and acetone may appear in the urine. In cases where, on account of coincident disturbances of nutrition (*e.g.* gastric ulcer, volvulus of duodenum, &c.), the emaciation proceeds very rapidly, a marked excretion of these substances has been noticed, and there can be little doubt that it is the excessive disintegration of the proteid tissues which furnishes their source. It should be remembered, further, that only a small fraction of the β-oxy-butyric acid formed in the tissues reappears in the urine, most of it being further oxidised in the organism, as is shown by the fact that 20 grm. sodium butyrate when given to a depancreated dog only reappeared in the urine to the extent of 0·4 grm. This latter fact will explain why in many cases of pancreatic, and other forms of severe diabetes, no β-oxy-butyric acid is found.

It will be noted that in our case β-oxy-butyric acid appeared in the urine only at a late stage in the diabetes. The index of this we have taken as the difference between the percentages of dextrose as estimated by reduction and by rotation of the plane of polarised light. β-oxy-butyric acid is levo-rotatory, and when present in the urine along with dextrose will diminish the amount of dextro-rotation which this latter should induce.

We have described the more important features of pancreatic diabetes, and it now remains to discuss what *the underlying cause of these may be, and especially of the glycosuria*. An examination of the blood reveals a high percentage of sugar—sometimes as high as 0·4 per cent.—and this, just as in puncture diabetes, is undoubtedly the immediate cause of the glycosuria. If, moreover, the kidneys be removed or the ureters ligatured in

[1] Which, however, was not complete, as revealed by microscopic examination of the duodenum.

depancreated dogs, the percentage of the sugar in the blood rises still higher.

This hyperglycæmia, as it is called, may be due either to a greater production of sugar, or to a lessened power of its destruction, by the organism. With regard to the former possibility —viz. greater production—the rapid disappearance of hepatic glycogen during the first few days of the condition and the excessive decomposition of proteid tissues which soon supervenes would seem to lend support. On the other hand, as we have seen, there is abundant evidence that the organism has almost entirely lost its power of utilising dextrose, the reappearance in the urine of almost all the ingested dextrose being sufficient proof of this.

We may for the present conclude, then, that *the primary cause of the accumulation of sugar in the blood is the withdrawal from the organism of some influence necessary for the utilisation of dextrose.* Now, the pancreas may influence the destruction of dextrose in the organism in one of two ways, either by the dextrose being brought in actual contact with the gland tissue, or by the gland secreting some substance—an internal ferment—into the blood which brings about the destruction of dextrose elsewhere in the organism. The latter possibility is the more probable one, since it is only when the gland has been almost entirely removed that glycosuria follows. If a small portion of the gland be left, or even if a portion of it (the free portion of the vertical part below the large duct of the gland) be pulled out and, without injury to its vessels, transplanted into the abdominal parietes and allowed to heal in there —an operation which is possible on account of the lower portion of the gland being free from the duodenum and lying in the mesentery—and then the main gland excised, no diabetes will follow. Only a very small fraction of the animal's blood can under such conditions come in contact with pancreatic tissue, and still there is no diabetes. It cannot, therefore, be necessary for the blood to actually transfuse the pancreas in order to be subjected to the glycolytic action. The diabetes is likewise shown by this experiment to be independent of injury to the nerve ganglia adjacent to the pancreas, which injury might quite possibly occur during the excision of the gland tissue.

There must, therefore, be some internal secretion furnished

by the pancreas which acts on dextrose independent of the pancreatic tissue. The small portion of transplanted gland is large enough to furnish this secretion, but when this is also removed diabetes immediately follows.

Nor has this internal secretion anything to do with the intestinal secretion of the gland. The transplantation experiment just mentioned demonstrates this, as do other experiments, such as ligature of the pancreatic duct and the establishment of a pancreatic fistula, neither of which operations induces any glycosuria.

There can be no doubt, then, that the cause of the non-utilisation of dextrose is the absence from the organism of some internal secretion of the pancreas. This being so, it might be expected that in the blood removed from a depancreated dog, dextrose would be less quickly decomposed than in normal blood (*i.e.* which contains the sugar-destroying internal secretion of the pancreas). We have already seen, however, that normal blood itself possesses no glycolytic power, and that the rôle of the internal pancreatic secretion is probably only that of an activating substance which activates a pro-ferment produced by the muscles.

In patients dead of Diabetes mellitus, as stated above, morbid changes have not infrequently been observed in the pancreas. These changes have often been noticed to be especially located in the isles of Langerhans, which are small rounded nests of epithelial-like cells embedded in the connective tissue lying between the true secreting acini. These isles have a richer blood supply than the rest of the pancreatic tissue. Dale ([21]) is of opinion that they are connected structurally with the secreting acini; for he says after he had completely exhausted the secreting cells of the acini by repeatedly injecting an animal with *secretin*, he could easily trace the connection between the two. He noticed, moreover, that many of the secreting acini by this treatment became very like the isles in appearance. In certain fishes, on the other hand, the isles are collected together as small nodules structurally isolated from the pancreas proper, the pancreas itself in such cases possessing no such isles. Rennie and Fraser ([22]), and, later, Diamare and Kubialiko ([23]), have investigated the physiological action of these isolated isle nodules, especially with regard to their dextrose-destroying action, but have not been able to discover any

marked power in this direction. The extracts, however, do not contain any amylopsin. The further results of their investigations have not yet been published.

To sum up, then, we see that the two most remarkable perversions of metabolism in depancreated animals are the disappearance of glycogen from the tissues (liver especially) and the loss of the power of oxidising glucose; lævulose, however, can still be oxidised and likewise form glycogen.[1]

Phloridzin Diabetes.—In a series of papers, published between 1886 and 1889, von Mering described the course of the diabetes

1 The explanation usually offered of this disappearance of glycogen from the liver in pancreatic diabetes is as follows: the tissues have to a large extent lost the power of oxidising dextrose; this oxidation is, however, necessary for life, and, in order that the organism may obtain more dextrose, the glycogen stored in the liver and elsewhere is called upon, and in this way becomes uselessly used up in the attempt of the tissues to try to oxidise sufficient dextrose by working on an excess of it. In the case of lævulose, on the other hand, the co-operation of the pancreas is not necessary for its oxidation; some of it is immediately oxidised, and what remains is converted into glycogen, which is only slowly converted into dextrose.

Such an explanation is unreasonable; it assumes that the remaining oxidative power of the organism (see p. 368) can be forced to greater activity by presenting a large excess of dextrose to the tissue cells, which presupposes that these cells are not already dealing with as much dextrose as they can under the circumstances—that their remaining powers are not being exercised to the full extent—an assumption for which there is not a particle of evidence; if it were the true explanation, then we should expect that where the organism is offered excess of carbohydrates—if, in other words, the excess of dextrose which, according to the theory, the tissues desire to have at their disposal, be increased from without—the glycogen would be saved, and could be found in the liver, which, as we have seen, is not the case. Is it not more probable that the internal secretion of the pancreas normally acts on dextrose in some way so as to render it capable both of oxidation and of transformation into glycogen? in other words, that as dextrose passes through the liver it is acted on by some ferment—the zymogen portion of which is derived from the liver, the activating portion from the pancreas, or *vice versâ*—which changes it in some way so as to render it capable of being oxidised, or, if not immediately required by the organism, of being stored away as glycogen. Chauveau and Kaufmann have indeed claimed that the production of sugar by the liver is controlled by an internal secretion from the pancreas; that normally this secretion inhibits sugar formation, so that when it is removed, the latter process becomes excessive, and glycæmia and glycosuria are the results. Markuse states that when the pancreas alone is excised in frogs diabetes results, but that there is no diabetes when both liver and pancreas are extirpated, and Montuori, that the same follows if the hepatic vessels be ligatured in depancreated dogs. Kausch found that although pancreatic extirpation in herbivorous birds did not cause diabetes it rendered the liver incapable of forming glycogen.

produced in dogs by the administration of phloridzin. This drug is a glucoside containing 38·1 per cent. of dextrose which can be split off from it by boiling with acids. The substance which remains after splitting off the dextrose is called phloretin.

If phloridzin be given by mouth to an animal (dog, rabbit, goose, &c.) in the dosage of 1 grm. per kilogr. body weight, glycosuria becomes established in a few hours, the percentage of sugar in the urine sometimes rising as high as 19·1. The glycosuria lasts for a few hours and then passes off, unless the drug be re-administered. The drug may also be injected subcutaneously (0·3–0·5 g m. per kilo body weight dissolved in warm water or in water made faintly alkaline with carbonate of soda), and this method is the more convenient when rabbits are under investigation.

In well-fed animals the amount of sugar excreted varies considerably, and the *D : N* ratio is very inconstant. During starvation, on the other hand, the sugar excretion becomes less but at the same time more constant, and there is a rise in the nitrogen excretion so that the D : N ratio comes to be the same as that found in pancreatic diabetes when no food or only flesh food is given. *To obtain this constant D : N ratio* certain points must, however, be borne in mind. The ratio obtained on the first day after the administration of the drug must be discarded, for it is always above the average which is subsequently obtained. The explanation given by Lusk and others of this higher initial quotient is that the first effect of the drug is to cause an excretion of a large part of the free sugar of the blood. Even when several days' starvation has preceded the administration of the phloridzin the same thing is seen. The following table, adapted from Lusk (71), will demonstrate this :—

TABLE VI

Day.	Phlor. (Dose).	Number of Injections per Day.	In 24 Hours.		Ratio.
			D.	N.	D : N
1	1 grm.	3	5·272	0·925	5·6
2	1 „	3	4·468	1·769	2·53
3	1 „	3	3·982	1·631	2·44
4	1 „	3	4·052	1·532	2·64

The amount of sugar excreted does not depend, within wide limits, on the dose of phloridzin injected, provided enough of the drug be

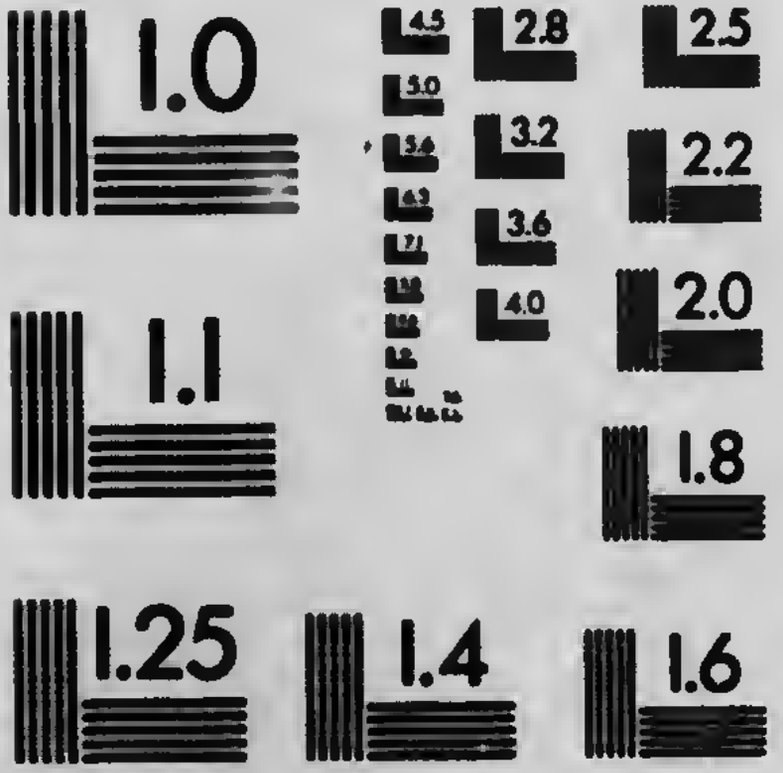
given to produce the maximal effect. The sugar in the phloridzin cannot therefore be of any account in influencing the urinary sugar. The drug should, however, be frequently injected, otherwise the influence of the first dose will have begun to disappear before the second dose has had time to act. The injections should be made three times a day. Observations similar to the above on six rabbits gave an average D : N ratio of 2·7 : 1. In general the quotient in starved dogs poisoned by phloridzin is about 3·5.[1]

We see that this ratio is very nearly the same as that found by Minkowski in starved depancreated dogs. This fact would seem to indicate firstly, that in phloridzin diabetes, as in pancreatic, the tissue proteid is the source of the sugar, and secondly, that the two forms of diabetes are identical in their causation. Concerning the *source of the sugar* in starved phloridzin-poisoned dogs, however, Pflüger has pointed out that in most of the hitherto recorded researches, an approximate estimate of the possible glycogen contents of the animal at the time the phloridzin was given shows that in them all, the sugar excreted in the urine —as a result of the action of the drug—might quite well have been derived from this source, and not at all from the tissue proteids. More recent work by Kraus (3) has, however, established beyond doubt that the glycogen stored in the tissues cannot be the only source of the sugar in phloridzin diabetes. A number of cats were similarly fed for some time. The glycogen in five of these was estimated by Pflüger's method and the following amounts (expressed as grape-sugar) were found per 100 grm. body weight : (1) 0·2637 ; (2) 0·3773 ; (3) 0·2414 ; (4) 0·4700 ; (5) 0·1985. The other cats were kept under phloretin (1·2 grm. per kg. body weight) and starved for from five to eight days ; during this time, the total amount of sugar excreted in the urine was carefully estimated, and at the end of the period the cats were killed and the glycogen determined. The total sugar excretions *plus* the glycogen (expressed as dextrose) remaining in the body per 100 grm. body weight (initial weight taken) were : (1) 0·5887 ; (2) 0·4356 ; (3) 0·3272 (leucin given in food) ; (4) 0·7724 (alanin given in food) ; (5) 1·2282 (alanin given in food).

[1] In dogs starved for three days, von Mering found that 20 grm. phloridzin given by mouth caused, in the first twenty-four hours, a *D : N* ratio of about 5 : 1, which afterwards fell to 3 : 1.

MICROCOPY RESOLUTION TEST CHART

(ANSI and ISO TEST CHART No. 2)



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By comparing these figures, it will be seen that far more sugar had been formed in the phloridzin cats than could possibly have come from the glycogen stored in their tissues, taking as an estimate of this the amount found in the normal cats.[1]

To determine whether *the cause of the appearance of sugar in the urine* in phloridzin diabetes is the same as in pancreatic diabetes, let us further compare the two conditions.

Although the obtaining of a similar *D:N* ratio suggests a possible common cause for the glycosuria in the two conditions, we shall see that such is far from being the case. At the same time this constant ratio shows us that a maximal diabetic state exists during which we can compare this form of diabetes with the others. Let us, first of all, see whether the organism has lost the power of destroying dextrose. We saw, in connection with pancreatic diabetes, that this question could be readily solved by administering a measured quantity of dextrose, and seeing how much of it reappeared in the urine. If 20 grm. of dextrose be given to rabbits rendered diabetic to the maximal extent by phloridzin, only a certain proportion of the dextrose will reappear in the urine, but, coincidentally, the nitrogen excretion will fall, and the result on the D:N quotient will be a rise (to about 7). In dogs it is usually stated that the larger proportion of the administered dextrose does reappear in the urine, but not to the full extent.

That only a proportion of the administered dextrose reappears in the urine shows us that in phloridzin diabetes *the organism has not lost the power of utilising dextrose* to the same extent as in pancreatic diabetes or in Diabetes mellitus (see p. 367); it shows us that the diabetes must be due to quite another cause. The fall in the excretion of nitrogen is another indication that the dextrose has been utilised by the tissues, and has so spared the proteid metabolism. Similar results are obtained when the other sugars are investigated in this way.[2] Lævulose reappears to a small extent partly as such and partly as dextrose, and the nitrogen excretion falls slightly, but the greater proportion of the

[1] It will also be noticed that when alanin (amido-propionic acid) was given to the phloridzin cats a larger amount of sugar than otherwise was excreted. This important result we will return to later.

[2] To obtain the average sugar excretion for the days on which the sugar in question is fed, the nitrogen excretion on these days should be multiplied by 2·8.

lævulose disappears in its passage through the organism. Lactose causes a slight rise in the dextrose excretion, and has also a feeble proteid-sparing action.

A single dose of phloridzin does not appreciably affect the amount of *glycogen* in the liver and muscles. The repeated administration of phloridzin, however, causes the glycogen stored in the liver and muscles almost to disappear. Thus Pransnitz (25) found the liver of a dog which had been kept under phloridzin for twelve days—meanwhile receiving no food—to be practically glycogen free; the muscles of the same dog contained 0·3 per cent. of glycogen. In another similarly treated dog 0·1125 grm. glycogen was obtained from the liver, and the muscles contained 2·0 per cent. The glycogen does not, therefore, become used up so quickly as in pancreatic diabetes.

Another important distinction between phloridzin and all other forms of diabetes is with regard to the amount of *sugar in the blood*. In eleven cats Pavy produced diabetes by injecting phloridzin subcutaneously; during the time that the maximal glycosuria existed, samples of blood were removed, and the percentage of sugar in them determined. The average amount was found to be 0·149 per cent. In six normal cats, the average was found to be 0·088 per cent. Similar observations on rabbits and dogs (Coolen and Kolisch) showed the same result, viz. little difference from the normal, if anything a slight increase, but never sufficient to cause hyperglycæmia.

In other forms of diabetes, too, ablation of the kidneys or ligature of the ureters causes the percentage of sugar in the blood to rise markedly; no rise, however, occurs under similar conditions in phloridzin diabetes.

From what has been detailed it will be obvious that the cause of the glycosuria which follows phloridzin administration must be quite different from that of the other forms of glycosuria. It cannot be due to hyperglycæmia, and there is no marked disappearance of glycogen as in the other forms. These facts led von Mering to suppose that the glycosuria was due to the fact that the kidney, as a result of the action of the phloridzin, had acquired an increased permeability towards dextrose, so that the normal sugar of the blood was drained into the urine. Minkowski still further elaborated this hypothesis by supposing that the phloridzin was picked up by the renal cells and decomposed in them into

dextrose and phloretin, the dextrose being passed into the urine and the phloretin back into the blood, where it combined with the blood sugar to re-form phloridzin, which again underwent decomposition in the renal cells. Meanwhile, some of the phloridzin would leak into the urine, so that renewed doses would have to be given to keep up the diabetic state.

That the action of phloridzin is located in the kidney has been pretty conclusively shown by Zuntz. This worker found that when phloridzin is injected into the renal artery of one kidney, the urine excreted by that kidney becomes saccharine before the urine from the opposite kidney.

Minkowski's theory supposes that it is the blood sugar which escapes into the urine. If, however, a surviving kidney be perfused through its blood-vessels with defibrinated blood containing phloridzin, the urine meanwhile excreted will be found to contain much more sugar than can be accounted for by what has disappeared from the blood (Pavy, Brodie, and Siau[27]). The sugar in the blood is derived from the gastro-intestinal tract and liver; when, therefore, the abdominal viscera, excepting the kidneys, are removed from the circulation the percentage of sugar in the blood falls. The dog can be kept alive[1] for several hours after this operation. If now, into such an eviscerated dog, phlorizdin be injected intravenously, sugar will be found to appear in considerable amount in the urine, but the percentage of sugar in the blood will fall no lower than if no phloridzin had been given. In the light of these observations it is impossible that the blood sugar can be the source of the urinary sugar. It is much more reasonable to suppose, as Pavy, Brodie, and Siau have done, that *the sugar is formed in the kidney itself out of some precursors contained in the blood*. In this respect, the kidney comes to act like the mammary gland; its cells form sugar out of the non-saccharine constituents of the blood. Phloridzin would seem to confer secretory powers on the renal cells, in other words, to make the kidney in part glandular in function.

The importance of this recently offered explanation of the action of phloridzin makes it desirable that some of the evidence on which it is based be briefly given here.

1. Diuretics like caffein, nitrate of soda, &c., cause an increase in the amount of sugar contained in the urine in those forms of

[1] In a state of unconsciousness.

diabetes in which hyperglycæmia exists (pancreatic diabetes, &c.), whereas in phloridzin diabetes, although diuresis is produced, no increased amount of sugar is excreted. The increased volume of blood which perfuses the kidney blood-vessels, as a result of the action of these diuretics, allows more sugar to pass the kidney filter when hyperglycæmia exists, but it does not stimulate the kidney cells to increased production of sugar when they are poisoned by phloridzin.

2. Injury to the kidney, whether mechanical or produced by drugs or by disease, markedly diminishes its power of secreting a saccharine urine when it is subjected to the action of phloridzin.

3. Histological examination of the uriniferous tubules after prolonged phloridzin administration reveals necrotic changes in the cells.

The mother substance of the sugar is undoubtedly the serum proteid. It will be remembered that when the animal is liberally fed with carbohydrate much sugar is excreted in the urine without there being any rise in the nitrogen excretion, but that when starved of carbohydrate the amount of sugar falls and that of nitrogen rises. This must mean that in the well-fed animal, the sugar can be split off from the serum proteid without there being any disruption of the proteid molecule, and hence no rise in the excretion of nitrogen. Chemical evidence that this is possible we have given above (p. 319). This sugar is loosely combined with the proteid in the same way that oxygen is combined with hæmoglobin in oxy-hæmoglobin.

Further evidence that such a sugar-carrying function belongs to the blood proteid has recently been furnished by Embden and Blumenthal ([2]). The former worker has found that if a glycogen and jecorin [1]-free liver is perfused with blood, sugar accumulates in it.

Blumenthal applied Bial's reaction for aldo-hexoses (see p. 318) to serum globulin prepared from the blood of animals rendered diabetic by phloridzin, and obtained a very faint reaction; whereas with serum globulin prepared from a well-fed normal animal a very distinct reaction was noted.

It is not supposed that all the dextrose in the blood is thus

[1] Jecorin is a compound of lecithin and dextrose, and has been described by Henriques and Bing as the substance in the blood in chemical combination with which dextrose is carried.

combined; some of it is almost certainly in a free state. What the proportion between the free and combined sugar may be is as yet an entirely unsolved question.

The proteid thus deprived of its sugar in phloridzin poisoning, becomes recombined with more of it during its circulation through the rest of the body. When, however, the animal is starved, and no fresh supply of carbohydrate is available for the reconstruction of this gluco-proteid, then the poisoned kidney cells attack the proteid molecule itself, and thereby liberate not only the sugar which is intimately bound up in it or which is derivable from certain of its decomposition products, but also the nitrogen. That excessive break-down of proteids does occur in advanced phloridzin poisoning during starvation is further proven by the fact that β-oxy-butyric acid, &c., make their appearance in the urine.

Adrenalin Glycoscuria.—The subcutaneous or intraperitoneal injection of suprarenal extract or adrenalin chloride solution causes glycosuria. An examination of the blood has shown that hyperglycæmia is the immediate cause of the glycosuria, so that increased production of dextrose in the organism, or its diminished destruction, must exist.

Starved dogs, from whose tissues most of the glycogen has been caused to disappear by repeated phloridzin administration, also react to suprarenal extract, so that increased production of dextrose cannot be the cause of the hyperglycæmia. This does not of course mean that the extent of the glycosuria induced by the injection is the same in starved as in well-fed animals; for the more glycogen there is available to convert into dextrose, the more marked will be the glycosuria. Diminished destruction of dextrose must therefore exist. Now, we have seen that the form of experimental diabetes which best typifies glycosuria due to want of dextrose destruction in the tissues, is that which follows extirpation of the pancreas. Can it be, then, that suprarenal extract acts on this gland in some way so as to diminish the influence of the latter on dextrose destruction? Herter ([32]) and his co-workers have shown that painting the pancreas with adrenalin chloride solution likewise induces glycosuria, so that the question would appear to be answered in the affirmative.

The best known physiological action of suprarenal extract is vaso-constricting. It might be thought that this is, indirectly, the cause of the hyperglycæmia. That such is not the cause

of the condition is, however, indicated by the fact that in guinea-pigs very marked vaso-constriction follows suprarenal extract administration, but no glycosuria, and, further, that the painting of the pancreas with many reducing substances is also followed by glycosuria (Herter).

Animals may after being repeatedly injected with the drug acquire a certain tolerance towards it (Noel Paton[iii]). The ammonia excretion is considerably increased during the glycosuria.

Before proceeding further, let us briefly consider whether these experimental forms of diabetes bear any analogy to the disease **Diabetes mellitus** in man. A description of the disease itself would be out of place here, but a consideration of its possible causes, deduced from the foregoing experimental observations, must be of very great interest and importance.

We have seen that glycosuria itself may be induced by—

(1) An overproduction of dextrose by the liver—*piqûre*, &c.;

(2) A want of destruction of dextrose in the organism—*pancreatic diabetes*;

(3) A production of sugar from the blood by the renal cells—*phloridzin diabetes*.

To which of these processes is the glycosuria in Diabetes mellitus due? If we can but answer this question, then some indication may present itself of the cause of the other symptoms. At the very outset we may delete the third possibility, for were D. mellitus analogous with that produced by phloridzin injection—which typifies glycosuria due to this cause—we should certainly expect to find the blood of a diabetic patient to contain a more or less subnormal percentage of dextrose, which, however, is not the case; there always being an excess.[1] Moreover, there is no evidence that the kidney tissue is in any way pathological at an early stage in the disease. These arguments do not, of course, preclude the possibility of some of the mildest transitory forms of glycosuria in man being due to renal trouble; the disease D. mellitus is, however, not due to it.

Either of the first two conditions enumerated above would cause hyperglycæmia, which always seems to exist. *This hyperglycæmia is the immediate cause of glycosuria.*

[1] The analytical data with reference to this question are, however, very meagre.

What, then, causes the hyperglycæmia in D. mellitus? Can it be due to an overproduction of sugar by the liver? No doubt, as we have seen, such is the cause of the transitory glycosuria which sometimes accompanies injuries to nerves, or neuralgias, or the presence of intracranial tumours; but it cannot be the cause of the glycosuria in the severer disease, else would we expect to find entire absence of hepatic glycogen after death, and a disappearance of the glycosuria after the store of glycogen had become used up. Neither of these conditions has been shown to exist in D. mellitus. The glycosuria persists till death; and in the few cases in which the liver has been examined for glycogen, some glycogen has always been found, even although, in most of the cases reported, the liver was not examined till some time after death, so that *post-mortem* hydrolysis of glycogen might have occurred. The histological examination of some liver cells from two diabetics, removed by puncture of the liver with a trochar during life, showed distinct glycogen masses in one case but only small amounts in another case (Bunge,[8] p. 432).

Some forms of temporary glycosuria, such as the so-called *alimentary glycosuria*, may be due to inefficiency in the glycogenic function of the liver—to a sluggishness in the process—so that the excess of sugar in the portal blood during digestion is not sufficiently retained by the liver, as a result of which some of it passes into the systemic blood and induces hyperglycæmia. It is only after taking considerable quantities of sugar (100–200 grm. dextrose) that glycosuria follows in this mild form of diabetes. Starches do not cause it, for they are more gradually absorbed and do not so markedly raise the sugar percentage in the blood that leakage through the kidney filter should occur. The sugar appears in the urine in from ¾–1 hr. after its ingestion. None of the severer symptoms of diabetes is exhibited.

By a process of elimination, then, we see that a *want of destruction of dextrose* in the organism must be the cause of the glycosuria. Diabetes mellitus in man must be very similar, in its chemical pathology, to pancreatic diabetes in animals, and indeed, as we have seen, morbid changes in the pancreas are not infrequently found in patients dead of this disease.

What direct evidence have we that in D. mellitus in man the power of dextrose destruction in the body has been depressed? The answer to this question is furnished by administering dextrose

to a diabetic patient on an otherwise constant diet, and seeing how much of it can be recovered from the urine (*vide* Pancreatic Diabetes, p. 351). It has usually been found that a large proportion of the administered dextrose can be recovered in this way, though not usually all of it. Von Noorden ([29]), however, records a case in which all the administered dextrose reappeared.

With regard to other sugars than dextrose, it has been noted that *lævulose* is much more readily utilised by the diabetic than dextrose is, especially in the milder forms of the disease, where it usually causes no increase in the glycosuria; in the severer forms of diabetes it may but cause a slight rise in the excretion of dextrose, while in other cases not only is dextrose increased, lævulose itself may appear in the urine. We have already seen that in pancreatic diabetes in the dog, lævulose is the only sugar which leads to glycogen formation. There can, therefore, be no doubt of the power of the diabetic organism of choosing between the two sugars; and it is interesting to note that this power is not confined to the animal body, but is exhibited by low forms of life: *e.g.* a pure culture of the yeast plant (*Saccharomyces apiculatus*) can ferment dextrose and lævulose, but is inactive on solutions of other sugars.[1] If cane-sugar be given to a diabetic the increase in the dextrose excretion in the urine will amount to about one-half of the administered cane-sugar, the dextrose portion of the cane-sugar is excreted but the lævulose portion is destroyed. This interesting fact recalls one of Pasteur's first discoveries: that *Penicillium glaucum*, a common fungus, when grown in a solution of inactive racemic acid, which consists of a double molecule of right and left rotating racemic acid, destroys the left-rotating molecule, but has no action on the right-rotating molecule.

The behaviour of other disaccharides and of polysaccharides will be readily understood if the above facts be borne in mind.

Further proof that the human organism in D. mellitus has largely lost the power of utilising dextrose—if indeed further proof be needed—is furnished by an examination of the respiratory quotient. This, it will be remembered, is a fraction representing the relative amounts of carbon dioxide (CO_2) expired and of oxygen (O_2) retained in the body. It is written $\frac{CO_2}{O}$. When

[1] Dextrose and lævulose have distinctly different chemical structures, so that the discrimination of them by the tissues is quite conceivable.

carbohydrates form the greater bulk of the food-stuffs undergoing combustion in the organism this quotient is nearly 1; for a monosaccharide contains within its own molecule sufficient oxygen to oxidise all its hydrogen, and requires oxygen from without only for the oxidation of its carbon. It contains oxygen and hydrogen in the same proportion as they exist in water. Now, when a certain volume of oxygen combines with carbon, the volume of carbon dioxide gas thus formed is equal to that of the oxygen used. The theoretical R. Q. on a carbohydrate diet is, therefore, 1.

In the case of fats and proteids, on the other hand, the molecule contains relatively more hydrogen, so that, for its oxidation, it requires oxygen for its hydrogen as well as its carbon; the volume of oxygen absorbed is therefore greater than the volume of CO_2 produced, and hence the R. Q. is less than 1. As a matter of fact, for fats the R. Q. is 0·707, and for proteids it is 0·8. On examination of persons suffering from severe diabetes it has been shown that R. Q. is about 0·7 even when dextrose is given in the food (Magnus Levy [30]).[1]

There can be no doubt, then, that the excess of dextrose in the blood in diabetes is due to the organism having, to a greater or less degree, lost the power of utilising the sugar.

We have already stated that the end products of the metabolism of dextrose are carbon dioxide and water. If we compare the structural chemical formula of dextrose with that of the end products in its metabolism, it will at once be evident that two chemical processes must be called into play in the break-down of the molecule, viz. a process of disruption and a process of oxidation of the disrupted groups; or, it may be that oxidation takes place first, and that the oxidised groups are then split off. It is possible, therefore, that the failure of *either of these processes might be the immediate cause of the want of break-down of dextrose in the diabetic organism*. Let us consider which of these processes is really at fault.

[1] This fact alone merely shows that carbohydrates are not being oxidised, but such might result from the elimination of carbohydrates from the diet, as in starvation, where a practically identical R. Q. is obtained. Magnus Levy [30] has recently shown that the R. Q. during rest and hunger in severe diabetes lies midway between what it would be were fat and the non-carbohydrate portion of proteid the only substances undergoing oxidation in the organism. Had the carbohydrate moiety of proteid been also oxidised, he shows that the R. Q. would have been distinctly higher than what he actually found it to be.

There is considerable evidence to hand that, in the earlier stages of diabetes at least, processes of oxidation proceed in their normal fashion in the organism: benzol is oxidised to phenol; fat is oxidised to carbonic acid and water; organic acids are oxidised to carbonates. Conversely, we can experimentally diminish oxidation in the organism—for example, by poisoning with phosphorus—without inducing any glycosuria. Deficient oxidation is therefore not the immediate cause of diabetes.

It is much more probable that a want of disruption of the dextrose molecule is the primary cause of its non-utilisation by the organism, and it is commonly assumed further that the disruptive process is an antecedent of the oxidative: that oxidation is only possible after the large sugar molecule has been broken into smaller groups.

In the severer forms of the disease, however, the oxidative processes are also depressed, for incompletely oxidised bodies make their appearance in the urine, such as β-oxy-butyric acid, acetone, and aceto-acetic acid, and the intake of oxygen becomes much less than in health, whereas it might be expected to be more, on account of its having to oxidise proteid and fat—which require relatively more oxygen for their oxidation than carbohydrates do—instead of dextrose, which cannot be utilised by the organism.

Being deprived of the power of utilising dextrose, the diabetic organism, in order to obtain the energy necessary for life, is compelled to live on fat and proteid. Sufficient of these food-stuffs is frequently not absorbed from the intestine (especially where, as is common enough, absorption is interfered with), so that the tissue fat and proteid are used up, and emaciation results.

Death may result from this, for soon all the available proteid and fat become exhausted, and the organism, incapable of oxidising carbohydrates, does not obtain the energy necessary to carry on its vital functions. Death, in such cases, is really due to acute starvation. Quite frequently, however, in Diabetes mellitus death is due to another cause than acute starvation, namely, to *diabetic coma*. This can best be described as a condition of gradually increasing collapse and mental dulness, frequently associated with a form of dyspnœa—"air hunger"—in which the respiratory movements

are deep and strong, in striking contrast to the muscular weakness of the rest of the body.

This coma is associated with the presence in the urine of certain organic acids, especially of β-oxy-butyric acid, CH_3-$CHOH$-CH_2COOH, and its oxidation products, aceto-acetic acid, CH_3-CO-CH_2COOH, and acetone, CH_3-CO-CH_3. These substances can usually be detected in the urine in diabetes previous to the onset of the coma, and during the coma they considerably increase in amount. It has, not unfrequently, been noted that during the comatous state the breath smells of acetone, and further, that when this state supervenes, the amount of acetone in the urine becomes less, while that of its precursors, β-oxy-butryic acid and aceto-acetic acid, becomes greater.

These facts would seem to indicate that the coma is due to an excess, either of acetone or of the acids in the organism.

The smell of acetone on the breath has been taken to point to an intoxication by this substance as the cause of the condition. Such a view would seem to be substantiated by the fact that when acetone is given to dogs in the dosage of 4 grm. per kg. body weight intoxication symptoms follow, like those produced by ethyl alcohol, and if this dose be doubled, death may result. A closer examination of this result, however, instead of proving the contention, at once disproves it, for if 8 grm. per kg. be the minimal lethal dose, then at least 500 grm. would require to be produced in a man of 70 kg. to cause death, and it is inconceivable how such could be the case, especially since, in most cases, it would all have to be derived from proteid and fat.

Let us then consider whether the *acids* may not be the cause of the intoxication. They increase in amount in the urine at the expense of acetone, just before, and during the coma, and if bicarbonate of soda be given by mouth to the patient, or if faintly alkaline intravenous saline injections be made, still more of the acids appears in the urine. Moreover, the condition of coma seems to be frequently alleviated by the administration of alkali, and there can be little doubt that, in many cases of diabetes, attacks of coma can be warded off by the regular exhibition of alkali. The alkali neutralises the excess of acid in the organism. All these facts indicate an excess of acid in the tissues as the possible cause of the coma.

What, then, is the effect of excess of acid in the organism?

If mineral acids be given by mouth, to rabbits, the animals lose the power of moving about, become comatose, and ultimately die of collapse; a symptom complex, at least, suggestive of diabetic coma. If alkali be administered to acid-poisoned animals these symptoms disappear.

If the urine of such poisoned animals be examined it will be found to contain an excess of ammonia. This must mean that, for its neutralisation, the acid has not only used up all the fixed alkali (Na, K, &c.) in the organism, but has appropriated some of the ammonia which normally would have been transformed into urea. The excretion of an excess of ammonia may, therefore, be taken to indicate the neutralisation of all the fixed alkali by the acid administered.

A direct estimation of the alkalinity of the blood in acid intoxication will show that most of the alkali has been neutralised. One of the most important functions of the fixed alkali, in the blood at least, is the carrying of carbonic acid, and since the mineral acids possess so very much stronger affinities for alkali than carbonic acid does, we would expect to find very little carbonic acid in the blood of acid-poisoned animals. As a matter of fact, Walter[31] found only 2–3 vols. per cent., the normal amount being 30–40 vols. per cent. The immediate cause of the coma may therefore be the accumulation of carbonic acid in the tissues; it remains where it is produced, for the blood, deprived of its alkali, can no longer transport it quickly enough to the lungs.

Is there, then, really an acid intoxication in diabetic coma? The indications of this in the experimentally produced condition we have seen to be: (1) Excess of ammonia in the urine. (2) Marked diminution in the percentage of CO_2 in the blood. (3) Diminution of alkalinity of blood. Do these conditions exist in diabetic coma?

It has been known for long that an excess of ammonia is excreted in severe diabetes. In normal human urine, the amount of nitrogen excreted as ammonia is only 3–6 per cent. of the total nitrogen; in diabetes, it may form 20–30 per cent. of the total nitrogen (Magnus Levy[39]). Since this represents ammonia diverted from its normal metabolism into urea for the purpose of neutralising the excess of acid, it would be expected that alkali administration to diabetics would cause it to diminish. Such has frequently

been found to be the case, although *all* the excess of ammonia cannot thus be made to disappear (Magnus Levy).

The percentage of carbonic acid in diabetic blood, especially during coma, is stated to be very small; thus Minkowski ([28]) found only 3·3 vols. per cent. in one case of diabetic coma shortly before death, and 15 per cent. in another case at the beginning of the coma, and a normal amount in two cases of severe diabetes not in the comatous state (Naunyn[31]). Kraus found 6·4 vols. per cent. (*vide* Magnus Levy[30]).

The alkalinity of the blood is likewise said to be diminished in diabetic coma. According to Kraus the normal alkalinity corresponds to 185–220 m.g. NaOH for 100 c.c. blood, whereas in diabetic coma it was found to be 125 m.g. NaOH.

The fact that the administration of alkali increases the excretion of acid in the urine would further corroborate the idea that an acid intoxication exists. Beddard, Pembrey, and Spriggs ([12]) have recently reported some determinations of the alkalinity and CO_2-content of the venous blood of diabetics: they have found that a rise or fall in the one is accompanied by a rise or fall in the other, but that a strict parallelism between the two does not exist. During diabetic coma the percentage of CO_2 in the alveolar air was found to be much below the normal. They conclude that the diminished CO_2-content of the venous blood in diabetic coma is the *result* of the hyperpnœa (*i.e.* of the increased ventilation of the lungs) rather than its cause.[1] This conclusion is based not only on the observations detailed above, but also on certain experiments which, however, cannot be given here.

The source of the β-oxy-butyric acid and its oxidation products is somewhat obscure. It cannot be derived from *proteid*, as the following observations show: in the urine of three days from a case of diabetic coma Magnus Levy ([30]) was able to separate 326 grm. β-oxy-butyric acid, and the nitrogen excretion showed that meanwhile 271 grm. proteid had undergone metabolism in the organism. There was an excess of β-oxy-butyric acid alone, to say nothing of the aceto-acetic acid and acetone which had also been excreted. Nor is it conceivable that all the carbon of proteid could have been thus excreted. This does not of course preclude

[1] They found the blood of a diabetic (in coma) could take up a normal amount of CO_2 on exposure to an atmosphere of this gas. The coma causes lessened production of CO_2. (*Editor.*)

the possibility of a part of the acids being derived from proteid. The acid cannot be derived from *carbohydrate* alone, for it is produced in abundance on a carbohydrate-free diet, and at a stage in diabetes when practically all the tissue carbohydrate must have been used up.

Two other possibilities remain to explain the source of β-oxy-butyric acid and its derivatives; the decomposition of fat and a synthetic process. For this *synthesis*, bodies with two and three carbon atoms would be available, and these might be derived from proteids or fats or carbohydrates; so that, indirectly at least, these bodies may come from any of the proximate principles of food.

Their derivation from *fats* would, for certain fats at least, be a very simple chemical process. Thus from butyric acid, $CH_3CH_2CH_2COOH$, the replacement by hydroxyl (OH) of one of the hydrogen atoms of the second methyl group of the chain is all that would have to occur—$CH_3CHOH\ CH_2COOH$. In the case of higher fats, with 16–18 carbon atoms (*e.g.* palmitic, $C_{15}H_{31}COOH$, and stearic, $C_{17}H_{35}COOH$), it is possible that a gradual breaking away, by an oxidative process, of the ends of the chain occurs, till at last an acid with a chain of four carbon atoms, viz. buytric acid, is left, and that, in the diabetic state oxidation can only proceed a short distance farther and convert this into β-oxy-butyric acid, aceto-acetic acid, and acetone, but not, as normally, into CO_2 and H_2O. By such a process only one molecule of β-oxy-butyric acid would be derived from one of fatty acid. It is conceivable, however, that the larger fatty acid molecule breaks down into several smaller fractions, each of which then changes into butyric acid.[1] By such a process, several butyric-acid molecules might be derived from each fatty acid molecule. Let us see now how far actual observation bears out these suppositions.

Schwarz ([30]) noticed a distinct increase in the amount of acetone excreted in the urine of a diabetic when large quantities of butter were added to a proteid, or proteid and carbohydrate diet. An increase likewise followed the administration of the various fat

[1] Such a disruption of a large molecule into several smaller molecules undoubtedly occurs in the case of carbohydrates (*e.g.* lactic acid fermentation). These contain, however, within each molecule sufficient oxygen for the disruption, and it is a question whether fatty acid with its lack of oxygen could undergo the same cleavage.

acids separable from butter, and this increase was most marked when butyric acid was given. Butyric acid is therefore undoubtedly one of the precursors of β-oxy-butyric acid, &c.; but it cannot be the only one, for an amount of these substances is often excreted far in excess of what could possibly be derived from this source (*vide* Magnus Levy[30]). The fact that Schwarz found the non-volatile acids of butter (palmitic, stearic, and oleic) also to cause an increase in their excretion further supports this contention.

No experimental or clinical data throws any light on whether only one oxy-butyric acid molecule is derived from each molecule of these higher acids, or several. To answer this question it would be necessary to know exactly how much fat is undergoing metabolism in the body and how much oxy-butyric acid, aceto-acetic acid, and acetone is meanwhile being excreted; and, until this question can be answered, it can scarcely be hoped that the exact nature of the chemical process by which fat yields these bodies can be understood. *At present it is uncertain whether fat is alone their source, or whether they may not be the products of some synthetic process.*

The large amounts of sugar excreted in pancreatic and phloridzin diabetes and in Diabetes mellitus cannot, as we have seen, be derived entirely from the carbohydrates stored in the body as glycogen. **Where, then, does this excess of sugar come from?** We have assumed, up to the present, that proteid must be its source, and yet we have not proven beyond doubt that such is actually the case, nor have we shown how, if this sugar be so derived, the proteid comes to yield it: we have not, in other words, explained the bio-chemical processes which must ensue to bring about such a transmutation of proteid into carbohydrate.

The discovery by Pavy that reducing bodies (sugars) are formed when egg proteid is boiled for some time with mineral acids, and the further chemical evidence along the same lines which we have already considered, seemed to point to a pre-formed carbohydrate group in the proteid molecule as the source of the sugar. The evidence for, or against, such a derivation we shall now consider in greater detail.

We shall see whether fat—at least the glycerine portion of it—may not contribute towards this sugar production in the body;

and, finally, whether proteid itself, apart from its carbohydrate group, may not, under certain conditions, behave similarly.

In this review some repetition will be unavoidable, and several observations from the clinical study of diabetes in man, not yet considered in this article, will be cited to assist us in our conclusions.

With regard to its derivation from a pre-formed carbohydrate group in the proteid molecule.

When other forms of proteid, and more especially the proteids of blood serum, were examined for carbohydrate groups by Pavy's method, much smaller yields were obtained than in the case of egg proteid, and indeed in some proteids (*e.g.* casein) no carbohydrate group at all could be detected.

The carbohydrate group separable from egg proteid and from serum proteid and from several other varieties of proteid is, moreover, not dextrose, but *glucosamin*. Before any rôle in glycogen or dextose formation could be ascribed to this substance, it would be necessary to show that it can give up its amido (NH_2) group in place of a hydroxyl (OH) group, so as to produce a nitrogen-free sugar—chitose. In the laboratory, this substitution can be brought about (see p. 316), but there is no certain evidence that it occurs in the animal body. Glucosamin, given by the mouth in large doses, passes in part at least into the urine unchanged, and does not seem to lead to the deposition of glycogen in the tissues. Such a result must not of course be considered as furnishing a final answer to our question, for, as Langstein points out, it is quite conceivable that the glucosamin liberated in the organism itself (*i.e.* of endogenous origin) is susceptible to quite a different metabolic process from glucosamin introduced from without (*i.e.* of exogenous origin).

In this connection it is also of interest to note that there is much evidence that the process of denitrification of amido bodies can be carried out by living cells; thus Hopkins and Cole have recently shown that if anaerobic bacteria be grown in the presence of tryptophane (skatol amido-acetic acid) they cause the amido group (NH_2) to be eliminated and leave behind skatol-acetic acid. Jones and others have also shown that amido-purins can be converted into oxy-purins by a denitrifying ferment, and as is explained elsewhere, there is no doubt that this denitrification of amido-purins is constantly going on in the body.

There is also some indirect evidence that glucosamin can form

glycogen, for Blumenthal and Wohlgemuth ([33]) have found that if frogs be fed on proteids, such as egg albumin, which contain glucosamin, glycogen becomes deposited in the organism; whereas when fed on such proteids as casein, which contains no glucosamin, glycogen is not formed.

It is possible, therefore, that, under some conditions, the glucosamin present in certain proteids may be converted into dextrose in the animal body; but for various reasons, glucosamin cannot be held accountable for the large amount of sugar excreted by starved diabetic animals. Let us then consider in this connection the other sugar groups which have been isolated from proteid.

We saw that *pentoses* were almost invariably found among the decomposition products of nucleo-proteid, and we know that nucleo-proteids are constantly being broken down in the organism (see p. 399); what then becomes of the pentose thereby liberated? does it serve to build up glycogen and so become converted into dextrose; or is it excreted unchanged in the urine? We know that when pentoses are given by the mouth they appear unchanged in the urine; they are not retained in the organism as glycogen, nor are they oxidised in their passage through it. Taking these facts into consideration, we might expect that the pentoses liberated in the break-down of nucleo-proteid would likewise pass unchanged into the urine, in which therefore traces at least of pentose sugar would be constantly present. Such, however, is not the case. Pentosuria is a rare condition, and the variety of pentose which does appear when it is present is chemically different from that which appears as a decomposition product of nucleo-proteid.[1]

The *dextrose group* recently found in serum globulin (see p. 317) is no doubt a direct glycogen former, but, at most, no more than 2 per cent. of dextrose can be separated from this proteid. Of the other proteids which are among the constituents of our food, casein and gelatin contain no carbohydrate groups, and in serum albumin there is much doubt whether the 0·5 per cent of carbohydrate which has been separated from it does not come from adherent mucin.

Pflüger ([6]) has recently brought forward the view that glycogen,

[1] It is beyond the scope of this article to discuss the interesting condition of pentosuria.

and hence dextrose, can be derived *only from carbohydrates*, and he explains the evident sugar *formation* which occurs in the animal body in diabetes as being derived from the carbohydrate groups in the proteid molecule. He asserts that if we allow 10 per cent. of carbohydrate as the average amount in all proteids, then the whole of the sugar which is excreted in diabetes can be accounted for as of proteid origin. We shall presently see, however, that even if we do admit this average it is impossible thus to explain the sugar production in many cases of diabetes; and, moreover, the premise cannot be granted, for, as we have just seen, none of the proteids which we take as food contains anything like this amount of sugar: it would be more correct, in fact, to state that the average amount is 1 per cent.

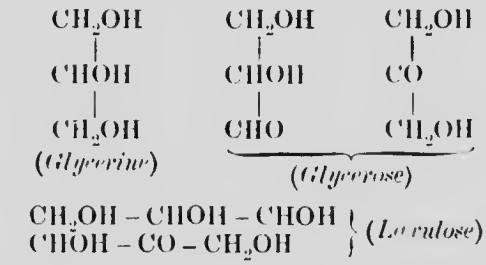
Besides the evidence of Lüthje and others that Pflüger's hypothesis cannot possibly hold in the case of depancreated dogs, there is a considerable amount of clinical evidence that in Diabetes mellitus too the hypothesis is untenable. The strongest evidence in this connection has been furnished by F. Kraus and by Mohr.

F. Kraus ([34]) kept a patient for several weeks on a daily diet of milk, eggs, flesh, some white bread, and 20 grm. butter, and found that for every 1 grm. of sugar which was obtainable by hydrolysis from the food, 1·655 grm. appeared in the urine. Mohr ([35]) estimated the total sugar excreted by two diabetic patients for four to five weeks, and found that over 1300 grm. carbohydrate appeared in the urine in excess of what could have been derived from the diet, supposing even that all the proteids of this contained 10 per cent. of sugar.

Granted, then, that *the sugar in diabetes cannot be derived from carbohydrates, either free or bound to the proteid molecule, we are driven to the conclusion that proteid itself or fat must be its source.*

Let us consider whether fat may not be the source of the sugar. Neutral fat, it will be remembered, is a compound of glycerine and fat acid. We have seen that *glycerine* feeding probably does not cause glycogen to be deposited in the liver (see p. 331). On the other hand, it has been found by Emil Fischer ([2]) that, in the chemical laboratory, glycerine can be readily converted into glycerose by mild oxidation with a mixture

of bromine and sodium hydroxide, and that two molecules of glycerose readily condense (aldol condensation) into a molecule of lævulose.



An index of whether the sugar in severe diabetes is derived from glycerine would be the obtaining of a *D : N* ratio higher than that which could exist were the sugar derived from proteid alone, at a stage in the diabetes during which the glycogen in the body had all disappeared. Von Mering calculates that 20 grm. of proteid or 100 grm. of flesh (*i.e.* 3·4 grm. N), might yield 27 grm. sugar, after allowing for the carbon of urea. This would give a D : N quotient of 8 : 1. Other observers, including Minkowski, consider that the D : N ratio obtained in depancreated dogs, starved or fed on flesh alone (viz. 2·8 : 1), represents the highest one possible when proteid alone is the source of the sugar.[1]

Another way of answering the problem would be to see whether the total sugar excreted by a diabetic animal during a week or two could be accounted for by the glycogen previously stored in the body (taking for the purpose of this calculation, Pflüger's average of 40 grm. of glycogen per kilo body weight),[2]

[1] In using this quotient as a criterion of the source of dextrose in diabetes, however, it must be borne in mind that the retention of nitrogen, as well as the increased excretion of dextrose, might cause it to rise. Now several clinical observers have shown that a considerable retention of nitrogen may occur in Diabetes mellitus, so that, for the quotient to be of any value in indicating the source of the sugar, the excretion of nitrogen should be at least as great as the intake, and the observations should extend over several days. In other words, the sugar and the nitrogenous excretory bodies formed in the organism, say when egg albumin is given in the food, may not appear in the urine at the same moment, the excretion of the sugar coming to an end before that of the nitrogenous bodies. To obtain the correct quotient, therefore, the examination must last as long as it is noticed that there is any effect on either the sugar or the nitrogen excretions.

[2] When the observation is made at such a stage in diabetes during which little glycogen could still remain in the body, a lower value than this would have to be taken.

and by the proteid which had meanwhile undergone metabolism as indicated by the excretion of nitrogen.

As a matter of fact, Hartohg and Schumm [36] have found in phloridzin-poisoned dogs, at a late stage of the diabetes, *D : N* ratios of 10·6 (for two days), and 13 (for one day); and Rumpf [37] records a case of D. mellitus in which during fifteen days on a limited diet the sugar excretion was 1169·8 grm., and that of nitrogen 98·8 grm. (D : N ratio 11·8). In neither of these cases could much glycogen have been present in the organism to account for the high sugar excretion.

The *fatty acid* portion of neutral fat cannot probably be converted into sugar, and this may possibly explain why volatile fatty acids occur in the urine in severe Diabetes mellitus.

These results would seem at least to suggest that fat may be one source of the sugar. *That it cannot, however, be the only source, will be evident after we have considered somewhat more fully how proteids themselves can be converted into sugar.*

Falta and Mohr [2] have noticed that the increased excretion of dextrose which often follows proteid feeding in diabetes depends largely on the ease with which the proteid in question can undergo metabolism in the organism. Falta found that casein caused a rise in the dextrose excretion in a case of Diabetes mellitus of medium severity, whereas egg albumin and serum globulin did not. Apart from showing us that the excretion of dextrose does not depend on the presence of a carbohydrate group in the proteid molecule —for casein contains no such group, whereas egg albumin and serum globulin do—this result shows us that the more easily the proteid is made use of by the body the more likelihood is there of its influencing the excretion of dextrose, casein being more easily assimilated than egg proteid. It would appear that the sugar derived from casein is too quickly produced to be converted into glycogen—the power of effecting this transformation being probably depressed in diabetes—so that it accumulates in the blood, causing hyperglycæmia; whereas, from egg albumin and serum globulin, this sugar is produced slowly enough for the organism to deal with it and convert it into glycogen.

In contrast to the small amount of sugar which can be obtained in the laboratory from proteid, we see that in the organism a large amount must be derived from this source. This fact compels us to consider the question of sugar formation in the

body from proteid from another point of view, namely, that the sugar is derived from some of the *decomposition products of the proteid molecule itself*—that is, from amido-acids, hexone bases, aromatic bodies, &c.

In considering the possibility of such a process we must admit at the outset that a liberation of the amido group (NH_2) is possible in the body. That such a process does actually obtain we have already seen in connection with glucosamin and amido-purins.

The amido-acids which we would naturally look to as possible precursors of dextrose are those which contain either three or six carbon atoms. Such amido-acids are leucin and alanin.

Alanin is amido-propionic acid and has the formula

$$\begin{array}{l} CH_3 \\ | \\ CH-NH_2 \\ | \\ COOH \end{array}$$

By the action of nitrous acid [1] (1) on this, the NH_2 group is substituted by an OH group, the resulting body being lactic acid or α-hydroxy-propionic acid:

$$\begin{array}{l} CH_3 \\ | \\ CHOH \\ | \\ COOH \end{array}$$

Now lactic acid is closely allied to glyceric aldehyde [2] (2), which by a process of condensation easily passes into dextrose, thus:—

$$\begin{array}{lll} CH_2OH & CH_2OH & CH_2OH \\ | & | & | \\ CHOH & + CHOH & = (CHOH)_4 \\ | & | & | \\ CHO & CHO & CHO \\ (\textit{Glyceric aldehyde}) & & (\textit{Dextrose}) \end{array}$$

[1] (1) When nitrous acid is allowed to act on any organic substance of the paraffin series containing an amido group, the amido group is replaced by hydroxyl, and free nitrogen is evolved.

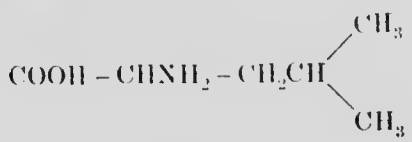
[2] (2) Glyceric aldehyde can be obtained from acrolein dibromide by treating the latter with baryta water.

$$\begin{array}{lll} CH_2Br & & CH_2OH \\ | & & | \\ CHBr & + Ba(OH)_2 = & CHOH \\ | & & | \\ COH & & COH \end{array} + BaBr_2$$

Confirmation of the possibility of such a process is further found in the case with which dextrose can be converted into lactic acid, for example, during the action of zymase (G. Buchner): $C_6H_{12}O_6 = 2C_3H_6C_3$.

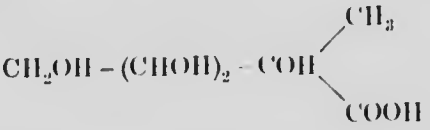
In the laboratory, then, we see that the conversion of alanin into dextrose is quite possible, and that, as an intermediate product, lactic acid is formed. By biological experiment, equally convincing results have been obtained by Langstein and Neuberg (2). Those workers starved rabbits for eleven days—by which time, as we have seen above, the tissues will be pretty well cleared of glycogen—and then fed them with 20–30 grm. of alanin; on killing the animals shortly afterwards, 2 grm. of glycogen were found in the liver, an amount greater than could possibly have been deposited had the alanin acted merely as a pseudo-glycogen former. Moreover, and this is the most convincing result of all, the urine contained lactic acid in considerable amount. That this lactic acid had actually come from the alanin, and not from the tissues of the animal, was shown by the fact that the administration of the oxyphenyl compound of alanin, namely, tyrosin (oxyphenyl-amido-propionic acid), was followed by the excretion in the urine of oxyphenyl-lactic acid. Kraus (2), as we have stated above, also found that cats, when starved and poisoned with phloridzin, excreted much more sugar when alanin was given them than when no alanin was given.

With regard to amido-acids containing six carbon atoms, if we place side by side the formula of *leucin*—the representative of this class—and that of dextrose, we shall see that no very great rearrangement of atoms would be necessary to convert the former into the latter body. Leucin is iso-butyl amido-acetic acid, and has the formula:—



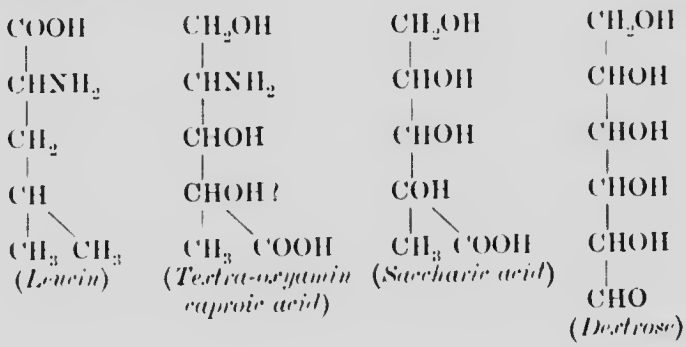
dextrose has the formula $CH_2OH-(CHOH)_4CHO$, and although the transformation would involve the production of a straight chain of carbon atoms out of one which is branched—*i.e.* of a *normal* out of an *iso*-compound—a process which is rarely met with in organic chemistry—there can be no denial on *a priori*

grounds of the possibility of the transformation. In fact, F. Muller has shown that by the action of KOH on dextrose, tetra-oxy-caproic acid—



is formed, and this contains a branched chain.

The recent discovery by Orgler and Neuberg that tetra-oxyamin caproic acid is produced from chondrosin by the action of baryta on it, furnishes us with direct chemical evidence of the possibility of sugar production from leucin, as is shown by the following equations :—



The evidence that leucin may form sugar in the animal body is by no means so convincing as the chemical results which we have just been considering might lead us to expect. Thus, Halsey ([38]) did not find any increase in the sugar excretion to follow leucin ingestion in dogs rendered diabetic by phloridzin; and feeding animals with proteids which yield much leucin has not been found, by various workers, to increase the glycogen deposits any more than do other proteids. On the other hand, Mohr has recently obtained positive evidence in a case of severe adolescent diabetes in man. The patient was for some time on a constant diet during which the daily sugar excretion varied only between 49 and 63 grm. By adding 20 grm. leucin, obtained from a pancreatic digest, to the diet, the sugar excretion rose to 72–75 grm., sinking again to 55–59 grm. after the leucin feeding was stopped.

It may be, as Langstein (2) has pointed out, that the cause of the discordant results obtained by different workers is that the same form of leucin has not been used. Felix Ehrlich has recently shown that leucin, as prepared by the usual methods, is not only a mixture of several isomeric forms of this body, but that there are also frequently mixed with it amido-acids containing fewer carbon atoms than six. In any case, this is a portion of our subject urgently requiring further investigation.

The invariable presence of *oxy-amino acids* amongst the decomposition products of proteids furnishes us with a further illustration of how sugar may be formed from this source. For example, serin, the oxy derivative of alanin, has been found by Emil Fischer (2) and his co-workers to be a very common decomposition product of proteid. Serin is oxy-amido-propionic acid, $CH_2OH.CH\ NH_2.COOH$; by substitution of the *OH* group by hydrogen, alanin is formed, which, as we have seen, easily passes into dextrose. Furthermore, the *thio*-derivative of serin is cystein, having the formula $CH_2SH.CH\ NH_2.COOH$ (protein cystein) or $CH_2NH_2.CHSH\ COOH$ (stein cystein). Cystein occurs plentifully in the body and is closely related to the taurin of bile.

The possible relationship of this to dextrose is best seen by a comparison of the following formulæ (after Neuberg):—

$CH_2SH.CH(NH)_2COOH$ (Proteincystein)
$CH_2NH_2-CHSH.COOH$ (Steincystein)
$CH_2OH-CHNH_2-COOH$ (Serin)
$\left[\begin{matrix}CH_3-CH(NH)_2-COOH\ \text{(Alanin)}\\ CH_3-CH(OH)-COOH\ \text{(Lactic acid)}\end{matrix}\right]$
$CH_2OH-CHOH-COOH$ (Glyceric acid)
$CH_2OH-CHOH-CHO$ (Glyceric aldehyde)
$CH_2OH-(CHOH)_4-CHO$ (Dextrose)

Of the other decomposition products of proteid there is not as yet a great amount of evidence that they may form dextrose. Neuberg, however, has shown that certain diamido-acids can be converted into oxy-amido-acids, which, as we have seen, may easily be transformed into dextrose.

Before concluding it should be mentioned that Seegen and others (39) have stated that in the *post-mortem* production of dextrose by the liver a greater increase of sugar occurs than

corresponds to the diminution of glycogen. On account of this result, Seegen thinks that there is in the liver some other polysaccharide besides glycogen. This polysaccharide contains nitrogen.[1] These workers also found that when peptone was added to a liver mush, reducing sugar was produced in increased amount as well as substances from which sugar could be produced by hydrolysis. Of course peptone is not normally carried as such to the liver, being converted, in its passage through the intestinal epithelium, into native proteid. The result is nevertheless of interest, because it opens up a new line of research, viz. to study the effect of adding the various decomposition products of proteid to active liver tissue.

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[2] *Cl. Bernard*, Leçons sur le Diabete et la Glycogenèse animale, Paris : Librairie J. B. Baillière et Fils (1877).

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[8] *Bendix*, Über physiologische Zuckerbildung nach Erweissedarreichung. Ztschr. f. phys. Chemie, Bd. 32, p. 179 (1901).

[9] *J. Frentzel*, Über Glykogenbildung im Tierkorper nach Fütterung mit Holzzucker. Arch. f. d. ges. Physiologie, Bd. 56, p. 273 (1894).

[10] *F. W. Pavy*, The Physiology of the Carbohydrates, London (1893).

[11] *Schöndorff*, Die Entstehung von Glykogen aus Eiweiss. Arch. f. d. ges. Physiologie, Bd. 88, p. 339 (1902).

[12] *Blumenthal* und *Wohlgemuth*, Über Glykogenbildung nach Eiweissfutterung. Berlin Klin. Wochenschr., Bd. 38, p. 391 (1901).

[1] This polysaccharide can be isolated from a decoction of liver by first of all precipitating all the proteid and glycogen with tannic acid, then removing the excess of tannic acid, evaporating *in vacuo* and adding alcohol to the residue. The resulting precipitate is largely soluble in water and yields on hydrolysis with acid from 70 to 70 per cent. of sugar. It contains 6 to 7 per cent. of nitrogen.

[14] *Hirsch* und *Balta*, Zur Frage der Entstehung von Glykogen aus Körper eiweiss. Deutsch. Arch. f. Klin. Med., Bd. 78, p. 380 (1903).

[15] *F. Pick*, Über das Glykogenspaltende Ferment der Leber. Beiträge zur phys. u. path. Chemie (Hofmeister), Bd. 3, p. 163 (1902).

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[16] *R. Chaus* und *G. Embden*, Pankreas und Glykolyse. Beiträge zur phys. u. path. Chemie (Hofmeister), Bd. 6, p. 214 (1905).

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[18] *J. M. Schiff*, Untersuch. über die Zuckerbildung in der Leber und den Einfluss der Nervensystems auf die Erzeugung des Diabetes, Würzburg (1859).

[19] *O. Minkowski*, Untersuch. über den Diabetes mellitus nach Extirpation des Pankreas. Arch. f. exper. Path. u. Pharm., Bd. 31, p. 85. (1893).

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[26] *Zuntz*, Zur Kenntnis des Phlorhizin Diabetes. Dubois Arch. (1895), 570–574.

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CHAPTER XIII

THE METABOLISM OF URIC ACID AND THE OTHER PURIN BODIES

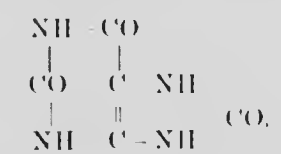
Nothing is perhaps so bewildering in the whole of bio-chemistry as are the various hypotheses regarding the metabolism of the purin bodies. The discovery by Scheele in 1776 of uric acid (lithic acid) in urinary calculi was followed, some years later, by a description of its chemical characters by Wöhler and Liebig ([20]). The main outcome of the studies of these chemists was to show the close relationship of uric acid to urea; for example, Liebig found that when uric acid was suspended in water and slowly heated with peroxide of lead (mildly oxidised), it yielded urea, allantoin, and oxalic acid. At this stage, and armed with little more than this fact relating to its chemistry, the study of uric acid metabolism in the animal was undertaken; and for many years this subject furnished a pet theme for physiological and clinical research. Most of the earlier work on uric acid metabolism is, however, of very little value, and for many reasons:—there was no accurate method for estimating uric acid in the urine; its chemistry was little understood; erroneous hypotheses by eminent men biased the interpretation which other workers put on their results, the hypotheses being considered more probable than the results when the results were contrary to expectation; no distinction was made between mammals and birds, the results obtained on birds—in which uric acid is abundant and easy of estimation—being directly applied to explain its metabolism in mammals, in which the uric acid excretion is small.

Recently, however, dating from the discoveries by Emil Fischer of the exact chemical structure of the purin bodies, and by Kossel of the composition of nucleins, a great advance has been made; and, with accurate quantitative methods, we are now in possession of a comparatively clear, though as yet fragmentary, insight into the metabolism of the purin bodies in the animal body.

In studying the metabolism of the purin bodies, as in other studies in metabolism, it is necessary that we possess a clear understanding of their chemistry: *i.e.* of their relationship to one another, of their methods of synthesis, and of their decomposition products in the laboratory. On this account, it may not be out of place to describe briefly some of these points at this stage.

Let us first of all consider the *decomposition products* of uric acid, for it is only after learning what these are that we can hope to understand the synthesis of this complex organic substance. The empirical formula for uric acid is $C_5H_4N_4O_3$. By treating uric acid with nitric acid *a double process of decomposition and oxidation ensues:* with *cold* nitric acid alloxan and urea are produced, and by further oxidising alloxan (as by treating it with *warm* nitric acid), parabanic acid and carbon dioxide gas are formed. By causing parabanic acid to take up a molecule of water (by heating it with alkalies), oxaluric acid is produced, and this can be still further hydrolysed (by prolonged boiling with water) when it yields *urea* and oxalic acid. From this we see that uric acid must contain two urea molecules (one easily liberated, the other liberated only by vigorous treatment), linked together by a chain of carbon atoms which is represented in the decomposition products by oxalic acid.

To explain, by structural formulæ, the chemical reactions involved in this process, it will be necessary to consider the constitutional formula of uric acid, although by so doing we are of course anticipating the deductions from its other reactions, for the structural formula of any substance is simply a diagrammatic expression of all its chemical reactions, and can be set forth only after all these have been considered. Medicus and Emil Fischer have shown this structural formula to be: —



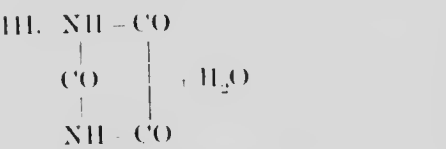
The reactions described above can then be easily demonstrated: —

I. 

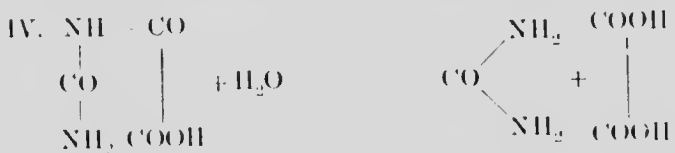
Uric acid (treated with cold HNO_3). *Alloxan.* *Urea.*

II. 

Alloxan (treated with warm HNO_3). *Parabanic acid.*

III. 

Parabanic acid (heated with alkali). *Oxaluric acid.*

IV. 

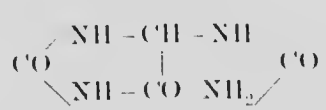
Oxaluric acid (boiled with water). *Urea.* *Oxalic acid.*

These intermediate bodies can be prepared by other means than those described above, and the accuracy of their structural formulæ thereby proven.

By using a *milder oxidising agent*, such as potassium permanganate, uric acid first of all forms a substance called allantoin. This has the empirical formula $C_4H_6N_4O_3$, and the reaction is as follows:—

$$\underset{\text{(uric acid)}}{C_5H_4N_4O_3} + O + H_2O = \underset{\text{(allantoin)}}{C_4H_6N_4O_3} + CO_2$$

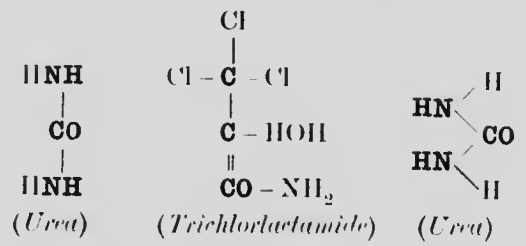
The structural formula for allantoin is



from which it is seen to be very like uric acid, possessing two urea molecules and a central carbon chain. By further oxidation

it yields urea and oxalic acid. Allantoin is, as we will see later, of peculiar interest from a physiological standpoint because it appears in the urine of certain animals (dogs and cats) when a large excess of uric acid is present in the tissues.

So far, then, we have seen that uric acid is a *diureid;* it contains in its molecule two urea radicles linked together by a carbon residue. Now any diureid can be *built up* by the condensation of oxy-acids with urea. To form uric acid, lactic acid is the most suitable oxy-acid, and, for the purpose of this synthesis, a substitution product of it, viz. trichlorlactamide, is used, because it is much more capable of uniting with such bodies as urea than the acid itself would be. (The chlorine atoms and the amido groups which this derivative contains are more easily replaced than hydrogen atoms.) If then trichlorlactamide and urea be heated together the following reaction ensues:—



(*Urea*) (*Trichlorlactamide*) (*Urea*)

The groups printed dark unite to form uric acid. Ammonia, hydrochloric acid, and water are evolved as bye-products.

Before leaving this subject of its chemical structure, another reaction of uric acid must be mentioned. If uric acid be hydrolysed (caused to take up water) by heating it in a closed tube to 170° C., it yields glycin (amido acetic acid), carbonic acid, and ammonia:

$$\underset{(Uric\ acid)}{C_5H_4N_4O_3} + 5H_2O = \underset{(Glycin)}{CH_2(NH_2)COOH} + 3CO_2 + 3NH_3$$

and if glycin and urea be melted together and heated to 220–230° C. uric acid is formed. Now glycin is abundantly present in the organism, and the above reactions might lead one to expect a relationship between it and uric acid. We will discuss this question later.

So far we have spoken only of uric acid. It must be

remembered, however, that uric acid is only the highest oxidation product of a whole series of organic substances, several of which occur in the tissues and are common ingredients of our food-stuffs. The most important of these bodies, besides uric acid, are hypoxanthin, xanthin, adenin, guanin, caffein, theobromine, and thein. The first four of these occur both in the tissues and the food, the last three only in food. These substances are called *purin bodies* because they all contain, as their nucleus of construction, a body called purin. This has the empirical formula $C_5H_4N_4$. By placing beside it the formulæ of the other members of the group, their relationships to one another are clearly seen: —

Purin, $C_5H_4N_4$

Purin bases:
- Hypoxanthin, $C_5H_4N_4O$
- Adenin, $C_5H_4N_4 - NH$
- Xanthin, $C_5H_4N_4O_2$
- Guanin, $C_5H_4N_4O - NH$

Thein and Caffein, $C_5H(CH_3)_3N_4O_2$

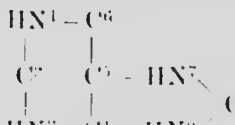
Theobromine, $C_5H_2(CH_3)_2N_4O_2$

Uric acid, $C_5H_4N_4O_3$

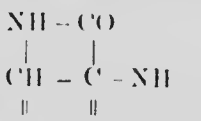
Purin is only of theoretical interest. Of the others, hypoxanthin, xanthin, and uric acid may be considered as oxides of purin, adenin as hypoxanthin in which the oxygen atom is replaced by an amido group, and guanin as xanthin with one oxygen atom replaced by an amido group. In thein and caffein all three of the hydrogen atoms of xanthin are replaced by methyl groups, and in theobromine two of them are thus replaced.

Adenin and guanin are constituents of nucleic acid, in which they are in combination with phosphoric acid and usually with a carbohydrate group. Nucleic acid is further combined with albumin to form the nuclein of the tissues. In the nuclein of the thymus gland adenin is most plentiful, whereas that of the pancreas contains almost exclusively guanin. By the break-down of the nucleins in the tissues, adenin and guanin lose their amido groups and become converted into xanthin and hypoxanthin. In their further passage through the organism these bodies are mostly oxidised to uric acid, and as this they are excreted in the urine. The methyl purins, when they are taken into the organism, lose their methyl groups and become converted into

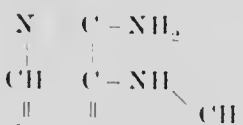
xanthin and hypoxanthin. The following structural formulæ demonstrates the chemical relationship of these bodies :—



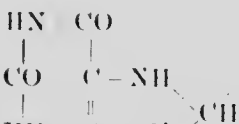
Purin



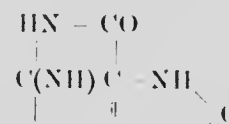
Hypoxanthin
(6 oxy-purin)



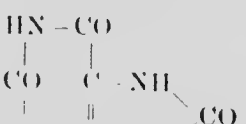
Adenin
(6 amino-purin)



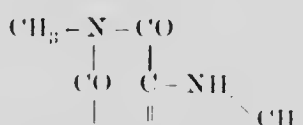
Xanthin
(2–6 di-oxy-purin)



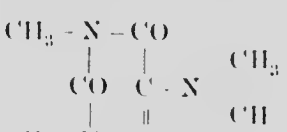
Guanin
(2 amino 6 oxy-purin)



Uric acid
(2 6–8 tri oxy-purin)



Caffein or theïn
(1 3 di-methyl-hypoxanthin)



Theobromine
(1 3 7 tri-methyl-hypoxanthin)

The atoms in purin are numbered so that we can indicate in the formulæ of the other members of the group the exact position of side groups.

We are now in a position to proceed with the **metabolism of the purins**.

In the case of mammals, the metabolism of the purin bodies is by no means so simple a study as it is in birds. In mammals, the purin bodies are excretory products of only secondary importance in comparison with urea, which forms the chief end product of proteid metabolism. Thus, in mammals, of the total nitrogen excreted each day in the urine about 86 per cent. appears as urea and only 3 per cent as purin bodies. In birds, on the other hand, the greater proportion of the nitrogen is excreted as purin bodies (uric acid) and only a trace as urea. If uric acid be given by mouth to mammals it reappears in the urine as urea : if urea be similarly fed to birds it reappears in the urine as uric acid. Before proceeding further, let us see wherein lies this difference

between birds and mammals. Why should the chief end product of proteid metabolism be uric acid in birds and urea in mammals?

In birds, uric acid is, almost entirely, produced by the synthesis of two urea molecules with a tri-carbon chain. If this synthesis be, from any cause, prevented, urea, and not uric acid, is excreted, and the bird behaves, so far as its proteid metabolism is concerned, like a mammal. The difference between the two groups of animals is therefore more apparent than real; in both cases urea is the end product of proteid metabolism, but in mammals it is excreted in the urine as such, whereas in birds it is excreted as uric acid.[1]

Our next question, therefore, is, whether or not the small amount of uric acid which mammals do excrete arises also by a synthetic process? Is it, as was at one time suggested, the remnant of an evolutionary process which betrays the development of birds and mammals from some common stock in which the synthetic process alone obtained? Or is the source of uric acid in mammals an entirely different one from that in birds? To answer this question is to anticipate much of what will follow in the succeeding pages, but it is necessary to do so briefly in order to understand what more immediately concerns us. The trace of uric acid in mammals is not, to any appreciable extent at least, produced by a synthetic process, but arises by the oxidation of other purin bodies in the organism; about one-half of it from purins given in the food and the other half from the purins which are set free in the tissues.

All the purins given in the food, or all that are liberated in the tissues, do not, however, appear in the urine. A certain portion (in

[1] This analogy between uric acid in birds and urea in mammals has been strikingly demonstrated in some experiments by T. H. Milroy ([37]). By making an artificial anus in large birds (geese, ducks, and turkeys), Milroy was able to collect urine unmixed with faeces, and so to estimate accurately in the urine how much total nitrogen, uric acid, purin bases, and ammonia were excreted. When nitrogenous equilibrium had become established and the excretion of purins constant in amount, he administered to the birds sufficient acid (hydrochloric or lactic) to produce mild symptoms of acid toxaemia, and found —besides a distinct diuresis— that the amount of uric acid in the urine became greatly diminished ($\frac{1}{10}$th its previous amount), but that of ammonia greatly increased. In mammals, acid intoxication greatly diminishes the excretion of *urea*, but causes the ammonia excretion to increase, and, no doubt, this is what had primarily occurred in Milroy's experiment; the acid had by combining with ammonia— prevented urea formation, and consequently also that of uric acid.

The acid administration did not, in the above experiments, influence the excretion of the purin bases, and it is possible that these may be produced by some process akin to that obtaining in mammals, viz., oxidation. No certain evidence of this could, however, be obtained by Milroy.

man about one-half) undergoes decomposition; the purin ring becomes disrupted, the urea molecules are liberated, and the connecting carbon chain becomes oxidised into carbon dioxide and water. The process is, therefore, exactly the reverse of that which obtains in birds. Urea in birds is a precursor of uric acid; in mammals, urea is an end product never forming uric acid but being in small part derived from uric acid.

If these facts be remembered, it will at once become evident that a knowledge of the metabolism of uric acid in birds will be absolutely valueless in explaining its metabolism in mammals; indeed, to try and do so would lead, and has in the past led, to very serious misconceptions. Let us confine our studies for the present entirely to *mammals*.

Our chemical introduction has shown us that uric acid is the oxidation product of xanthin and hypoxanthin; as we would expect, therefore, these two bodies may appear in the urine along with uric acid. To form a true estimate of purin metabolism we must, therefore, measure the total purin excretion, and, in order to investigate further those purins which are derived from the tissues (*endogenous purins*), we must eliminate the purins taken into the body in the food (*exogenous purins*). This could, of course, easily be done by giving food which contained no purin. Such a precaution would, however, be unnecessary did we know exactly how much purin we were giving in the diet and what proportion of this reappeared as purin in the urine.[1]

From these preliminary remarks we see that the questions to be considered first of all are these:—what food-stuffs contain substances capable of influencing the urinary purin excretion and what is the nature of these substances? what proportion of these substances reappears in the urine and in what form? (that is, as uric acid or some other purin). In answering these questions, it will be necessary to consider a method for estimating endogenous purins. We will then be in a position to see whether or not the amount of endogenous purins excreted in the urine bears any con-

[1] In fact, to be certain of maintaining everything in a perfectly physiological state, the latter diet would probably be preferable, at least in the case of carnivora, since these animals are habituated to a diet containing purin bodies, and it might conceivably be subjecting them to abnormal conditions, were we to place them on one devoid of purins. As we shall see later, this hypothetical purin starvation is of no account, since nucleins are built up out of purin-free bodies and not out of purins themselves.

stant proportion to the amount of purins set free in the tissues, in which case it would be possible, by multiplying the excreted amount by a certain factor, to determine how much purin formation is actually occurring in the body. It is only after the above points have been cleared up that we can expect to understand what the conditions are which influence this tissue formation of purins.

The Chemical Nature of the Food-Stuffs which Influence Purin Excretion

It is not so very many years ago since it was universally believed that the urinary uric acid was derived solely from the proteid given as food (¹). As proof of this, it was pointed out that the quantitative relationship between urea and uric acid in the urine is a constant one. This we would of course expect to find were both these bodies derived from the same source (from proteid.)

As a matter of fact the quotient $\left(\frac{\text{urea}}{\text{uric acid}}\right)$ which expresses this relationship was found to be fairly constant (viz. 45 65) when flesh diet alone was given; but when the diet was flesh-free (composed of milk, pepton, and vegetable proteid) a quotient quite different from that observed on flesh food was obtained. On such a flesh-free diet, it was found that the uric acid excretion became much smaller in amount, and remained at this low level however much the amount of flesh-free proteid might be increased or diminished in the diet, whereas on the other hand, the urea excretion rose and fell with the amount of proteid ingested. For example, Camerer (¹⁰) was able to double the total nitrogen excretion without there being any change in the uric acid nitrogen, so that a high quotient was obtained when much proteid was taken $\left(\frac{\text{urea (raised)}}{\text{uric acid (constant)}}\right)$ and a low one when there was only a small amount of proteid $\left(\frac{\text{urea (lowered)}}{\text{uric acid (constant)}}\right)$. It is scarcely necessary to dwell longer on this supposed relationship. The enormous variations which it undergoes under different diets even in the same person, a variation between 23·2 and 122·4 in some cases collected by von Noorden (²), shows us, once and for all, that *the uric acid excretion can bear no relationship to the extent of proteid metabolism.*

One fact, however, stands out clearly in the results of the numerous researches which were recorded in connection with the influence of diet on the uric acid excretion, and this is that flesh contains *something* which influences the excretion. What could this be? The chemical relationship between the xanthin bases, and uric acid, already described in the chemical introduction, suggested these bases as possible precursors of uric acid, and experiments were accordingly undertaken in which purin bodies were given in the food to animals, and their influence on the uric acid excretion observed. The first experiments along these lines yielded, however, entirely negative results; the cause of which was later shown to be that unsuitable animals (dog, rabbit) had been chosen for the experiments, *i.e.* animals in which an extensive break-down of the purin ring ensues. More recently by experimenting on man, it has been definitely established that hypoxanthin at least causes quite a distinct increase in the excretion of uric acid, and that meat extract, which contains a considerable amount of this oxy-purin and also of xanthin, had the same effect (2). *There can be no doubt then that xanthin and hypoxanthin can cause a marked increase in the excretion of uric acid in man.*

There are certain animal food-stuffs (thymus gland, &c.), however, which do not contain, in simple extracts of them, any large amount of these oxy-purins, and which nevertheless cause, even in dogs, a distinct increase in the purin excretion. A clue to the influence of these foods on the uric acid excretion was furnished in 1882 by Kossel (1). By this worker it was shown that certain xanthin bases are among the chief decomposition products of nucleins, which are very abundant in these food-stuffs, and the idea was suggested by him that uric acid might be derived from these, hypoxanthin being formed as an intermediate product. It was, however, several years later before it could be conclusively shown that this supposition of Kossel was correct. This Horbaczewski (3) succeeded in doing. He allowed a mixture of one part of spleen pulp and eight parts of water to stand at a temperature of about 50° C. for eight hours. After this time, moderate putrefaction had set in. By filtering he obtained a fluid from which—after precipitating various substances with lead acetate, and separating the proteid by boiling—he was able to isolate a large amount of xanthin and hypoxanthin. On

the other hand, no xanthin and hypoxanthin or only a trace could be prepared from a fresh extract of spleen. If the incubated, slightly putrid mixture were supplied with oxygen as by shaking it with air, or adding H_2O_2 or blood to it, he obtained uric acid instead of hypoxanthin and xanthin.

That these purin bodies were actually derived from the nuclein of the spleen was demonstrated by the fact that nuclein, prepared from *any* organ or tissue by the usual methods, gave similar results when treated in the same way as described above for the spleen. Horbaczewski concluded from these experiments that nuclein must contain some precursor from which, on its decomposition, either xanthin and hypoxanthin *or* uric acid was produced; the former bodies when no oxygen was present in the mixture, the uric acid in the presence of oxygen. He further stated that it was impossible to convert xanthin bases directly into uric acid.

Spitzer (6) repeated Horbaczewski's experiments and found that uric acid could be produced by merely bubbling air through a watery extract of spleen pulp, in which putrefaction was prevented by adding chloroform or thymol. Putrefaction is, therefore, not necessary for the process. This worker further found that xanthin and hypoxanthin could be directly converted into uric acid by *adding* these bodies to an extract of spleen or liver and bubbling air through the mixture at a temperature of 50° C. Extracts of other organs, such as kidneys, thymus, and pancreas could not affect this oxidation to anything like the same extent as could those of the liver and spleen.

Burian (35) has recently continued Spitzer's work on the conversion of xanthin and hypoxanthin into uric acid by the liver. By macerating the minced liver of the ox with ice-cold chloroform water this worker has been able to prepare an extract, containing only traces of nucleo-proteids and purin bodies, but possessing quite a marked power of oxidising xanthin and hypoxanthin into uric acid when these bodies are added to the extract, and the whole incubated at body temperature in the presence of an excess of oxygen. He has been able to show that this action is due to an oxidising ferment—*xanthin-oxydase*—which itself does not become used up during its action, and which does not show any reversible action (*i.e.* cannot reduce uric acid to xanthin bases).

Xanthin-oxydase cannot, however, convert guanin and adenin

—the amido-purins which exist in nuclein—directly into uric acid; and yet, as we have seen above, nuclein undoubtedly is an important mother substance of uric acid in extracts of spleen and liver. It will be remembered that guanin is amido-xanthin and adenin amido-hypoxanthin—that is to say, that by the removal of an amido group (NH_2) the transformation into the corresponding oxy-purin would occur. Jones [36] and his co-workers and others have shown that the spleen and liver of oxen possess another ferment—*guanase* or *adenase*—which effects this removal of the amido group.

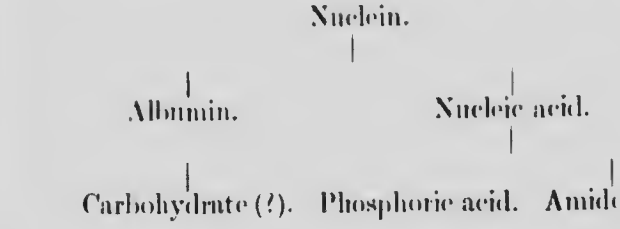
The process of uric acid formation from nuclein entails, therefore, first of all a decomposition of the nuclein whereby, among other things, amido-purins are produced; a denitrification of these amido-purins by guanase (and adenase); and then an oxidation of the resulting oxy-purins into uric acid by means of xanthin-oxydase.

These laboratory experiments undoubtedly prove that *oxy-purins can be derived from some group present in nuclein.*

With regard to the evidence that feeding with nuclein influences the amount of urinary purins, Horbaczewski added 5 grm. of nuclein prepared from spleen pulp to the diet of a *man* and found an increase of 0·28 grm. in the urinary uric acid. Various observers confirmed Horbaczewski in this result, and showed further that *all nuclein containing food-stuffs influenced the uric acid excretion according to the amount and nature of the nuclein which they contained.*[1]

In connection with the effect of nuclein feeding, our next inquiry is *whether or not the purin substances which the nuclein contains can, when given in an uncombined state in the diet, influence the purin excretion in the same way as nuclein itself does!* The chemical structure of nuclein is demonstrated by the following scheme:—

[1] These researches concerned almost without exception the uric acid excretion; the coincident behaviour of the other urinary purins was not, for want of reliable methods, clearly demonstrated. Horbaczewski explained the results which followed nuclein feeding by supposing that an excessive destruction of leucocytes was produced, and that it was from the liberated nuclein of these and not from the nuclein ingested, that the uric acid was derived. That such an explanation could not hold was shown by Weintraud [7], who found that a thymus diet did not cause any greater an increase in the number of leucocytes in the blood than an ordinary diet did, but, nevertheless, increased the uric acid excretion to a much greater extent. Weintraud therefore concluded that the *administered nuclein itself furnished the urinary purin.*



The amido-purins contained in nucleic acids of different sources (prepared from different nuclein) vary in their nature. The nucleic acid of the thymus contains mainly adenin, that of the pancreas guanin alone, and that of a salmon sperm both guanin and adenin. The amount and nature of carbohydrate in the molecule also varies considerably.

Knowing, then, that nuclein contains amido-purin bodies in its molecule, we might naturally assume that feeding with these purins in a pure state (*i.e.* with guanin and adenin) would influence the purin excretion just as nuclein itself does. This is, however, not the case, neither adenin nor guanin having much effect on the purin excretion, at least in dogs, and, in man, so far as they have been tried, only having a doubtful effect.

The last group of food-stuffs which contain purin bodies are such substances as thein, caffein, and theobromine, which it will be remembered are methyl-purins. These do not raise the excretion of uric acid, when they are given in the food, but, as we shall see later, they do raise that of the purin bases.

So far, then, we have fairly answered our first question. We have seen that exogenous urinary purins may be derived from *certain free xanthin bases, but not from all, and from nucleins.* No other nitrogenous food-stuff in any way influences the amount of the urinary purins. We see why it is that the urea and purin excretions cannot be expected to run parallel, for, by feeding with purin-free nitrogenous food in varying amount, the urea can be made to swing up and down at will, the uric acid remaining, meanwhile, absolutely stationary.[1] These facts show us how hopeless it was in the older researches to try and strike a constant average for the normal purin excretion of man; and they

[1] For example, Hess and Schmoll (*) found no increase in the urinary purins by adding twenty-four eggs to a fixed diet, although the urea excretion rose enormously.

explain why some observers believed the purin excretion to depend on the diet whilst others denied any connection between the two.

Our next question, what proportion of ingested purin reappears as such in the urine? cannot be answered until we have become acquainted with a method for estimating the endogenous moiety (that portion of the purins derived from the tissues), and it is with this problem that we will concern ourselves now.

The Determination and Fundamental Characteristics of the Endogenous Purin Excretion

If the daily purin excretion be determined during starvation, it will be found to fall gradually during the first few days, and then to gain a level at which it remains practically constant. The gradually lessening excretion during the first few days is no doubt due to exogenous purins being drained out of the system, and the constant level afterwards attained must mean that all this store of exogenous purins is exhausted, and that the tissues are themselves furnishing purins. Is this starvation excretion, then, not an accurate measure of endogenous purin? Probably not, for starvation is no normal state, there being, during it, a greater break-down of the tissues, amongst others of muscle, than in health, and, consequently, an abnormal liberation of purins. To measure the endogenous moiety, therefore, we must disturb the general metabolism as little as possible; we must estimate the purin excretion whilst the organism is living on a diet containing an adequate amount of nitrogen and a sufficient number of calories to maintain the tissues, but no purin bodies. Is such a diet obtainable? Considering analytical data alone, the only food-stuffs which contain absolutely no trace of purins are carbohydrates, pure fats and eggs. There are other food-stuffs, however, which contain only minute traces of purin bodies (milk, 0·004–·006 per cent.; potatoes, 0·0005–·0006 per cent.; white bread, minutest traces), and since Burian and Schur (9) have shown that these latter foods do not have any appreciable influence on the urinary purins, we may, for all practical purposes, consider them also as purin-free food-stuffs.

The following experiment by Burian and Schur (9) will make clear *how the endogenous moiety of purin excretion may be measured*. The experiment was divided into four periods. During these

periods the following diets were taken: *Period I.*—A diet of 250 grm. beef, and 120 grm. ham, with potatoes, bread, cheese, butter, &c.—total nitrogen, 16·8 grm. *Period II.*—Instead of flesh, ten eggs and a litre of milk were taken; the other constituents were the same as in Period I., the quantities being slightly varied. This diet contained no purin-yielding food—total nitrogen, 16·8 grm. *Period III.*—The diet contained the same substances as that of Period II., only much less milk and eggs, so that the total nitrogen content was 9·3 grm. Rice was added to bring the calories to the same level as in Period II. *Period IV.*—A purely vegetable diet (500 grm. potatoes, 100 grm. rice, bread, butter, and sugar), containing the same amount of nitrogen as Period III.

For convenience we may describe these diets as follows:—

Period I.—Meat diet of high nitrogen content.

Period II.—Milk, cheese, and egg diet, of equally high nitrogen content.

Period III.—Milk, cheese, and egg diet, of low nitrogen content.

Period IV.—Vegetable diet of equally low nitrogen content.

Each of these periods lasted four days, and during them the total nitrogen and total purin excretions were measured. The following table shows the results.

TABLE I

Period.	Nature of Diet.	Nitrogen of Diet.	Nitrogen of Urine and Fæces.	Nitrogen of Purins excreted in Urine.
		Grm.	Grm.	Grm.
I.	Mixed diet with large amount of flesh	16·8	16·13	0·3462
			16·85	0·3111
			16·82	0·3382
			16·67	0·3390
II.	Flesh-free diet, with large amount of milk, eggs and cheese	16·2	16·59	0·2112
			15·36	0·2076
			10·13	0·1991
			10·02	0·1997
III.	Diet similar to II., but with portion of milk, eggs and cheese replaced by rice	9·3	10·91	0·2072
			9·91	0·2058
			9·07	0·1971
IV.	Vegetable diet with same amount of nitrogen as III.	9·1	8·94	0·1973
			9·28	0·2011
			9·23	0·2082
			9·13	0·1981

It will be noticed that there was no appreciable change in the *nitrogen excretion* between the first and second periods. Between the second and third periods there was, of course, a temporary disturbance lasting for three days, more nitrogen being excreted than administered, but by the fourth day equilibrium had again been attained, and on changing to the vegetable diet (fourth period) no further disturbance occurred. The necessary conditions for a rational measurement of the endogenous purins were thus obtained in Period II., there being here no disturbance in metabolism.

Turning now to the *excretion of purins* during these four periods, and taking for this purpose the average of the last three days of each period (since during the first day the previous diet will still have an influence on the urine), we obtain the following values:—

Period I.,	total purin nitrogen,		0·339	grm.
„ II.,	„	„	0·202	„
„ III.,	„	„	0·203	„
„ IV.,	„	„	0·203	„

The endogenous moiety here is obviously 0·202 grm. purin nitrogen; and the exogenous moiety during the first period 0·137 grm. purin nitrogen. *The endogenous moiety*, it will be seen, *is uninfluenced by the nature and amount of the diet, provided always that this is purin-free* and is sufficient in amount, and of proper composition to prevent starvation. Chemical analysis has already shown us that the food-stuffs given in the last three periods of this experiment are practically purin-free, and now we have the proof that from the physiological point of view they may be considered entirely so, for did the food-stuffs during these periods contain any appreciable amount of purin, there obviously would have been variations in the amount of purins excreted between Periods II. and III. and Periods III. and IV.

These findings of Burian and Schur were almost simultaneously confirmed by Siven, in whose experiments longer periods of time were allowed and greater variations in nitrogen intake brought about, and still with the same constancy in endogenous purin excretion.

In a series of very complete metabolism observations on men, recently recorded by Folin, it has been noted, among other things,

that this constancy in the amount of endogenous *uric acid* on changing from a purin-free diet of high nitrogen value eggs and cream—to one of low nitrogen value—starch and cream—is not so marked as Burian and Schur state it to be. In several of the investigations recorded by Folin, the change in endogenous uric acid following the change of diet was indeed very slight, but in two cases it was quite marked. This worker, while accepting in general Burian and Schur's contention, would, therefore, not consider it as infallible. In other observations recorded by Folin, it was noted that on changing from a pure starch diet to one of potatoes a very distinct increase in the endogenous uric acid excretion occurred. This would seem to show that the amount of purins in potatoes, though quantitatively small, is yet sufficient to influence the exogenous excretion.

Burian and Schur have collected together from the literature a considerable number of estimations of the endogenous purin excretions by this direct method, and have found that it *may vary considerably in different individuals*. Taking all the results hitherto obtained, Burian and Schur give the following figures:—

0·12 grm. purin nitrogen as lowest endogenous quotient (1 cases).						
0·16	„	„	„	„	„	(10 cases).
0·20	„	„	„	„	„	(4 cases).

These "levels" do not depend on any known physiological condition of the individual. Walker Hall ([13]) has tried to show that body weight and endogenous purin excretion ran parallel, but, even from his tables can no simple relation be seen. It is possible, however, that it may depend on muscle weight, for, as we will see later, much of the endogenous moiety probably comes from the muscles. Nor does the average *daily* level bear any relation to the amount of work which is performed, provided this be neither too excessive nor too slight. We have already seen it to be uninfluenced by the diet, provided this be purin-free and sufficient in amount to prevent starvation.[1]

[1] It should, however, be pointed out in this connection that the purin excretion is not so large in complete starvation as when proteids alone are withheld in the diet.

That the excretion of endogenous purins is variable for different individuals, has been accepted by most of the modern workers, but is denied by Loewi [14, 15, 16]. His conclusions are based mainly on observations on three persons who were of about the same age, and each of whom received the same mixed diet. The uric acid excretion in the three persons was the same, and from this Loewi concludes that similarly fed men in similar conditions of metabolism excrete the same amount of uric acid, and that this latter is dependent on the food alone, and does not show any difference due to individuality. It must be evident, however, that it by no means disproves Burian and Schur's statement, that Loewi should have happened to observe three persons excreting, on the same diet, the same amount of uric acid. In the collected cases, which we have given above, ten out of eighteen had the same endogenous purin excretion, and if to any three of these ten persons the same amount and kind of purin were given in the food, their total purin excretion would be the same, for, as we shall see later, exogenous purins reappear in the urine to the same extent in all individuals.

Loewi states further that it is incorrect to try and measure the endogenous uric acid on a purin-free diet, because the tissues, in such a case, will be starved of nuclein, and will, consequently, not destroy so much of it as they would were an adequate supply furnished them from without. This presupposes, of course, that tissue nuclein is regenerated from food nuclein, and, in such a case, Loewi's argument would be correct enough: Burian's endogenous purins would represent a starvation amount, and on giving nuclein in the food the first thing to happen would be that the starving tissues appropriated some, at least, of the nuclein for reconstruction, and the endogenous factor would fall. Loewi's contention is, however, absolutely groundless, for tissue nuclein is not built up out of food nuclein, but out of a nuclein-free substance, even when nuclein rich food-stuffs are also present (Burian and Schur).

Although variable for different individuals, *the endogenous purin excretion seems to be constant for any one person, provided he be living under the same physiological conditions.* Even a considerable disturbance of nitrogen equilibrium appears to have no

influence on the amount; if, however, there be a fundamental change in the mode of life, if there be a much higher or lower caloric exchange in the tissues, then will there be a variable endogenous excretion. After excessive muscular work there also occurs a temporary rise. That the endogenous quotient remains constant under similar conditions is demonstrated in the following table:—

TABLE II

Investigated Person and Investigator.	Date.	Endogenous Purin Nitrogen in Grm.
Burian (recorded by Burian and Schur)	July 1899	0·203
,,	May 1899	0·199
,,	November 1899	0·200
,,	December 1900	0·199
		Uric Acid.
A (recorded by Rockwood)	December, January 1903	0·308
,,	January, February 1903	0·305
,,	March 1903	0·315
,,	May 1903	0·321
,,	July 1903	0·313
,,	November 1903	0·298
C (recorded by Rockwood)	January 1903	0·478
,,	March 1903	0·452

In these observations by Rockwood the diet, although purin free, varied considerably in composition at the different periods.

Having described how the endog[illegible]us quotient may be directly determined, and the fundamental characteristics of this portion of purin excretion, we are now in a position to consider what *proportion of the purin administered in the food, i.e. exogenous, reappears as such in the urine, and whether this portion which reappears varies with the kind of purin administered!*

As has been shown above, food stuffs which contain purin bodies in greatest amount are the cellular tissues (glands) and the muscles. These tissues contain their purin bodies partly in a free state (xanthin and hypoxanthin in muscle) and partly combined in nuclein. It will be remembered, however (see p. 399), that free adenin and guanin seem to exercise a different influence on the purin excretion according to whether they are given free or built up into the nuclein molecule. In

estimating the purin contents of an organ it is of importance, therefore, to determine independently the *combined* and the *free* purins. It would be beyond the scope of this article to describe the methods used for these estimations; the details are fully given, and the accuracy of the methods proven, in a recent paper by Burian and Walker Hall ([12]). Of importance for our purpose, however, are the results obtained for different animal food-stuffs. These are given in the following table.

TABLE III

100 *Grm. Flesh Substance contain*:—

Organ.	Total Purin N.	Free Purin N.	Nuclein Purin N.	Kind of Purin.
	Grm.	Grm.	Grm.	
Flesh . .	0·036	0·045	0·015	Xanthin and Hypoxanthin
Thymus of calf .	0·45	0·05	0·40	Adenin
Liver of calf .	0·12	—	—	—
Spleen of calf .	0·16	—	—	—
Pancreas of pig .	0·123	—	—	Guanin
Pancreas of ox .	0·183	—	—	Guanin

Only in the case of flesh and thymus gland is the proportion of free to nuclein purins given. It will be noticed that in flesh most of the purin is present in a free state as xanthin and hypoxanthin, whereas in thymus most of it (adenin) is combined. The methyl-purins represented by the alkaloids caffein, thein, and theobromine must also be reckoned as purin-containing food-stuffs.

As already pointed out, in estimating the purins in the urine, a distinction has to be made between the completely oxidised purin uric acid, and the less completely oxidised basic bodies xanthin and hypoxanthin. This is necessary, as it is, on *a priori* grounds, improbable that the three groups of purins, the oxy-purins (xanthin and hypoxanthin), the amido-purins (adenin and guanin), and the methyl-purins (caffein, &c.), will all undergo the same changes in the organism and be excreted in the urine in the same form. To convert the oxy-purins into uric acid, oxidation alone has to occur, but before oxidation of the

others can take place a preliminary disruption, of CH_3 in the case of methyl-purins, and of NH_2 in the case of amido-purins, has to occur.

Confining our considerations for the present to *man*, it will be seen that for the purpose of our experiment it is unnecessary to determine the endogenous excretion. All that is necessary is to keep him on a constant diet, and, when the purin excretion is constant, to administer a carefully weighed amount of the purin, or purin containing food-stuff under investigation. As a result of investigations on this plan it has been found that of *hypoxanthin* given with the food, one-half of that administered reappears the urine as uric acid. The same result is obtained if flesh, liver, or spleen be given, for, as our table shows, these contain mainly hypoxanthin. Thus in an experiment of Burian and Schur's 100 grm. of meat (containing therefore 0·06 grm. purin nitrogen) caused an increase of 0·03 grm. in the purin nitrogen content of the urine: 100 grm. calf's liver (containing 0·12 grm. purin nitrogen) caused an increase of 0·06 grm., and 100 grm. calf's spleen (containing 0·16 grm. purin nitrogen) an increase of 0·08 grm. In all these cases it was uric acid which was increased, and the increase was present for two or three days succeeding the day on which the purin was given.

Taking the *amido-purins* next. It has already been pointed out (p. 399) that adenin and guanin, the purin bodies present in nuclein, when added in a pure state to the diet do not cause any change in the *uric acid* excretion. This does not mean that the purin excretion is unaffected, for it has been found a certain amount of these purins—of guanin at least—reappears unchanged in the urine. It passes through the organism intact. When presented in a combined state as nuclein, however, quite another result is obtained: there is a distinct increase in the uric acid excretion. Thus 100 grm. of the thymus of the calf (containing 0·40 grm. nuclein-purin nitrogen) gave an increase of 0·10 grm. urinary purin nitrogen, the increase being in uric acid. In the case of the *methyl-purins*, 100 grm. coffee containing 0·20–0·22 caffein nitrogen yielded 0·075 grm. purin nitrogen.

To sum up, then—*about one-half of hypoxanthin-*N *reappears in the urine as uric acid, about one-fourth of nuclein purin-*N,

also as uric acid, and of caffein-N, about one-third as basic purin-N.

The amount of this exogenous urinary purin remains constant for any given dietetic purin, and—a very important fact—*it is the same in all individuals of the same species.* (To obviate subsequent confusion, it may not be out of place to mention here that, although constant for all individuals of the same species, it varies enormously for different species. For example, as we have seen above, of hypoxanthin man re-excretes one-half as uric acid, a dog on the other hand excretes only one-twentieth.) Burian and Schur have collected together a large number of experiments, in which hypoxanthin and hypoxanthin-containing organs were fed to different individuals, and have shown that the variations in the percentage of purin which passes into the urine lies between 63·2 per cent. as a maximum, and 46·2 per cent. as a minimum. These differences are probably due to errors in estimation and technique, the most carefully conducted estimations lying near 50 per cent.

We have seen so far: (1) that, by placing a person on a purin-free diet, the endogenous moiety of urinary purins can be directly estimated; (2) that any given purin body, when given in the food, raises the purin excretion always to the same extent; (3) that the exogenous moiety depends solely on the chemical nature of the purin given in the food. From these facts it follows, then, that *the endogenous moiety can be calculated indirectly.* To do this we must know the exact amount of purin-containing food ingested and the nature of the purins which it contains. By deducting from the total purin excretion the amount of purin which we know—by applying the results detailed above—must have been derived from the food, we obtain the endogenous purin excretion. The accuracy of this method can, of course, very easily be put to the test. A person is placed on a diet containing an accurately known amount of purins; after a few days on this diet, the purins are removed and a purin-free diet is given. By subtracting from the purin excretion of the first period the amount of exogenous purins which it ought to yield, a product is obtained which should be identical with the directly estimated endogenous moiety of the second period. Is this the case? A table arranged on this plan, and compiled from various sources, by Burian and Schur, shows

that it is. It may be of interest to give this table in its entirety here.

TABLE IV

Grm. of Uric Acid N or total Purin N excreted per Diem.[1]

A. On purin-rich Diet.	B. Calculated amount of Exogenous Purin which the Diet of A could yield.	C. On Purin Free Diet (*Direct Endogenous Value*).	D. Difference between A B (*Indirect Endogenous Value*).	Observers.
0·339	0·133	0·203	0·206	Bucian and Schur
0·244	0·091	0·153	0·153	
0·393	0·230	0·155	0·163	
*0·283	0·069	0·214	0·214	Herringham, Davies and Groves
*0·230	0·084		0·146	Loewi
*0·192	0·045	0·142	0·147	
*0·202	0·060		0·142	
*0·241	0·084	0·173	0·160	
0·239	0·075	0·169	0·164	Krüger and Schmidt (29)

In *other mammals* than man, the proportion of ingested purin which reappears in the urine may vary considerably. For example, in dogs the purin excretion is much less proportionally, for about ten times as much purin disappears in its passage through the organism in the case of this animal as in the case of man; in rabbits three times as much disappears. Indeed, we have every reason to believe that the amount which disappears, and therefore the amount which passes into the urine, is not the same for any two groups of animals, although it is probable that closely related animals (cat and dog) excrete about the same fraction.

We are now in a position to consider *whether or not the purin bodies produced in the tissues themselves* (from nuclein break-down and from the muscles) *behave according to the same laws which govern those of exogenous origin*. In other words, does the amount of endogenous purin excreted in the urine bear the same relation to the amount of purin produced in the tissues, as the exogenous purins in the urine bear to the purin of the food?

In answering this question, we must first of all see whether

[1] Those marked * are calculated as uric acid N.

the increase in the purin excretion which follows purin ingestion really is due to some of the ingested purin reappearing in the urine, or whether it may not be the result of a stimulating effect which exogenous purins might conceivably have on the production of purins in the tissues—in other words, on the endogenous production of purins. The former explanation is made probable by the fact that in dogs the subcutaneous injection of a solution of uric acid and feeding with thymus gland or with hypoxanthin, all increase the purin excretion to about the same extent.[1] The following table demonstrates this:—

TABLE V

Average purin-*N* in dog's urine on an endogenous diet	0·0156 grm.			
Increase of purin-*N* after feeding with 0·4 grm. purin-*N* in thymus	0·0157 grm.	39	of the purin administered.	
Increase in purin-*N* after subcutaneously injecting 0·4 grm. uric acid-*N*	0·0160 grm.	1	„	„
Increase in purin-*N* after feeding with 0·4 grm. hypoxanthin-*N*	0·0185 grm.	= 1·6	„	„

In man, too, the addition of hypoxanthin to the diet raises the excretion of uric acid but has no influence on that of phosphoric acid (Krueger and Schmidt, *vide* [20]). If the higher purin excretion, in this case, had been due to an increased endogenous production, the phosphate excretion would probably have been raised as well as that of the purin bodies, since phosphoric acid is also an ultimate metabolic product of nuclein, and nuclein is, in part at least, a source of endogenous purins. There can be no doubt, therefore, that a portion of administered purin passes unchanged into the urine.[2]

The above table furnishes us also with data from which our

[1] The difference in the increase of exogenous purins following ingestion of amido- and oxy-purins is not evident in the case of the dog.

[2] A point of difference between the dog and man must be noted here, viz. that thymus purins are excreted in the dog's urine to the same extent as hypoxanthin, whereas in the case of man this is not the case.

leading question can be answered: from it we see that, in the dog at least, feeding with thymus gland and with hypoxanthin raises the exogenous excretion to the same extent as does the subcutaneous injection of an equivalent amount of uric acid. Now adenin, the purin in thymus nuclein, differs from hypoxanthin in having an amido group in place of oxygen (see p. 392). Since both adenin and hypoxanthin are easily oxidised into uric acid, and all the three, *i.e.* adenin, hypoxanthin, and uric acid itself, raise the purin excretion to the same extent, it is highly probable that in the blood they are all converted into the same substance, viz. uric acid, which is then partially destroyed. In other words, any oxy- or amido-purin, in whatever way it gets into the systemic blood of the dog, is probably converted into uric acid,[1] of which one-twentieth part (5 per cent.) is excreted unchanged in the urine. We must naturally assume, then, that the endogenous purin bodies, produced by nuclein disintegration or from the muscles, will be excreted in the urine to the same extent. If experiments of this type be repeated at different periods on the same dog, or on different dogs, similar results will invariably be obtained, one-twentieth to one-thirtieth of the uric acid present in the blood being excreted in the urine; and this is true from whatever source, *i.e.* exogenous or endogenous, the uric acid is derived. Subcutaneous injection, feeding with hypoxanthin or with nuclein and decomposition of tissue nuclein, all yield the same result ([32]).

In cats, Mendel and Brown ([15]) found that of pancreas purin-*N* 4·5–5·7 per cent. reappeared as uric acid-*N* in the urine; figures, in general, very like those which Burian and Schur obtained for dogs.[2]

If, then, the actual uric acid excretion in these animals (cats, dogs) be multiplied by 20–22, the product will represent the total amount of uric acid produced in the tissues. This figure Burian and Schur call the *integral factor for purin excretion*.[3]

[1] This is brought about by an oxidising ferment, xanthin-oxydase. Uric acid given by the mouth is all converted into urea.

[2] Intravenous injection cannot be employed for the experiments because it produces profound depression.

[3] Kanger ([31]) has recently tried to make out that in cats the amount of uric acid excreted bears no numerical relationship to the amount of uric acid given in the food. If, however, his results be calculated out on Burian and Schur's method, they will be found to agree very well with the laws formulated by these workers. This is true until a dose of ·5 grm. uric acid is given, when poisoning symptoms appear.

Similar experiments on herbivorous animals yield quite a different integral factor. For example, in the rabbit subcutaneous injection of hypoxanthin or of uric acid causes an increase of purin-*N* excretion amounting to 17·1 17·7 per cent. of the injected purin-*N*. The integral factor in rabbits is therefore 6.

To prove that the integral factor is the same for exogenous and endogenous purins in the case of man it is, of course, impracticable to perform all the injection experiments which have been practised on dogs. Several such experiments have, however, been performed, and, so far, they have yielded positive results. Schur, for example, injected subcutaneously into himself 1 grm. of uric acid, in sterile alkaline solution, and found an increase in urinary uric acid amounting to 19·85 per cent. of the injected uric acid. In a second experiment 47·95 per cent. was recovered. We have already seen that, in man, feeding with xanthin or hypoxanthin raises the purin excretion to a similar extent, viz. 50 per cent.[1] That these two modes of purin assimilation should raise the urinary purins to an equal extent renders improbable any supposed influence the exogenous purins might conceivably have on the liberation of endogenous purins, for it is unlikely that both modes would effect the metabolism of the latter just to the same extent. *The integral factor for man, then, is 2.*

What becomes of that portion of food purin which disappears in its passage through the organism?

There are two possible answers to this question. It might be stored up somewhere in the organism or it might be decomposed, the purin ring being ruptured and excreted as something else. The former possibility, that uric acid may be stored up somewhere in the tissues, has been expounded as the true state of affairs mainly by Haig (82); but, despite the large number of observations which he has recorded in support of this view, it is not generally accepted. It is disproved by the following, among other experiments: if uric acid be injected subcutaneously into a dog, one-twentieth part of it reappears as such in the urine; according to

[1] This average (50 per cent.) for purins which reappear in urine in man is only applicable to healthy individuals with full absorption, since, if absorption be disturbed, the purins may pass into the faeces (Walker Hall). The purins of meat are readily absorbed, whereas thymus, guanin, and nucleic acid are apt to raise the purin percentage in the faeces. On an ordinary diet the purins of the faeces amount to about ·05 ·06 grm. N in the twenty-four hours.

Haig, part at least of that which disappears becomes deposited in an insoluble form in certain of the tissues, to be very gradually removed by leucocytes. If this be a correct explanation, a much larger amount of similarly injected hypoxanthin, which is readily soluble in the tissue fluids, should be excreted in the urine. Experiments show, however, that in this case also one-twentieth of the injected hypoxanthin appears in the urine. *We must conclude, therefore, that it is destroyed.*

By what chemical process does this destruction take place? In the laboratory, uric acid may be decomposed by various agencies, and the end products are different according to which of these is used. The most important agencies, it will be remembered, are: (1) *Hydrolysis*—heating to 170° C. in a closed tube with hydrochloric acid, yields glycin, carbonic anhydride, and ammonia. (2) *Mild oxidation*—treating with potassium permanganate or with peroxide of lead, yields allantoin and carbonic anhydride. (3) More *powerful oxidation* with nitric acid yields as ultimate products oxalic acid and urea. To determine by which of the processes destruction of the purin ring takes place in the organism, it will be necessary to find out which of the possible decomposition products becomes increased in the tissues or excreted in excess after extensive purin break-down.

Of the decomposition products above indicated, urea, allantoin, and oxalic acid are normal constituents of the urine, although the latter two only in minute traces, and glycin is a constituent of the tissues. An increase in urea excretion could result from purin destruction by any of the three ways indicated, for glycin, produced by the first process, and possibly the allantoin, produced by the second, might be converted into urea before being excreted. An increase of allantoin could only result from a process akin to the second reaction, whereas an increase of oxalic acid might mean processes like the second and third, for oxidation of allantoin in the laboratory yields urea and oxalic acid.

With regard to *glycin*, most authors have considered this rather as a precursor of uric acid than as one of its decomposition products. As a matter of fact, however, Wiener [18, 20] has definitely shown it to be a decomposition product, at least in the rabbit. In these, as in all herbivorous animals, a large part of the glycin in the organism unites directly with benzoic acid to form hippuric acid. If a large

amount of benzoic acid be given to rabbits so that an excess is present in the tissues, the hippuric acid excretion will become maximal and of constant amount; all the available glycin in the organism being used up to form hippuric acid. In such a condition any added glycin, by uniting with the excess of benzoic acid, will at once raise the hippuric acid excretion. By feeding such rabbits with uric acid Wiener obtained an increase in the hippuric acid excretion, showing plainly that glycin had been formed in the organism. Wiener has confirmed this result by finding that glycin is formed when uric acid and a saline extract of ox's kidneys are incubated for some time. As we shall see later, the kidneys are the site of uric acid destruction in herbivora. *Glycin, then, is undoubtedly a decomposition product of purin disruption in herbivora*, and is most probably also so in man, although, in this case, experiments similar to those of Weiner on rabbits have not, so far as I am aware, been performed.

With regard to *allantoin*, there seemed for long to be undoubted evidence of its being the chief intermediate product of uric acid destruction in the body. Salkowski found it present in considerable amount in the urine of dogs to which large amounts of uric acid had been given, and the same result has been obtained in similar experiments on cats [17]. Thus Mendel, Underhill, and White have shown that in whatever way nucleic acid is administered to cats and dogs it causes a very distinct increase in the allantoin excretion, and that intravenous injection of lithium urate also raises the allantoin excretion. Allantoin has also been found present, under normal conditions, in the urine of several animals, but never, in any amount at least, in the urine of man, even after copious purin ingestion. Nor has it been observed in the urine of herbivorous animals under similar conditions. These results [3] seem to show that the uric acid produced by purin decomposition in the body is oxidised only as far as allantoin in dogs and cats, but that, in man, the allantoin is further converted into urea. It was further observed (by Minkowski) that in feeding dogs with allantoin all of it passed unchanged into the urine, whereas in man only one-fourth could be thus recovered. This being so, one could expect, were allantoin really produced in the tissues, its presence in considerable amount as a normal constituent of dog's urine, which it is not; and, in human urine, we would expect its appearance after extensive purin katabolism, which has also never been

observed (*cf.* Wiener).[1] *The question as to whether, by purin destruction, allantoin normally is produced in the organism, cannot, therefore, be at present definitely answered.*

Still less is known regarding the possible derivation of *oxalic acid* from purin break-down. It is probable that even were it thus derived it would be as quickly oxidised as it was produced, and would thus escape detection. It has, however, been found increased in the urine of rabbits fed with allantoin (Luzzatto). On the other hand, Salkowski [23] has recently shown that a dog's liver in which uric acid is actively destroyed (as we will see later) contains no more oxalic acid than does a calf's liver in which no destruction of uric acid ensues.

In man the urea excretion, as well as that of uric acid, rises after feeding with pure nuclein (prepared from salmon sperm or yeast, &c.), and the relationship between total *N* and urea-*N* seems to remain practically unchanged, showing that all the decomposed purins are thrown out from the body as urea. Very few researches have, however, been undertaken by the use of modern accurate methods to confirm this important point.

The only conclusion we can come to, from a consideration of these observations, is that *glycin is produced, in certain animals at least, as an intermediate product of purin break-down; and that allantoin and oxalic acid may be produced during the same process under certain not clearly defined conditions.*

We come now to a problem in purin metabolism which has, for long, defied all solution, and can, even now, be considered only as partially solved. The problem is this:— *why, if the organism be capable of destroying purins at all, does it not entirely destroy them so that the urine is purin-free?* The answer to the problem is all the more difficult because no uric acid or other purin body can, under normal conditions, be detected in a free state in the blood. The following three hypotheses have been offered in solution of the problem:—

(1) *Garrod's hypothesis:* that (endogenous) uric acid is produced only in the kidneys from precursors carried to it by the

[1] Furthermore, Podnecka and Swain [21], on repeating Salkowski's experiments of adding uric acid to the food of dogs, found allantoin to appear in the urine only after very large amounts (3 grm.) of uric acid were given; whereas, with small amounts (1 grm.), none of it appeared.

blood, and that, as it is produced, it is excreted into the urine. All other purins in the body (*e.g.* of exogenous origin) are destroyed in the various organs and tissues, so that the blood is purin-free.

(2) *Hoppe-Seyler's hypothesis:* that most of the organs in the body can both form and destroy uric acid, the balance of these two processes being such that no uric acid escapes into the blood. The kidney is also endowed with the power of forming uric acid which, instead of destroying, it excretes.

(3) *Von Noorden's hypothesis:* that endogenous uric acid is shed into the blood as some compound, which is more stable than uric acid itself (*i.e.* exogenous uric acid), and which is, therefore, not decomposed by the tissues.

None of these hypotheses, however, is correct, and some other explanation must be offered to satisfy all the observed facts. In our foregoing description of the behaviour of different animals to purin administration we have assumed that the endogenous moiety of purins has the same integral factor as the exogenous. If this be correct, it must follow that both endogenous and exogenous purin substances are intermediary bodies in metabolism, and it cannot be true, as von Noorden has suggested, that endogenous purins differ from exogenous in that they are more stable, and so are not further decomposed. We must before going further, therefore, retrace our steps and see *what direct evidence is to hand, showing that endogenous purins are destroyed in the body, i.e.* that they are not end products of metabolism but only intermediary ones.[1] After joining the portal vein to the vena cava in dogs, and so preventing the portal blood from directly perfusing the liver (21) it has been stated that the uric acid excretion is higher than normal, even when the dogs are fed on a purin-free diet.[2] As will be shown later, the liver is the main seat of uric acid destruction in dogs, so that its partial removal from the systemic circulation, as in the above experiment, might have caused less endogenous purins to be destroyed than normally. Of course, it could be advanced as an objection to this proof, that the

[1] We have already had occasion to consider this question from another point of view.

[2] Haskins, Herrick, and the author have been unable to confirm this high endogenous purin excretion in two Eck's fistula dogs.

production of (endogenous) purins had been raised by the operation, not their destruction diminished. That such is improbable, however, was shown by the total N-excretion (which is a measure of tissue break-down) remaining constant.

By the injection of oil into the lung and by poisoning with hydrazine sulphate ([20]), allantoin makes its appearance in considerable amount in the urine of dogs fed meanwhile on a purin-free diet. Allantoin is a decomposition product of uric acid in dogs. In both these experiments, the endogenous production of purins must undoubtedly have been raised, and the increased amount further destroyed, and excreted as allantoin. Neither of the two latter experiments furnishes us, however, with absolute proof, for it is possible—though in view of Mendel's recent work not probable—that the allantoin might be derived from something other than a purin substance.

Burian and Schur ([19]) have recently removed all doubt of the intermediary nature both of exogenous and endogenous purins. Briefly, their experiments are as follows:—The kidneys were excised in several dogs, of whom some were previously fed with purin-containing food, and others with purin-free. After periods varying from one to three days, the dogs were killed and the blood carefully examined for uric acid. In no case, even where large quantities of purin had been contained in the diet, was the minutest trace of uric acid found present in the blood. Two interpretations of this result are possible: (1) that uric acid becomes entirely destroyed somewhere in the body, unless some of it be drained off in the kidneys before it is destroyed (*i.e.* that Hoppe-Seyler's hypothesis is correct); (2) that the endogenous uric acid is formed in the kidneys themselves from some non-purin precursors, the exogenous portion being, however, capable of destruction in the body (*i.e.* that Garrod's hypothesis is correct). Excision of the kidneys, according to Garrod's hypothesis, would mean want of formation of endogenous purins, and complete destruction of the exogenous, because of their repeated circulation through the destructive organs.

To ascertain which of these explanations is the correct one it was necessary to remove from the circulation not only the kidneys but also the purin-destructive organ or organs. If, then, on a purin-free diet, purins should accumulate in the blood, the kidneys could not be the site of formation of endogenous purins.

Before going further with a consideration of Burian and Schur's work it will be necessary to learn something about *the site of purin destruction* in the body. Nencki and Hahn's observations on the purin excretion in Eck's fistula dogs have already been referred to as indicating the liver as the possible seat of purin destruction, in this animal at least.[1] In the same year Richet ([10]) showed that extracts of dog's liver, by standing at body temperature, were capable of producing urea or some closely allied body, and a few years later it was shown that the precursors of urea in these experiments were not ammonium salts or amido-acids but uric acid.[2] Jacoby ([10]) carried Richet's discovery a stage further by finding that there were two agencies in the liver extract which caused this decomposition of uric acid, one a ferment-like body easily destroyed by heat, the other a heat-resistant body, and present only in small amount compared with the ferment. Jacoby supposed that allantoin—and not area—was produced from the uric acid, and pointed out that the ferment must, therefore, be an oxidase (an oxygenating ferment). Ascoli ([10]) demonstrated the same facts another way: he perfused the excised liver of a dog with defibrinated blood in which some uric acid had been dissolved, and found a decrease of uric acid and an accumulation of allantoin[3] in the blood after perfusion, whereas by allowing a mixture of blood and uric acid to stand for some time at body temperature without perfusing it through the liver, the uric acid remained undiminished. Wiener ([18]) showed further that saline extracts of the livers of the dog and pig destroyed uric acid, whereas similar extracts of the kidneys possessed no such power. There can be no doubt, then, *that the liver of the dog and pig can oxidise uric acid.* Whether or not any other organ of the dog can do the same thing remains an open question, and it is also undetermined whether the liver of every carnivorous animal is possessed of the same power.

With regard to *herbivorous animals,* the most important experiments concerning the site of purin destruction are those of

[1] Hahn and Nencki thought the increase to be due to increased alkalinity of the urine.

[2] The supposed urea in Richet's experiment, however, was most probably allantoin.

[3] Ascoli thought it was urea.

Wiener.[1] He found that extracts of the kidneys and, to a less extent, of the muscles of herbivorous animals are capable of decomposing uric acid, the end products being not allantoin but glycin. Similar extracts of the livers of the same animals were found to possess no power of destroying uric acid but rather of producing it,[2] for, after incubation for some hours, uric acid was found to have accumulated in the extracts. As we have already seen, Horbaczewski and Spitzer (100) had previously observed the same accumulation of uric acid when a mixture of liver pulp and blood of oxen was allowed to stand some time. These latter facts are mentioned here to indicate how undefined at present is our knowledge of the site of uric acid formation and destruction in animals of different habits. So much, however, we may take as certain, that *in dogs the liver is the organ possessed of any considerable power of destroying uric acid*, and this is all that we require to know for our present purpose.

To return to Burian and Schur's experiments regarding the intermediary nature of (endogenous) purins: we are now in a position to see which of the two explanations offered on p. 417 to explain the disappearance of uric acid from the blood of nephrotomised dogs is the correct one. If, in these dogs, the liver as well as the kidneys be excised, and we find uric acid to accumulate in the blood, we can, with confidence, discard the hypothesis which supposes endogenous uric acid to be produced in the kidneys, and must accept that which supposes it to be, like the exogenous moiety, capable of further destruction in the body. To exclude both the kidneys and the liver from the circulation, Burian and Schur placed a ligature on the aorta immediately above the coeliac artery. Such a ligature cuts off from the general circulation any blood coming from the intestines, and so excludes any absorption of exogenous purins. As it was shown in a control experiment that there might be some exogenous purins in the blood before the ligature was applied, and as the experiment concerns the endogenous moiety, the precaution had to be taken to feed the dog on a purin-free diet for some time before the

[1] Weiner made extracts of various organs and tissues with isotonic salt solution, added uric acid to these extracts and placed them on a shaker in the incubator for several hours.

[2] Burian has recently shown, however, that the liver of the ox does possess some power of *destroying* uric acid, especially if the uric acid be in solution

ligature was applied. In two experiments done as above described, and in which the dogs were killed three hours after the ligature was applied, uric acid was detected in the blood. This ligature, it must be remembered, will cut out from the circulation the intestines as well as the liver, and it might be argued that the accumulation of uric acid in the blood in such a case was due to the occlusion of the intestines from the circulation, thereby preventing any excretion of uric acid by that path.[1] That such is not the explanation was shown by a separate experiment in which the ligature was applied between the coeliac axis and the sup. mesenteric artery—the intestines were occluded, the liver not so—and in which after several hours the blood did not contain a trace of uric acid: the liver had destroyed it.

How, then, does it happen that **any** *purin bodies are excreted in the urine of dogs when there is such an active destruction of them in the liver?* The answer to this question was first of all suggested by Lüthje ([30]), and has been supported by Burian and Schur ([10]). By these observers it is supposed that the urinary purins represent that portion of the total purins which the kidneys have removed from the blood passing through them before the purin-destructive organs have had time to complete their action. The process of purin destruction in the organism is, therefore, not a complete one; a certain amount of purin escapes from the blood before it can be destroyed. The incomplete destruction is not due to any feebleness in the action of the destructive organs, but is due to some of the purin being removed from their influence by its being excreted in the urine. We have already seen that after ligature of the renal arteries no purins can be detected in the blood even after copious feeding with nuclein, and moreover, that the fraction of purins which passes into the urine after purin feeding is the same whether large or small amounts of purin be administered. There is no question, therefore, of the ability of the destructive organs to do their work, and they would invariably destroy all the purins of the blood did some of this blood not traverse the kidneys and so allow some purins to escape.

This hypothesis may be stated in another way: of the blood entering the abdomen, a certain fraction perfuses the liver and a certain fraction the kidneys; this blood contains purin bodies

[1] The question of intestinal excretion of purins is fully discussed by Walker Hall ([13]).

(mainly as uric acid), which are destroyed in the liver but excreted by the kidneys, the relative amounts destroyed and excreted depending on the relative amount of blood circulating through these two organs.

This explanation can at once be accepted for purins injected into a peripheral vessel, but at first sight it seems difficult to understand on the same basis why exogenous purins, which during their absorption *must* pass through the liver before getting to the kidneys, should behave quantitatively, with regard to excretion, like endogenous ones. The fact that the same fraction of purin-N reappears in the urine of dogs after thymus or pancreas feeding, hypoxanthin feeding and hypoxanthin or uric acid injection, but that, when uric acid is given in the food none of it can be recovered as purin-N in the urine, seems to show that, before their destruction in the liver, the purins must be converted into uric acid. Thus, when uric acid is given in the food, it becomes entirely destroyed before it enters the systemic circulation; when injected subcutaneously, however, part of it is carried to the kidneys and excreted, the rest to the liver and is destroyed. When purins other than uric acid are given by the mouth, they of course also pass through the liver, in which however they are not destroyed,[1] but pass on to the tissues, where they are converted into uric acid, and then the same fractions are destroyed and excreted as if uric acid itself had been injected subcutaneously.

In support of this view, Burian and Schur have performed some experiments in which the amount of blood circulating in the kidneys was increased in proportion to that perfusing the liver (*e.g.* by administering diuretics); and, as a result, have obtained an increased purin excretion. As it is possible to cause the dilatation of the renal vessels only for short periods of time, the urine in these experiments had to be examined every hour; in twenty-four hours the hourly increase would have been followed by a succeeding decrease, and very little difference from the daily excretion under normal conditions could be noticed. Grape-sugar and urea in amounts varying from 3–8 grm. and

[1] In this connection it should be mentioned that an alcoholic precipitate of one liver extract when added to another liver extract causes a rise in the urea produced. Chassevant and Richet argue from this that the liver can also destroy purin (nuclein) bases, for the proteid in the precipitate could be no precursor. This purin is, however, most probably oxidised to uric acid before being destroyed.

dissolved in from 25–50 c.c. isotonic NaCl were injected intravenously into dogs[1]—the purin excretion, total N, and, in some cases, the P_2O_5 and NH_3 excretions, being determined every one or two hours. In all cases a marked rise in purin-N was observed during the hour or two following the injection of the diuretic. This increase might, of course, have another cause than that described above, viz. the injected substance might induce an increased decomposition of tissue nucleins and a consequent rise in endogenous production. That such was not the case was proved in the dextrose experiments by the fact that the hourly NH_3 excretion was also raised; and, in the urea experiments, by the fact that the P_2O_5 excretions remained constant though the purin-N rose. In the former case, the rise of NH_3 was probably due to the same cause as the rise of purin-N, viz. that a larger fraction of blood than normal had been carried directly to the kidneys before it had traversed the liver, and that as a consequence the precursors of urea which it contained (viz. NH_3 salts), and which are converted into urea by the hepatic cells, were excreted in large amount. The fact that the P_2O_5 excretion remained constant when urea was injected shows that no increased nuclein disintegration could have occurred.

The same type of experiment was attempted on man, diuretics being given in the diet. No distinct increase could, however, be observed in the hourly excretions of purins. The integral factor in man is only 2, so that it would take an enormous hyperæmia of the kidneys (to divert sufficient uric acid to them) before any difference could be observed in the urinary purins.

Wiener (19) has suggested that the rise following the injection of these substances indicates a synthesis of uric acid, and is not due to the cause ascribed above. This possibility we will discuss later. Siven (25) and Pfeil (26) have both noticed that on a purin-free diet the purin excretion is greater in the forenoon and least during night. It is greatest, therefore, just after the erect posture is assumed, and when there is, conceivably, more active circulation through the kidneys.

[1] These animals are very suitable for this experiment, as their integral factor is high (*i.e.* their uric acid destructive power is great), and consequently a marked difference would be expected to follow the diverting of uric acid from the destructive organ.

We have seen that by multiplying the endogenous urinary purins by a certain factor, varying in different species of animals, an estimate may be made of the purins actually set free in the tissues. So far as is possible from the small amount of work which has been done since the dual origin of urinary purins was clearly indicated, let us *inquire into the exact source of the endogenous purins:* and how their excretion *behaves* under various physiological and pathological conditions.

First of all let us see *whether any of the endogenous moiety of urinary purins in mammals is produced by a synthetic process analogous with that occurring in birds.* The fact that the endogenous moiety remains constant, even when the diet (purin-free) undergoes enormous variations, would tend to show that the synthetic production, if it does exist, can be only of very secondary importance. On the other hand, there are certain chemical substances which are not purins, but which, when given to animals (mammals) on a purin-free diet, can cause a slight increase in the endogenous purin excretion. These same substances cause a very marked increase in the purin excretion of birds where uric acid synthesis undoubtedly obtains. It is obvious, therefore, that before going farther, we must study *the synthetic process in birds*, and then, after we are familiar with this, see in how far the knowledge can be applied to what occurs in mammals.

That uric acid in birds is produced by a synthetic process is shown by the fact that if urea be given them in their food it all reappears as uric acid in the urine. The same thing results if these substances which cause increased urea excretion in mammals, such as ammonia salts and amido acids, are given to birds, an immediate increase of uric acid results. Chemical investigation has shown us that uric acid consists of two urea molecules linked together by a central chain of carbon atoms. What substance in the organism furnishes this central carbon chain, and in what organ does the synthesis ensue? To answer these questions Minkowski (3) extirpated the liver in geese.[1] This operation in birds, and especially in large birds, is, technically, not very difficult, because no artificial anastamosis of portal vein and vena cava has to be created, as in

[1] That it is in the liver that this synthesis occurs in birds has been shown by T. H. Milroy, who found that galvanic stimulation of the liver of geese caused an increase in the uric acid excretion (37).

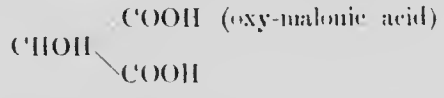
mammals, there being in birds an easy natural anastamosis between these two through the vein of Jacobson which joins together the portal vein and the vena advehens (running from the tail and pelvis to the kidneys). By ligaturing the portal vein above this anastamotic branch, the portal blood does not traverse the liver, and this latter can now be excised, except the portion of it which immediately surrounds, and is intimately adherent with, the hepatic vein. To excise this latter part would mean too much bleeding, but it can be crushed so as to render it functionless. In Minkowski's experiments the geese lived from six to twenty hours after the liver extirpation, during which time this experimenter found the amount of uric acid in the urine to be markedly diminished and ammonium lactate to be correspondingly increased. The replacement of uric acid by ammonium lactate pointed to lactic acid as the substance which furnishes the carbon chain; the ammonia increase being of course also due to the absence of any synthesis of uric acid.

Before Minkowski's explanation of this result could be accepted, however, it had to be shown that the lactic acid was not produced in some other way. Lactic acid in the urine may occur in a variety of conditions, such, for example, as in severe liver disease in man, in phosphorus poisoning, and also in conditions associated with deficient tissue oxidation. In fact, Hoppe-Seyler ([20]) suggested that deficient oxidation, due to the operative interference impeding the movements of respiration, might be the cause of its appearance in Minkowski's birds. That such was not the case has, however, been shown by Minkowski himself; for the same lactic acid excretion occurred after all the blood-vessels going to the liver were tied but no liver extirpation practised. It might, however, be possible, as Bunge ([38]) suggested, that lactic acid had been produced by the removal of the liver, not because uric acid formation was inhibited, but from some other unknown cause; that this free acid acted like any other free acid in the tissues would (see art. Diabetes, p. 372), in that it used up all the available alkali, until there was no alkali left but ammonia, with which it then combined, and thereby prevented from being transformed into urea and uric acid (see also Milroy's expt., p. 393). Minkowski, however, showed this explanation to be incorrect—and the want of synthesis to be the main cause of lactic acid excretion—by feeding liverless geese on urea; no increase was thereby produced in uric acid excretion,

from which he argued that synthesis had disappeared. Salaskin has confirmed Minkowski by showing that when lactic acid is perfused through the liver of a goose uric acid is formed.[1]

Wiener ([19]) has carried this subject of uric acid synthesis farther, and has shown that not only lactic acid but a whole series of organic acids and related substances can furnish the carbon chain which links the two urea molecules together. To demonstrate clearly what these substances are, Wiener used geese whose tissues contained an excess of urea. If urea alone be fed to birds in moderate amount it all becomes transformed into uric acid, but if an excess be given (subcutaneously) all is not thus synthesised — because all the available store of substance which yields the carbon chain becomes used up—so that some is excreted unchanged in the urine. If now, to a goose, thus treated with urea till some overflows into the urine, lactic acid be given, an immediate increase in uric acid excretion follows, for the lactic acid furnishes the necessary tri-carbon chain for the synthesis. Experimenting in this way, Wiener has shown that any oxy, ketone, or dibasic acid of the fatty acid series with a chain of three carbon atoms, or any of the higher organic acids or their derivatives[2] which, by their metabolism in the tissues, produce one of these, is capable when given with the food of furnishing the connecting link for the synthesis.

Working on artificially perfused livers on the other hand, Wiener found that the only one of these bodies mentioned above which caused any marked increase in uric acid formation was a dibasic acid called tartronic. This has the formula:—



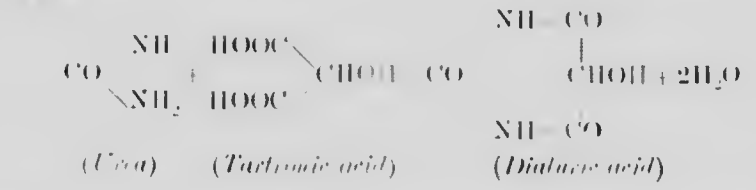
and is the lowest possible dibasic oxy-acid.

The urea residues attach themselves to this in two stages, the first formed compound—of tartronic acid with one urea mole-

[1] There is, however, some truth in Bunge's contention, for Long ([20]) has found that if alkali be administered to liverless fowls there follows a diminution in the ammonia excreted.

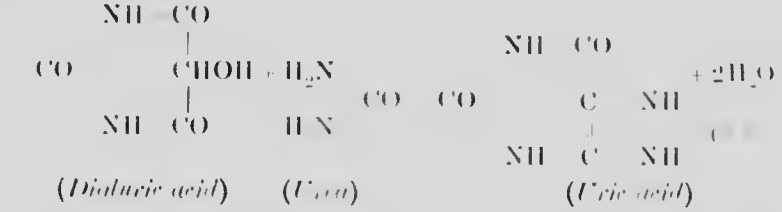
[2] The only one of these higher acids which Wiener found to cause any marked increase in uric acid was β-oxy-butyric. Propionic acid, which is produced when most of the higher acids break down in the tissues, does not enter into the synthesis.

cule—being a body called an *acyclic*, in this case dialuric acid, thus:



(*Urea*) (*Tartronic acid*) (*Dialuric acid*)

Dialuric acid then unites with a second urea molecule forming uric acid, thus:—



(*Dialuric acid*) (*Urea*) (*Uric acid*)

Wiener, therefore, thinks that all the above bodies which when given *per os* under the conditions described above—cause uric acid to be excreted in excess must first of all become converted into tartronic acid or into dialuric acid, before they form uric acid.

Can similar bodies produce uric acid synthesis in mammals? We have already incidentally mentioned that in mammals such a synthetic process is improbable under normal conditions. If, however, certain of these bodies, which aggerate the synthesis in birds, be administered to men, an increase, small indeed but still perceptible, is seen in the uric acid excretion (Wiener); hence there is reason to believe that lactic acid produced as a by-product of metabolism in the tissues synthesis of uric acid in mammals is quite conceivable. This present open and requires further investigation.[1]

[1] Wiener has tried to prove his contention of synthesis existing mammals by an experiment in which he perfused liver with a substance contained in the alcoholic extract of various and which contained no purin bodies), and found a production of uric synthesis of uric acid exists at all under normal conditions in mammals only obtains where an excess of the carbon residue is present, is unlikely. The latter is the view of Burian and Schur, and is the more probable. Burian has further shown that tartronic and dialuric acids have the power of accelerating the action of xanthin-oxydase, which would account for the increase of uric acid in the ox's liver when the bodies are perfused through it.

In passing, it may be mentioned that a synthesis of uric acid from glycin and [illegible] been supposed to exist in mammals. The two substances can be combined into uramil in the laboratory. Horbaczewski's Gly (amido acetic acid, CH_2NH_2COOH). As will be remembered, it enters into yet other synthetic processes in the organism; it unites with benzoic acid to form hippuric acid, so that, if benzoic acid be present in excess in the tissues an increased excretion of hippuric acid results. If uric acid is also formed from glycin, we would expect its excretion to be diminished when the available glycin in the organism combined with benzoic acid to form hippuric acid as by union with benzoic acid. In other words, we would expect the uric and hippuric acid excretions to bear an inverse ratio to one another. Such is however not the case. Feeding with substances yielding benzoic acid or which yield it in the process of their metabolism, cause a marked increase in hippuric acid excretion without causing any change in that of uric acid.[1] That urea and glycin when given in excess to mammals do not influence the uric acid excretion has moreover been shown by Horbaczewski and Weiss (30).

The excretory purins of mammals are practically all produced by some process different from that obtaining in birds. This process we have seen to be an oxidative one, and the substances which are thus oxidised to be other purin bodies. The purin bodies which are constituent parts of the animal tissues are the oxy-purins (xanthin and hypoxanthin) most abundant in muscle, and the amido-purins (guanin and adenin), integral parts of nuclein. *From these two sources, then—from nuclein and from muscular tissue—endogenous purins might be derived.*

Among the tissue nucleins which are constantly undergoing disintegration are those of the leucocytes; an increased leucolysis—cell destruction elsewhere remaining constant—should consequently run parallel with raised endogenous purin excretion. Now, increased leucolysis occurs in certain forms of leucocythaemia, and one of the first indications of the origin of endogenous purins was furnished in 1866 by the discovery of Bartels (20), that a large

[1] A diet of fruits has been said to diminish the excretion of uric acid and increase that of hippuric acid, because of an organic acid which they contain. This acid is converted into benzoic acid in its passage through the tissues, hence the increase of hippuric acid. The supposed diminution of uric acid, however, has recently been shown to be incorrect.

amount of uric acid is contained in the urine of leucocythæmic patients (20). Some years later (in 1889) Horbaczewski (5), after showing that by blowing air through a mixture of spleen pulp and blood uric acid is formed, brought forward the hypothesis that all the uric acid in the urine is derived from the nuclein of leucocytes. According to this view even nuclein-containing food increases the excretion only because it induces an increased leucolysis.

To prove this hypothesis, Horbaczewski made estimations of the number of leucocytes per cubic millimetre of blood removed from a peripheral vessel, and compared this result with the two-hourly uric acid excretion. He found them to run parallel. Where leucocytosis (increased number of leucocytes) existed there was increased uric acid excretion (*e.g.* in children, during the absorption of food, after the administration of pilocarpine, &c.); where, on the other hand, the number of leucocytes was subnormal there was a subnormal uric acid excretion (after quinine and atropin). He considered his doctrine confirmed by numerous clinical observations in which a high purin excretion was associated with a peripheral leucocytosis. Even where extreme and acute tissue disintegration existed, as in inanition and phosphorus poisoning, and a hyper-excretion of purins was present, none of the latter was supposed to come from the nuclein of the tissue cells, but all of it from leucocytic decay induced, according to Horbaczewski, by the liberated tissue nucleins. The liberated tissue nucleins acted as stimulants of leucocytic disintegration.

This hypothesis was accepted as correct by nearly all workers. Even where the number of leucocytes per cubic millimetre of blood did not run parallel with the uric acid excretion; even although some observers found no increase in the latter in marked cases of leucocythæmia, and others a normal uric acid excretion where the leucocytes were much diminished in amount, no one doubted the truth of the hypothesis. By the exercise of a vivid imagination it was possible to explain away all difficulties: if the uric acid excretion were normal, but the leucocytes increased in the blood of a peripheral vessel, the increase of the latter must be entirely due to increased production, their destruction remaining constant: the former process must have been more active for some time and the destructive forces not able to keep down the level.

Amongst those who first questioned Horbaczewski's hypothesis was Mares ([20]). He admitted that uric acid was a product of the metabolism of cells, but pointed out that *all* tissue cells, leucocytes included, contributed their share to its production. He agreed with Horbaczewski in supposing that nuclein given in the food only indirectly caused increased purin excretion by stimulating cellular metabolism.

Horbaczewski's hypothesis in its entirety is held nowadays by only a few workers. The fallacy of assuming increased or diminished destruction of leucocytes from the number in a cubic millimetre of blood from a peripheral vessel has been pointed out by Goldscheider and Jakob ([20]). These workers found that where the number of leucocytes fell in a peripheral vessel from any cause, there was a corresponding increase of leucocytes in the capillaries of the organs (*e.g.* lungs). Such drugs as atropin cause a peripheral hypoleucocytosis but a central leucocytosis. Of course, no one denies that some—it may be a large proportion—of the endogenous purins are derived from the nuclein of leucocytes, but that other cell nuclei also contribute is shown, among other things, by the increased endogenous excretion in phosphorus poisoning (where the liver cells are breaking down), and by the fact that there are some cases of leucocytosis unassociated with a raised purin excretion, and *vice versâ* cases of leucopenia (diminished number of leucocytes) with a normal excretion ([35]).

Doubtless a parallelism between the number of leucocytes in a peripheral blood-vessel and the uric acid excretion is a common experience, not because the two are related, but because both are the result of a common anomaly in some organ of the body. Even granting that tissue nucleins are a source of purins, cellular metabolism can scarcely be considered as active enough to account for more than a small fraction of the 0·3–0·6 grm. of endogenous purins excreted by a man in twenty-four hours. This amount of purin would correspond to nearly 100 grm. of such glands as the thymus or pancreas, and it is highly improbable that cellular break-down to such an extent could ensue in the animal body.

We are compelled, therefore, to conclude that nuclein is not the main source of endogenous purins.

Siven has shown that excessive muscular work causes a distinct rise in the endogenous moiety, pointing to *the muscles*

as a possible source of purins, and very recently Burian has brought forward evidence which places beyond all doubt this muscular derivation, and which indeed would seem to show that most of the endogenous moiety is thus derived.

Two types of experiment were employed by Burian to study this question. In the one, an estimation of the endogenous purins was made in the urine excreted every hour from 6 A.M. to 2 P.M., during all of which time the person (Burian himself) lay perfectly quiet in bed except for one hour (9–10), when strenuous muscular exercise was practised. For some time previous to the commencement of the observation (from 2 P.M. on previous day) and during it, no food was taken. In some of the observations a day of complete muscular rest, in others a day of considerable muscular exercise (a long bicycle ride), preceded the actual observation day.

It was found that the urine collected during the work hour already showed a small but yet distinct increase in the purin bases, the amount of uric acid being, however, as yet not increased. During the hour following the work hour, this increase in the basic purins had very greatly increased, and the uric acid had also begun to show some increase. During the second hour following the work, the basic purin excretion had begun to decrease, but that of the uric acid had now become markedly raised. After this time both the basic and acid purin excretions became less, until, in about four hours after the work, the normal had been reached, beyond which the excretion was *subnormal* for some time, lasting even till the next day. On account of this ultimate diminution in the excretion, it was found that the early morning purin excretion was always less when muscular work had been practised on the day previous to the observation than when there had been muscular rest. It is no doubt on account of this diminished excretion following the increase that very little change has been found by other workers to occur in the *daily* purin excretion when muscular work was practised: the increased excretion due to work being followed by a subsequent diminution, the total effect on the daily excretion would be masked.

In the second type of experiment, Burian perfused the muscles of the hind limbs of a dog through the abdominal aorta (von Frey's method) with defibrinated dog's blood diluted several

times with Ringer's fluid. He examined the perfusing fluid for uric acid before and after perfusing it.[1] In some of the experiments, the muscles were kept at rest, in others they were thrown into tetanus by stimulating the spinal cord with an induced current.

It was found that before perfusion no purins could be detected in the perfusing fluid, but that after perfusion purins appeared, and that these were more abundant when the muscles had been active. Of great interest was the further discovery that the purin increase due to muscular activity existed not only during the actual working time but for some time after it, and, further, that the increase was largely in basic purins during the work, and in uric acid in the following periods. These results entirely agree with those obtained by an examination of the hourly purin excretions. An examination of the muscles themselves also showed an increase in the purin bases (hypoxanthin) as a result of contraction.

From these experiments Burian concludes that hypoxanthin must be continually produced by the muscles, and that this production is increased by muscular contraction. Before it gains entrance into the blood, however, it is oxidised, by xanthin-oxydase, into uric acid. Of the uric acid thus produced, a portion is destroyed and a portion excreted, the proportion varying in different species of animals (integral factor, p. 111). The destruction is brought about by the destructive ferment.

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[4] *A. Kossel*, Zeitschr. f. physiol. Chemie, Bd. 7, S. 19 (1882).

[5] *Horbaczewski* (vide *Spitzer*, No. 6).

[6] *W. Spitzer*, Arch. f. d. ges. Physiolog., Bd. 76, S. 192.

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CHAPTER XIV

HÆMOLYSINS AND ALLIED BODIES

If non-fatal amounts of certain bacteria, or of certain toxins produced by bacterial growth,[1] be repeatedly inoculated into susceptible animals, a day or so intervening between each inoculation, it will be found that the animal acquires the power of withstanding larger and larger doses, and ultimately can tolerate an amount many times greater than that which would have proved fatal before the inoculations began. These facts have been known to bacteriologists for several years, the animal thus treated being described as immunised against the bacteria or toxins in question. It has further been shown that it is the blood which acquires this immunising property, and that the process can to a large extent be studied *in vitro*—for example, by mixing some immunised blood serum with a suspension of bacteria and observing the bacteria under the microscope, or by mixing the serum with some toxin, and then seeing whether the mixture is still toxic when injected into a susceptible animal.

These means of studying the antibacterial or antitoxic action of serum require, however, patient observation, and the effect produced can only be measured quantitatively by extremely laborious methods.

Within the last two or three years, however, it has been shown that substances which behave like bactericidal and antitoxic bodies can be produced in the tissue fluids by the injection into an animal of various cells other than bacteria, and poisons other than those of bacterial origin. Included amongst the toxic cells are the red blood-corpuscles of an animal belonging to a different species to that from which the corpuscles are derived. The destruction of the red corpuscles, or erythrocytes, is indicated by

[1] Bacterial toxins may be excreted (exotoxines) and pass in the blood to parts of the body remote from the seat of inoculation, or the poisons may be closely bound with the microbe (endotoxins) and be set free only after dissolution of the microbe. The efforts of the host to combat the two forms of intoxication are different in manner.—(*Editor's Note.*)

their being no longer capable of retaining hæmoglobin, the latter leaving the corpuscles to become dissolved in the serum. This *laking* of the blood can be easily observed in a test-tube as the normally opaque suspension of corpuscles changes to a transparent solution. By this means, then, we are furnished with a ready method for studying the laws which govern, and the conditions which influence, the production of anti-bodies towards foreign cells in general.

It will, of course, be impossible, in this lecture, to deal with the general question of immunity in all its aspects; all that can be attempted is to discuss briefly the laws which govern the production and action of anti-bodies in general, taking hæmolysis as a type; and to indicate, here and there, by way of example, some of the more important applications of these laws to Physiology and Pathology.

On thoroughly understanding the process of hæmolysis, no difficulty will be experienced in understanding such processes as those of bacteriolysis (destruction of bacteria), cytolysis (destruction of cells), agglutination, and præcipitin formation. In studying the process of hæmolysis we will incidentally find it advantageous to consider antitoxin formation.

Hæmolysis.—A red blood-corpuscle is composed of an envelope or membrane filled with hæmoglobin.[1] The envelope (and stroma) consists chemically of three substances: the phosphorised fat lecithin, the mon-atomic alcohol cholesterin, and a nucleo-proteid; and it prevents the hæmoglobin from leaving the red blood-corpuscles and becoming dissolved in the blood plasma. This envelope is possessed of a certain amount of vitality, for if it be killed, it can no longer prevent the diffusion of hæmoglobin into the surrounding fluids, so that laking of the blood results. Any protoplasmic poison will kill the envelope and cause hæmolysis, because the osmotic pressures of serum and corpuscular contents are not quite the same, that of the serum being a little less than that of the corpuscular contents. Physiologists have for long recognised this as the cause of the hæmolysis, which is produced by the repeated freezing and thawing of the blood, or by the addition to it of certain chemical poisons. Other poisons, *e.g.* sapotoxin, seem to damage the envelope by uniting with the cholesterin and possibly with the

[1] Some workers think that there is also a stroma or sponge-work in the interior of the corpuscle.(1)

lecithin to form a substance that is soluble in water. Others, like ether and chloroform, probably directly dissolve out these substances. Cholesterin (and lecithin) being thus removed, the permeability of the envelope is increased, and the normal, very slight difference in molecular concentration between plasma and corpuscular contents, become sufficient to determine diffusion of the latter into the former.[1] Lecithin and cholesterin are normally very insoluble in water, and no doubt form the most impervious part of the envelope.

In the case of biological poisons which we are about to describe, the same explanation is possible; something is changed in the envelope so as to increase its permeability. Thus Baumgarten has noticed that the first stage of hæmolysis, as produced by biological poison, is the swelling of the corpuscles, just as occurs when the serum is made hypisotonic. He finds, further, that heating hæmolytic serum to 55° C. does not in any way influence its osmotic pressure (freezing-point and electrical conductivity remain unaltered), whereas, as we shall see later, its hæmolytic power is destroyed. We must suppose, therefore, that the hæmolysin affects the permeability of the envelope—perhaps by combining with the lecithin or cholesterin—and the slight an-isotonic condition of corpuscles and serum is then sufficient to determine a diffusion of hæmoglobin. By observing, under a microscope, the behaviour of the nucleated erythrocytes of amphibians towards specific hæmolysins, it has been attempted to determine whether, or not, hæmolysin really kills the corpuscles. The nucleus does not undergo destruction in the process (Landau [3]), so that it would appear as if the permeability of the envelope had alone been affected.

Hæmolysis may also be produced by an entirely different cause than that described above, viz. by the disturbance of the osmotic equilibrium of the plasma and corpuscles. So long as the osmotic pressure of the fluids in which the corpuscles are suspended is almost the same as that of the corpuscular contents

[1] As Stewart [2] has pointed out, blood "lakers" are divided into two groups, according to whether they cause the hæmoglobin alone to leave the corpuscle—*mild lakers*, *e.g.* hæmolytic sera, freezing and thawing—or also cause inorganic salts, *i.e.* electrolytes, to be extracted—*severe lakers*, *e.g.* heat, water, &c. The latter group cause the electrical conductivity of the suspending medium (serum) to be increased when hæmolysis is produced, the former cause no such increased conductivity.

(that is, that they have the same molecular concentration) and the envelope is uninjured, no hæmolysis will result. Any such solution is said to be *isotonic* with the corpuscles. If, however, the osmotic pressure of the surrounding fluid be less than that of the corpuscles (hypisotonic), then the corpuscles will absorb water, and become swollen and pale, and ultimately the envelope will burst and so let loose the hæmoglobin. This law of osmotic equilibrium does not, however, hold for all salts or neutral bodies: thus ammonia salts, in whatever concentration, cause the corpuscles to lake, and the same is true for urea.

We must distinguish sharply between these two classes of hæmolytic agencies: those that poison the envelope but do not alter the osmotic relationship of corpuscles and serum, and those which primarily alter the isotonic condition so that certain molecules (water, &c.) diffuse into the corpuscle, causing the latter to distend till it bursts, and so lets loose its hæmoglobin.

Included in the group of toxic hæmolysins are a number of peculiar bodies which, under certain conditions, may become developed in the blood serum, or may be present in normal serum, or in various tissues or fluids of animal or of vegetable origin. These, in their general behaviour, are so like antibacterial bodies and cell poisons in general, that it is of great importance they should be thoroughly understood.

It is to Bordet (1) that we owe the discovery of **hæmolysins**. The blood serum of a guinea-pig added to a 5 per cent. suspension of the erythrocytes[1] of a rabbit in isotonic salt solution produces no laking. If, however, 3–5 c.c. of defibrinated rabbit's blood be injected several times into the peritoneal cavity of a guinea-pig, a few days elapsing between each injection, the serum of the injected guinea-pig will, when added to a suspension of rabbit's erythrocytes, be capable of quickly laking them. A hæmolysin, capable of killing the stroma of the rabbit's erythrocytes, and so allowing the hæmoglobin to diffuse through it, is thus produced in the guinea-pig's blood. If, instead of rabbit's erythrocytes, the erythrocytes of any other animal be mixed with this hæmolytic serum, no hæmolysis will result, or only a slight amount. The hæmolysin is therefore *specific* in its action.

[1] Hereafter, the term erythrocyte will be used instead of red corpuscle.

Similarly, if frog's blood be injected into a rabbit, the serum of the latter will lake frog's erythrocytes; although, in this case, the specific nature of the hæmolysin is not so definite, for the corpuscles of the salamander, triton, the toad, &c., are also laked by this serum.

It may, therefore, be stated as a law that *the blood serum of an animal of a species A, when that animal is repeatedly treated with the blood of a species B, acquires the property of causing hæmolysis of the erythrocytes of species B*, and to a less extent of other species of the same genus as B. Not only intraperitoneal, but also subcutaneous or intravenous injection of the blood, will lead to the production of the hæmolysin. Hæmolysins may also be produced by feeding an animal with the blood of another species.

This laking can be most conveniently studied in a test-tube, the erythrocytes to be tested (of B) being first of all washed free of adherent serum by shaking them with isotonic salt solution and centrifugalising until the washings no longer give a proteid reaction, then suspending in 20 parts of isotonic saline. Into each of a series of narrow test-tubes of about 5 c.c. capacity is placed 1 c.c. of the corpuscular suspension. Different amounts of the serum (1 c.c., ·5 c.c., ·25 c.c., ·1 c.c., ·05 c.c., ·025 c.c., and so on) to be tested are then mixed with the erythrocytes, the fluid in each test-tube is brought up to a definite amount—say 2 c.c.—the test-tubes are inverted several times so as to mix their contents, and are then placed in the incubator for a few hours. Laking is indicated by the salt solution becoming red and transparent on account of the hæmoglobin dissolving in it.

Hæmolysis may also occur in the blood-vessels on the injection of a specific hæmolysin into an animal. The liberated hæmoglobin in such cases appears in the urine, and various other symptoms are produced, being usually followed by the death of the animal. This fact was discovered by Belfanti and Carbone. Before Bordet's article appeared these authors injected rabbit's blood into horses, and found that the horses' serum, when injected into a rabbit, acted as a violent poison, producing hæmoglobinuria and being followed by death.

Bordet further discovered that heating the hæmolytic serum of a guinea-pig to 55° C. for half-an-hour deprived it of its hæmolytic power towards rabbit's erythrocytes. It had become *inactivated* by heating; therefore something necessary for hæmolysis

had been destroyed. The inactivated serum could be reactivated, *i.e.* made again hæmolytic towards rabbit's erythrocytes, by mixing it with some normal (guinea-pig's) serum. To make this clear, we will take an actual experiment. A guinea-pig receives several peritoneal injections of rabbit's defibrinated blood until its blood serum becomes markedly hæmolytic towards rabbit's erythrocytes, *i.e.* until a fraction of a cubic centimetre can lake 1 c.c. of a 5 per cent. suspension of rabbit's erythrocytes. The hæmolytic guinea-pig's serum is then inactivated by placing it for thirty-five minutes in a water-bath at 55° C. After cooling, an excess of the inactivated serum, added to the suspension of rabbit's erythrocytes, produces no laking: but, if the inactivated serum be mixed with a minute quantity (a fraction of a cubic centimetre) of a normal guinea-pig's serum, the mixture very quickly hæmolyses. The amount of inactivated serum necessary for laking when normal serum is also present is exceedingly small—0·005–0·001 c.c. being sufficient for the complete laking of 1 *drop* of blood.[1]

Two things must therefore be necessary for hæmolysis; one of them present in inactivated serum (can withstand heating), the other in normal serum (is destroyed by heating). It must be the former of these (*i.e.* the heat-resistant substance) which is produced by injecting the erythrocytes into a different animal. Regarding the other (the unstable body, which is present in normal serum), Nuttall in 1888 had shown normal serum to possess antibacterial properties, and Buchner, later, showed it to be capable of partially destroying foreign erythrocytes as well as bacteria. This latter worker ascribed this power to the presence in the serum of a substance which he called *alexin*, and this, Bordet considered as the agency in normal serum which reactivated hæmolytic serum, previously inactivated by heat. The substance which inactivated serum contained, and which had evidently been produced by injection (since it was contained only in the serum of injected animals), Bordet thought to be of the nature of a mordant. According to him, this mordant, or *substance sensibilatrice*, as he named it, acted on the erythrocytes by making them sensitive towards the alexin. Furthermore, the sensitising substance was shown to be specific in its nature, it would sensitise only the erythrocytes of an animal of the same species as that from which the blood for injection had been obtained. On the

[1] Quoted from Hans Sachs.

other hand, it was noted that the alexin of *any* animal's serum could complete the reaction. If in the above experiment, for example, some rabbit's serum had been added to the inactivated serum instead of guinea-pig's serum, hæmolysis would have followed; and if the rabbit's erythrocytes had not been thoroughly washed free of adherent serum (containing alexin), the mere addition of inactivated serum would have laked them.

To these observations of Bordet on erythrocytes, might be added, for the sake of interest, parallel ones by Pfeiffer, Metchnikoff, and Bordet on the behaviour of cholera vibrios in the tissue fluids. Under normal conditions these bacteria very quickly kill a guinea-pig. It was found, nevertheless, that a normal guinea-pig's serum can destroy *a very small amount* of cholera vibrios. This, the serum does in virtue of an alexin which it contains. The most important discovery from our point of view, however, is that a sensitising substance could be developed in the serum and the bactericidal power of the latter thereby greatly raised, so that the cholera vibrios soon become immobile, and die when mixed with it. If repeated sub-lethal doses of cholera culture were injected in gradually increasing amount into the peritoneal cavity of a guinea-pig, the serum of such an immunised animal became able to destroy quantities of cholera vibrios which would otherwise have killed many guinea-pigs; and, even if the immune serum were injected into a normal guinea-pig, could endow the latter also with resisting powers towards cholera infection. If the serum were warmed, or left standing in the air for some time, it became inactivated, and could be reactivated by the addition of normal serum, or by placing it in contact with the peritoneum; that is, by adding an *alexin* to it. The cholera vibrios, in this process, behave like erythrocytes. This important discovery in immunity is given here to exemplify how close is the relationship between hæmolysis and bacteriolysis, and to show how important it is for the bacteriologist to become acquainted with the former process, which can be studied with ease in the test-tube, in order that he may be able to infer the laws which govern the more practical, but much more obscure process of bacteriolysis.

Before proceeding further, it may be advantageous to summarise briefly what we have already learned. The facts so far, then, are as follows. The organism is normally endowed with a certain amount of destructive power towards all cells foreign to

its tissues. This *cytolytic power* may be increased in any animal by the repeated injection into it, in non-lethal but gradually increasing dosage, of these foreign cells. This increased cytolytic power depends, not on an increase of the destructive agency, but on the development in the tissue fluids of some substance which increases the sensitiveness of the invading cell towards the normally existing destructive agency of the tissues.

Bordet's explanation of the facts of bacteriolysis and hæmolysis does not, however, satisfactorily or completely explain all the observed phenomena of the formation of anti-bodies, (*e.g.* why the sensitising substance should be so remarkably specific in its nature, why there should be so great an increase of the substance in immune blood, &c.). It is to Ehrlich and Morgenroth that we owe a conception which seems to explain all the observed facts. These workers have evolved a theory which has become well known as the **side-chain theory**. This theory permits of considerable speculation, and by it a large number of possible conditions can be predicted and all the observed facts of immunity explained. It is of fundamental importance for us at this stage, therefore, to thoroughly master the details of this theory, and the experiments on which it is founded.

Let us first of all consider the simplest conception of the side-chain theory. This applies to *antitoxin formation*. If a bouillon culture of the diphtheria or tetanus bacillus be filtered through a Pasteur-Chamberland filter, the filtrate will contain tetanus- or diphtheria-toxin, and will, if injected into susceptible animals, cause death. If, however, the toxin be injected at intervals of a few days apart, at first in sub-lethal doses, and the doses gradually increased, the animal will become immune. A dose of the toxin, many times in excess of what would originally have killed the animal, comes to have no action on it. Moreover, the serum of such an immune animal will, if injected into another normal animal, protect the latter also against subsequent injection of the toxin. There must, therefore, be something developed in the blood of the immunised animal which can *neutralise* the toxin. This is called antitoxin. Ehrlich says that toxin and antitoxin unite with one another in definite quantities, and in multiples of these quantities;[1] that cold retards the union; and that concentrated solutions react quicker than dilute ones.

[1] The work of Danysz, Craw, &c., show this is not so, and afford evidence in favour of Bordet's theory.—(*Editor's Note.*)

To explain the production of this antitoxin, the side-chain theory supposes that the protoplasm or biogen of the animal tissues consists of a central nucleus or functionating centre (*C* in Fig. 21), attached to which there are innumerable arms or side-chains which are of various shapes at their free ends, all of them slightly different (*A A*). These side-chains are concerned physiologically in

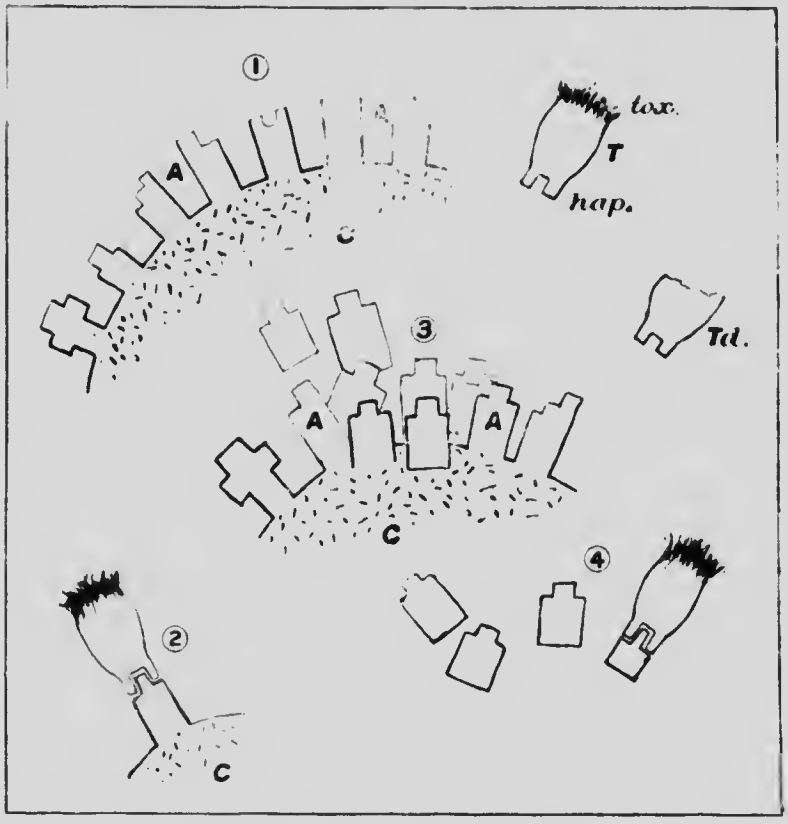

FIG. 21.—Receptors of First Order (Ehrlich's theory); Mechanism of Antitoxin Formation.

the absorption of food molecules, which must, therefore, be likewise provided with side chains, some, or all of which, have mouldings which fit on to some of the mouldings of the biogen side-chains. It is only by becoming locked on to the functionating centre of the cells, by means of side-chains, that the food molecule can be incorporated with the protoplasm of the cell and the potential energy which the food contains made available to the tissues.

After this assimilation is complete, the food molecule drops off, as it were, from the side-chains, and so leaves these free to link other similar food molecules to the biogen.

The molecule of a toxin (*T*) also possesses, besides the actually toxic portion of it (*toc.*), a combining side-chain [1] (*hap.*), and, if it so happen that the mouldings of this fit one of the mouldings on the side-chains of the tissue cells, then will the toxin unite with the cell and produce the symptoms of the disease [2] ((2) in Fig. 21). The most efficient *antitoxin* would, therefore, be one which covered over the combining moulding of the toxin molecule; the union of the toxin with a side-chain of a tissue cell would thereby be prevented. This process would best be accomplished by the presence in the blood, in a free state (*i.e.* disconnected from the biogen), of the same side-chains which normally anchor the toxin. Ehrlich supposes that a setting free of side-chains actually takes place when antitoxin is formed ((3) in Fig. 21). Antitoxin, according to him, is nothing more or less than disrupted tissue side-chains. By the presence of these in the blood, the toxin molecule will have its moulding fitted, in other words, its combining power satisfied, before it gets to the cells, and will no longer be able to poison these ((4) in Fig. 21). Ehrlich compares the mechanism to the attraction of lightning by a mass of iron. If the iron be placed in a house it will increase the risk of the house being damaged by the lightning; but if placed outside the house will save it, by attracting the lightning elsewhere. The house is the tissue cell, the lightning the toxin, the iron the antitoxin.

The cell side-chains are called *receptors*, the combining portion of the toxin molecule the *haptophoric group*, and the actually toxic portion of the toxin the *toxophoric group*. An antitoxin acts by fitting on to the haptophoric group of the toxin; the toxophoric group then becomes functionless because it is not chained to a cell.

How, now, does the repeated injection of toxin in gradually increasing dosage lead to the liberation in the blood of these same receptors, which, when attached to the cells, attract and fix the toxin to them, and thereby cause cell destruction? To answer

[1] Under certain conditions, the toxic portion of a toxin molecule may become destroyed, but the combining portion remain active. Toxoid (*Td.* in Fig. 21) is the name given to such a body.

[2] Owing to the ability of the toxines to turn toward some particular cell, Wright calls them "tropines." Thus tetanus toxin, which has an affinity for nerve protoplasm, may be called "neuro-tropic."—(*Editor's Note.*)

this, Ehrlich and Morgenroth take advantage of an observation of Weigert's, who showed that when a tissue is injured, the organism is not content with merely making good the defect, but reproduces the lost part in over excess. If some cells be destroyed, repair does not stop short when these have been replaced, but the repairing process goes on until a large excess of cells has been produced. Applying this observation to antitoxin formation, we see that if some of the receptors of a cell be rendered functionless—through their free ends being capped by uniting with the haptophoric group of a toxin—the cell will reproduce the lost receptors in over excess, and a large number of them will be thrown into the blood, where they will float free, and will, on coming in contact with toxin molecules, fix on to their haptophoric groups, thereby rendering the toxin incapable of exercising its toxic action.

Let us now see how the facts of *hæmolysis* can be explained by this theory. The receptors which we have just considered are comparatively simple in nature, they possess only one combining group. Ehrlich calls them *receptors of the first order*. To explain hæmolysis by a similar process, such simple receptors will not, however, suffice; for in this process, as a result of the union of the disrupted biogen receptors with the invading elements (erythrocytes), there is formed, not merely an inactive body, as in the reaction between toxins and antitoxins, but a new compound provided with a combining group to which becomes linked the ferment which destroys the erythrocyte. We must, therefore, have receptors which combine with the erythrocytes[1] on the one hand, and with the actual dissolving, or *lysogenic agency*, on the other. These, Ehrlich calls receptors of the third order.

The nature of such receptors is explained in Fig. 22, where *C* represents the tissue cell carrying a receptor, *A*, which has at each end a moulding, the exact form of which is varied as represented in No. 1; one of these mouldings fits on to a receptor, *E*, of an injected erythrocyte, and the other unites with a ferment, *F*. When thus combined, the ferment acts on the erythrocyte and destroys or hæmolyses it, and the tissue cell being deprived of receptors, which physiologically it makes use of to assimilate food, proceeds to reproduce the lost receptors. This it does in over-excess, so that a large number of receptors (*aa*), with two combining groups, are set free in the blood. This overproduction of receptors

[1] The erythrocytes must likewise be provided with receptors through which the combination can occur.

is represented in (3) of Fig. 21. It is to the presence of these that the blood serum of an animal comes to be able to hæmolyse erythrocytes having receptors with the same mouldings as these of the erythrocytes used for injection, that is, the erythrocytes of the same animal or of one closely related to it.

This double receptor (*Aa*) is called an *amboceptor*, because it

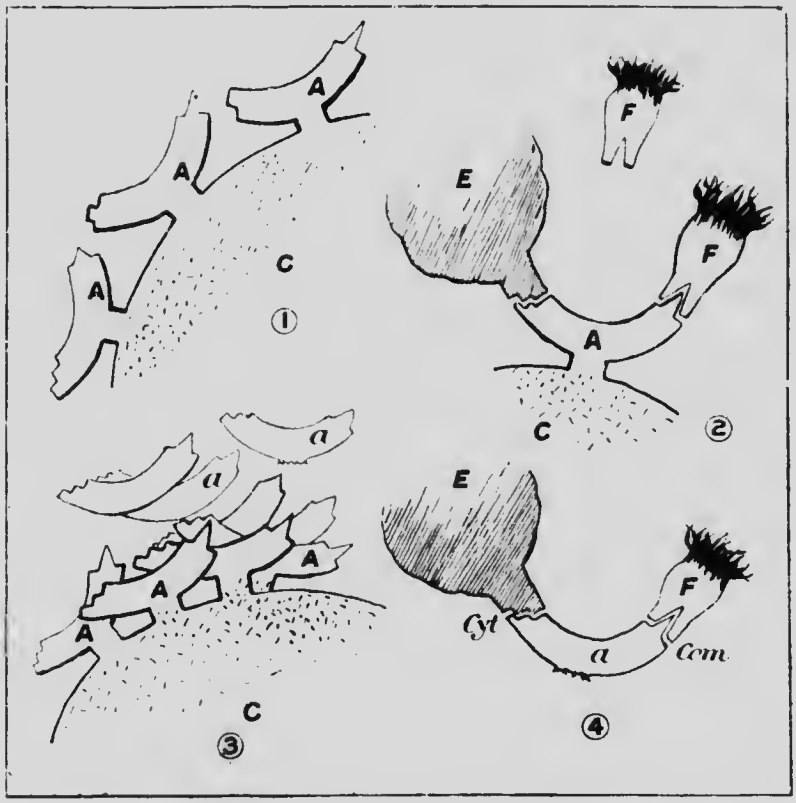

FIG. 22.—Receptors of Third Order (Ehrlich's theory); Mechanism of Hæmolysin Formation.

has two combining groups. The ferment-like body which becomes attached to one end of it is, according to Ehrlich and Morgenroth, nothing more or less than what Bordet called the alexin, which, it will be remembered, is present in the tissue fluids as a physiological constituent, and which is easily destroyed by heat or by contact with the air. Since the amboceptors cannot act on the cells (*e.g.* produce hæmolysis) unless they be combined with

alexin, the latter is called by Ehrlich a complement (*F* in diagram), and the haptophoric group of the amboceptors, on to which it fits, is called the complemento-philic group (*Com.* in (4) of Fig. 22), the opposite haptophoric group being called cytophilic (*Cyt.* in (4) of Fig. 22), because it fits the receptors of the invading cell. It is, according to this theory, the amboceptors which are produced by injection, the complements being present in normal blood. The complements act on the erythrocyte, when suitable amboceptors are present to chain them together.

We must now discuss the experiments on which Ehrlich and Morgenroth base this theory. They had already learned, from Bordet's observations, that two substances were concerned in hæmolysis: the one of these—called an alexin—being a normal constituent of the tissue fluids, easily destroyed by heat, and possessed of digesting properties; the other—called *substance sensibilatrice*—being produced by inoculation, endowed with much greater stability than the alexin, and which acted by sensitising certain cells towards the alexin. In order to explain hæmolysis by the side-chain theory, Ehrlich and Morgenroth had first of all to show that the *substance sensibilatrice* of Bordet actually combined in definite chemical union with the cell, and that the so-called alexin then attached itself to the resulting compound, this being the only possible way by which alexin and cell could unite so as to permit the digesting or lysogenic power of the alexin to assert itself.[1]

For their experiments, Ehrlich and Morgenroth injected sheep's blood into a goat, the goat's serum being thereby rendered hæmolytic towards sheep's erythrocytes. The experiments concerned themselves with the following questions:—

I.—*Does the amboceptor become combined with the erythrocyte?*

Hæmolytic goat's serum was inactivated by heating it to 55° C. After cooling, it was mixed with thoroughly washed sheep's erythrocytes. This mixture after standing some time was centrifugalised, and the sediment and supernatant fluid separately tested for the presence of amboceptors. The supernatant fluid was, for this purpose, mixed with sheep's erythrocytes and

[1] To prevent confusion of terms we will, from this stage on, adopt Ehrlich and Morgenroth's nomenclature, calling Bordet's alexin the *complement* and the *substance sensibilatrice* the *amboceptor*.

some normal serum, *i.e.* containing complement: no hæmolysis resulted. The sediment (erythrocytes) was suspended in isotonic saline and some normal serum—*i.e.* containing complement added: complete hæmolysis resulted in two hours. *The erythrocytes had therefore united with the amboceptors.* It was further shown that this union was not merely a mechanical adherence; for, repeatedly washing the sediment with isotonic saline did not influence the above result, the washed erythrocyte-amboceptor compound hæmolysing, when complement was added, as readily as when it had not been washed. Moreover, the union was *a specific chemical one*, for a repetition of the experiment with any erythrocytes other than those of a sheep gave a sediment containing no amboceptors (*i.e.* it did not hæmolyse when complement was added to it).

II.—*Does the complement also become attached to the erythrocytes, or can it only become attached to erythrocytes already united with amboceptors?*

Some serum of a normal goat was mixed with sheep's erythrocytes, which had been washed free of adherent serum, and, after some time, the mixture was centrifugalised. The corpuscular sediment, after washing, was found to contain no complement, for the addition to it of inactivated hæmolytic serum (*i.e.* containing only amboceptors) gave no hæmolysis. *The complement cannot therefore unite directly with erythrocytes.*

III.—*How, then, does the complement act on the erythrocytes?*

We have seen that it cannot combine with erythrocytes directly, whereas the amboceptors can. From which fact we might at once assume that during hæmolysis the complement acts on the erythrocyte through the intermediation of the amboceptor. To ascertain if this be so, Ehrlich and Morgenroth studied the behaviour of complement and amboceptor on erythrocytes when both were present in the mixture. (*a*) To prevent the rapid hæmolysis which would follow at ordinary temperature in this case, the mixture was cooled to 0·3° C., at which temperature hæmolysis is very slow. After leaving the mixture for some time at this temperature, it was centrifugalised, and sediment and supernatant fluid independently examined for the

presence of amboceptor and complement. The amboceptor was found confined to the sediment (that is, bound to the erythrocytes); the complement was confined to the supernatant fluid. This experiment shows us, so far, that the amboceptor has a very marked avidity for the erythrocytes, combining with them even at 0° C. This is then evidently the first stage in the process of hæmolysis, combination of amboceptors and erythrocytes. (*b*) To study the relation of the complements to this compound, it was necessary to work at a temperature at which hæmolysis might result, and to stop the process short in its middle, that is, when the amboceptors had united with the erythrocytes, but some of the complement was still free. Now, Ehrlich and Morgenroth found that hæmolysis was completed in fifteen minutes, at 37° C. If they stopped the process after ten minutes and then quickly centrifugalised the mixture, the resulting sediment, suspended in saline, was found to contain all the amboceptor and sufficient complement to produce moderate hæmolysis, this being readily completed by adding some normal serum. The supernatant fluid, on the other hand, was found to contain complement but no amboceptor.

From these results Ehrlich and Morgenroth argued that the amboceptors must possess two binding groups, one of which, the cytophilic group, has very marked combining affinities for erythrocytes—the combination occurring even at 0° C. (Expt. 3*a*); and the other, the complemento-philic group, only weak affinities for the complement—no union resulting at 0° C. and only partial union after ten minutes at 37° C. (Expt. 3*b*)—the latter union only occurring when the cytophilic group had been fitted to an erythrocyte.

It should be pointed out here that these experiments, although they can be best explained by Ehrlich's theory, and indeed were devised to prove this, do not disprove Bordet's. When we enter into the subject more fully, however, we shall encounter results (the production of anti-bodies, &c.) which can be explained best if Ehrlich's theory be accepted.

Perhaps the strongest evidence in favour of the side-chain theory is that furnished by Preston Kyes (6) and Hans Sachs in their studies on snake venom. As we shall see later, cobra venom, regarded as a hæmolytic agent, contains amboceptors alone. If it be mixed with the thoroughly washed erythrocytes of the

ox or goat (which contain no endo-complement), no hæmolysis will result, unless some complement (normal serum, lecithin, certain erythrocytes, &c.) be also added. If, however, snake venom and complement (*i.e.* serum or erythrocytes containing endo-complement) be mixed together so that there is a *large excess of amboceptors* in the mixture, then the erythrocytes will not undergo hæmolysis. This is due to *deviation* (ablenkung) *of the complement*, all the complement available for the activation of the erythrocyte-amboceptor compound having become united with those amboceptors which are floating free and are not anchored to the erythrocyte. The complement and amboceptor become united, but the combine cannot become fixed to the erythrocyte.

Deviation of the complement was first noticed by Neisser and Wechsberg in certain bactericidal sera. Such a result could not be explained on Bordet's theory. If we follow Kye's work further, we find that the complement, which unites with the venomous amboceptor, is the complex fat *lecithin*, for if a chloroformic solution of lecithin be shaken for two hours with cobra venom—and treated as described in the footnote[1]—a chemical compound, viz. *cobra-lecithid*, is formed, which is actively hæmolytic towards ox's erythrocytes. The presence of this compound furnishes a further argument against Bordet's theory.

For a full discussion on the two theories see Hans Sachs ([5]).

The next question of interest is whether the amboceptor and complement are simple or compound bodies.

Taking the amboceptor first; is the amboceptor, produced, for example, by the injection of ox's blood into a series of animals, always exactly the same; or does it vary in nature according to the animal in which it is produced? Ehrlich and Morgenroth hold the latter view, and ascribe this difference in

[1] The snake venom and chloroformic solution of lecithin are shaken for two hours, the mixture then centrifugalised and the chloroformic and watery solutions separately pipetted off. Ether is then added to the chloroformic solution, when a precipitate of *cobra-lecithid* is thrown down. This is soluble in water, and after being freed of adherent lecithin by washing with ether is capable of producing the immediate hæmolysis of ox's erythrocytes. In its chemical reactions it is quite different from lecithin.

its nature to the variable structure of the cytophilic and complemento-philic groups. To demonstrate this for the *cytophilic group*, let us take an actual experiment: the serum of a rabbit, inoculated with *ox's* erythrocytes, can lake both ox's and goat's erythrocytes. The erythrocytes of these two animals can also be laked by the serum of another rabbit previously treated with injections of goat's erythrocytes. If, then, the cytophilic group of the amboceptors be a simple body, it must fit the receptors both of ox's and goat's erythrocytes equally well, and by mixing the immune blood with *either kind* of erythrocyte it should be possible to remove the amboceptors to an equal extent. Experiment proves this is not the case.[1] For if rabbit's serum, immunised against ox's erythrocytes, be mixed with *ox's* erythrocytes its hæmolytic power, both for the ox's and the goat's erythrocytes, will be greatly reduced; while, if it be treated with *goat's* erythrocytes, the original hæmolytic power for ox's erythrocytes will remain almost as before, but there will be a very marked fall in hæmolytic power towards goat's erythrocytes. The amboceptors which react with these two kinds of erythrocytes cannot, therefore, be exactly alike, else, in the latter part of the experiment, would the fall in hæmolytic power of the serum affect ox's and goat's erythrocytes to the same extent. An exactly similar result follows if rabbit's serum, immunised against goat's erythrocytes, be used; goat's erythrocytes binding the amboceptors for both kinds of erythrocytes, but ox's erythrocytes binding only those which take part in the hæmolysis of ox's blood. The serum of both these rabbits must therefore contain a certain amount of amboceptors common to both kinds of erythrocytes, and, *besides this*, each kind of serum must contain an amboceptor reacting only with the erythrocytes used for injection. In other words, *the amboceptor cannot be a simple body*, but must be compounded out of a number of partial amboceptors, each of which has slightly different cytophilic groups.

The production of these compound amboceptors is easily explained on the side-chain theory. An erythrocyte contains a number of different receptors: some of these, when the erythrocyte is injected into an animal, will anchor on to suitable cell receptors. There will accordingly be a liberation into the blood of various

[1] *i.e.* rendered hæmolytic towards.

types of biogen receptors, all of them fitting the receptors of the erythrocytes used for injection, but some of them also fitting the receptors of other erythrocytes. Erythrocytes which do not become hæmolysed when a certain immune serum is added to them do not possess receptors capable of fitting any of the cytophilic groups of the amboceptors. We have seen, then, that the cytophilic group of the amboceptors varies somewhat in structure.

As stated above, there is reason to believe that the *complemento-philic group* also varies; for equal quantities of the same erythrocytes, mixed with equal quantities of *different* normal sera, require a very variable amount of the same amboceptors to induce hæmolysis. In this experiment, erythrocytes and amboceptors are the same; they will unite equally in each case, so that any difference in hæmolytic power must be due to a difference in the union of complement and amboceptors.

This complex nature of the amboceptors is of importance clinically. In the treatment of certain bacterial diseases it is customary to inject anti-serum derived from one animal only. It may happen, however, that the amboceptors of this serum do not possess complemento-philic groups which will fit the complements of human blood. To increase the chance of there being suitable complemento-philic groups, it would be desirable, therefore, to use a mixed antitoxic serum, *i.e.* one containing various amboceptors. Such a serum could be obtained by injecting the same bacillus into various animals.

As has been indicated above, *the complement also is probably a compound body*—that is, there are various complements normally present in the blood serum. The fact that the complemento-philic group of the amboceptor is compounded so that it can fit different complements would itself imply the plurality of complements. This has been further proven by a series of elaborate experiments by Ehrlich and Morgenroth and others. (A summary of these experiments will be found in Hans Sachs's article, *Die Hæmolysine*, pp. 58–63.) We will content ourselves here with two of these experiments. I. If goat's serum be warmed to 56° C., only a *portion* of the complements disappears—that is, the heated serum can still activate *certain* amboceptors. The separation of these two fractions of complement in goats' serum can also be effected by filtering the serum through a Pukall's filter. By this latter method, two complement fractions could also be demonstrated in horse's serum. II. Complement fractions behave

differently towards different reagents. By treating goat's serum with four different reagents (digestion with papain, action of alkali, heating to 50° C., and uniting with various erythrocytes) Ehrlich and Sachs were able to separate four varieties of complement.

This fact concerning the complex nature of the complement has also a clinical bearing. Certain diseases (cholera and typhoid) are produced by bacterial growth in the tissues (in contrast to diphtheria and tetanus, which are due to bacterial poisons or toxins). These bacteria may be killed by injecting anti-bactericidal serum into the patient. This is obtained from an animal into which cultures of the bacillus in question have been inoculated in gradually increasing dosage. Now, such inoculations will only increase the amboceptors; they will not affect the complement. The immune (*i.e.* anti-bactericidal) serum will, therefore, contain plenty of amboceptors, but the complement present may not be of sufficient variety to react with these. This complement will, moreover, gradually diminish in amount if the anti-bactericidal serum be kept some time. Wasserman (7) has, therefore, suggested that fresh normal serum should be injected into the patient along with the bactericidal serum. Experimenting on animals, he found that this very materially assisted the action of the bactericidal serum. When applied to clinical therapeutics, however, the large amount of serum necessary seems to have an unfavourable action, and the method has not been fully elaborated.

It will be gathered from what we have learned about hæmolysins so far, that they are, in all their reactions, exactly like toxins. We have also seen that by the gradual inoculation of toxins into an animal, antitoxins are produced; and, if our analogy is to hold, we must expect that hæmolysins will similarly produce anti-hæmolysins. What evidence have we then of this?

Anti-Hæmolysins

As we shall see later, the serum of an eel's blood can produce very active hæmolysis when mixed with the erythrocytes of most other animals. This is because it contains a hæmolysin. If, now, the eel's serum be injected intravenously into a susceptible animal, in small non-lethal doses at first, and the injection repeated in increasing dosage, the blood serum of the latter will, after a few weeks, become endowed with the property of uniting this

hæmolysis; that is to say, if mixed with the fresh serum of eel's blood, and the mixture added to susceptible erythrocytes, no hæmolysis will follow. Just as the injection of a toxin causes a specific antitoxin to be produced in the injected animal's blood, so does the injection of hæmolysin produce an anti-hæmolysin.

The same thing happens if an artificial hæmolysin be employed. Thus, if the serum of a guinea-pig which has received several injections of rabbit's blood be repeatedly injected into a rabbit, in gradually increasing dosage, this rabbit's serum will, after some time, acquire the property of inhibiting the hæmolytic action of immunised guinea-pig's serum on rabbit's erythrocytes. These results are quite what might be expected from what we have learned above; curiously enough, however, the strongest anti-hæmolysin can be produced by injecting the hæmolytic serum into an animal whose erythrocytes are not readily hæmolysed *in vitro* by that serum. Thus a very strong anti-hæmolysin can be produced in a rabbit by injecting it with a serum rendered hæmolytic towards ox's erythrocytes. The receptors which form the anti-hæmolysin in this case must be derived from cells other than the erythrocytes (*i.e.* from extravascular cells).

As indicated by the side-chain theory, there are three possible ways by which anti-hæmolysins may be produced: there are three haptophoric groups concerned in hæmolysis, one belonging to the complement and two to the amboceptors; each of these may anchor on to receptors in the organism, and so lead to the liberation into the blood of similar receptors which, as we have seen above, will be capable of neutralising one or other of the three haptophoric groups. This is explained in Fig. 23.

Anti-amboceptors (AA in Fig. 23) have been described by several workers. According to Ehrlich it is the cytophilic group which, in this case, enters into the reaction. Of much greater importance, however, are *anti-complements* (AC in Fig. 23), *i.e.* bodies which cover over the haptophoric group of a complement and prevent it from uniting with an amboceptor. Such anti-complements are produced by inoculation of the complements of one animal's blood into another animal. Since there are as many complements in normal as in immune blood, anti-complements can be readily produced by employing normal serum. The complement leads to the production of anti-complement in that its haptophoric group unites with receptors in the injected animal. These are then produced

in over excess, float free in the blood, and can, when they come in contact with complements with haptophoric groups like those which produced them, cap over these and thereby prevent their union with amboceptors. The toxophoric group of the complement plays no part in this mechanism.

It is of interest in this connection to mention that Schütze (*) noticed that inactivated serum could, when injected into an animal, produce anti-complement as readily as could normal serum, from

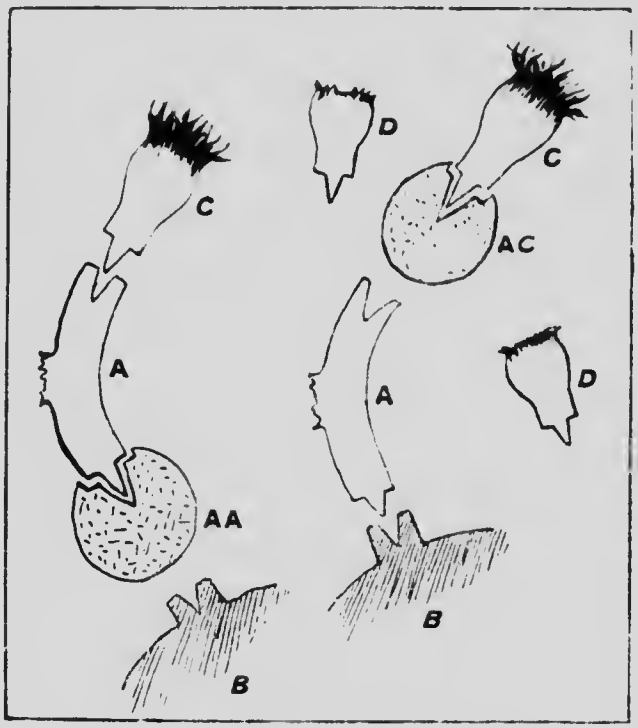

FIG. 23.—The Formation of Anti-hæmolysins.

which Ehrlich and Morgenroth argue that heat destroys only the toxophoric end of a complement, leaving the haptophoric intact. In other words, a body like a toxoid (see p. 442, footnote) is formed. This is called a *complementoid* (*D* in Fig. 23).

Arrhenius (*) has, however, recently brought forward evidence which seems to indicate that the anti-body which is produced when complements are injected into an animal is not strictly an anti-complement but an anti-lysin. In other words, the anti-body thus produced combines with the hæmolysin (*i.e.* amboceptor + comple-

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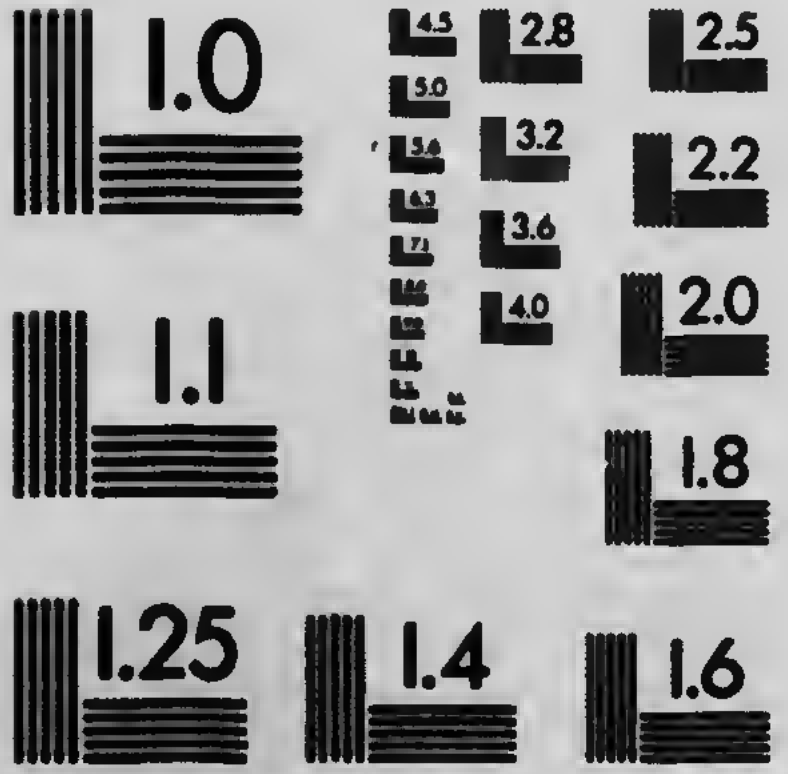

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ment in Ehrlich's sense) to form a new compound which does not act as a hæmolytic agent. The structure of this compound would therefore be hæmolysin + anti-hæmolysin.

Like anti-bodies in general, anti-complements can withstand a temperature of 55°–60° C. For their detection, therefore, the serum in which they exist is heated to the above temperature so as to inactivate any complements which it contains, and which might react with any suitable amboceptors in the blood with which it was mixed, and thereby mask the action of the anti-complement. For example, suppose we wish to study the anti-complements produced in rabbit's blood by the injection into the rabbit of guinea-pig's serum rendered hæmolytic towards rabbit's erythrocytes. If the anti-complement containing serum were directly mixed with a hæmolytic serum (produced by injecting rabbit's erythrocytes into a guinea-pig) the complements which that serum contains would unite with some of the amboceptors present in the immunised (guinea-pig's) serum, and would, consequently, mask the full action of the anti-complement—*i.e.* a certain amount of hæmolysis of the rabbit's erythrocytes would ensue. Whereas, if the anti-complement containing serum be heated no hæmolysis will follow.

Anti-complements are specific bodies; the anti-complements developed in rabbit's blood by the injection of guinea-pig's serum can only inhibit the action of the complements of guinea-pig's serum, unless, as a coincidence, it should happen that some other blood possesses complements with haptophoric groups which fit those anti-complements. Now we have seen that there are various complements present in serum. On injecting the serum of one animal into another, certain of these complements, but not all of them, will meet with receptors on to which they can anchor, and the liberated receptors, *i.e.* anti-complements, will inhibit the action only of some of the complements of the blood used for injection.

With regard to the exact nature of the receptors which form the anti-complements, Ehrlich and Morgenroth consider them as similar to amboceptors (*i.e.* of the third order), with the difference that the complemento-philic group has acquired greatly increased combining powers.

When anti-complements are present in the blood along with complements, whose action they can prevent, they are called *anti-auto-complements*. They never appear spontaneously in the blood, but, as Ehrlich and Morgenroth have shown, they can be experimentally produced. Thus, as we have seen above, normal

rabbit's serum can hæmolyse, to a certain extent, the erythrocytes of a guinea-pig. If goat's serum be injected into the rabbit, however, the serum of the latter loses its hæmolysing action on the erythrocytes of the guinea-pig; and even if some normal rabbit's serum be added to the mixture, no hæmolysis will occur. To explain the production of anti-complement, we must suppose the complements of the goat and rabbit to be very much alike, but in so far different that the former find, in the tissue cells of the latter, receptors with which their haptophoric groups unite, and these receptors, being rendered useless to the tissue cell, become regenerated in over excess and float free in the blood. The complement normally present in the blood cannot, of course, unite with receptors in the same animal's tissues, unless some other body—*e.g.* a foreign erythrocyte or a bacterium—be present to combine with the cytophilic group of the amboceptors. Nor could the injection of complements very unlike those normally present in blood lead to the production of anti-complements; because their haptophoric groups would not fit complemento-philic endings.

Anti-complements, then, are nothing more or less than free receptors, and, as we might expect, these receptors can be disconnected from their cells by other means than the injection of complements like them. Thus they can be liberated when the cell is disintegrated, as in phosphorus poisoning. For example, the serum of a rabbit, two days after receiving a poisonous dose of phosphorus, could not produce any hæmolysis of guinea-pig's erythrocytes (Ehrlich and Morgenroth).

Chronic suppuration may lead to the production of anti-complement from the same cause. Even emulsions of fresh organs can bind a certain amount of complement. As would be expected, the presence of anti-complement materially lowers the natural resistance of serum towards injections (Wassermann[7]).

According to the side-chain theory, any substance containing amboceptors which fit on to one or more of the receptors of an erythrocyte will be capable of producing hæmolysis, provided that the other combining group of the amboceptors is united with a complement or complements. As a matter of fact, there are a number of substances, some of vegetable and some of animal origin, which, when added to a suspension of erythrocytes in isotonic saline, can cause hæmolysis. The more important of these,

we shall study presently. Before doing so, however, another property of many of these substances, viz. their power of producing *agglutination of the red corpuscles*, must be studied. This frequently goes hand in hand with hæmolysis, although we have reason for believing that the two processes are produced by entirely different agencies. Bordet first discovered that the serum of an animal *A*, which has been gradually injected with the blood of an animal *B*, when added to some defibrinated blood of *B*, caused an immediate agglutination of the corpuscles, so that clumps of them, suspended in the serum, were formed. This clumping is very evident on shaking the test-tube. Other signs of agglutination are the rapidity with which the clumped corpuscles settle down, and the fact that, on filtering the blood through paper, the corpuscles remain behind as a precipitate, whereas in normal blood they filter through.

These hæmagglutinins are analogous with bacterioagglutinins, discovered by Gruber, Durham, and Widal in the blood serum of animals injected with certain organisms; for example, the serum of a typhoid patient agglutinates typhoid bacilli. With regard to their mode of action, it is necessary to somewhat modify the side-chain theory as applied to the process of hæmolysis. The receptor which produces the clumping is supposed to be furnished at one end with a functionating or agglutinophoric group, the other end being a haptophoric or combining group. This latter, when agglutination occurs, is supposed to anchor on to a suitable receptor of the erythrocyte or bacterium. When thus united, the agglutinophoric end of the agglutinin amboceptor acts in some, not yet clearly described, manner on the cell, whereby it causes these cells to clump together. Such receptors Ehrlich called *receptors of the second order*, and the essential difference between them and hæmolysin receptors (*i.e.* third order) is that the complemento-philic group is replaced by a zymophoric or acting group, which does not require to become combined with complements before it can act.

These agglutinins are quite distinct from hæmolysins or bacteriolysins, although some observers have thought them to be identical. Thus Gruber thinks agglutination to be the preliminary stage of bacteriolysis, and Baumgarten thinks the agglutinin to be the same thing as the amboceptor, the apparent difference between them being, according to this observer, merely a quantitative one.

That the two are different processes, however, is shown by the following facts: (1) agglutinated bacteria can multiply as well as can

unagglutinated bacteria; (2) certain sera are able to agglutinate the corpuscles of one species without effecting their hæmolysis, and to both agglutinate and hæmolyse the blood of other species; (3) agglutinins are more resistant to heat than hæmolysins, so that, if a serum containing both be warmed to 55° C., the

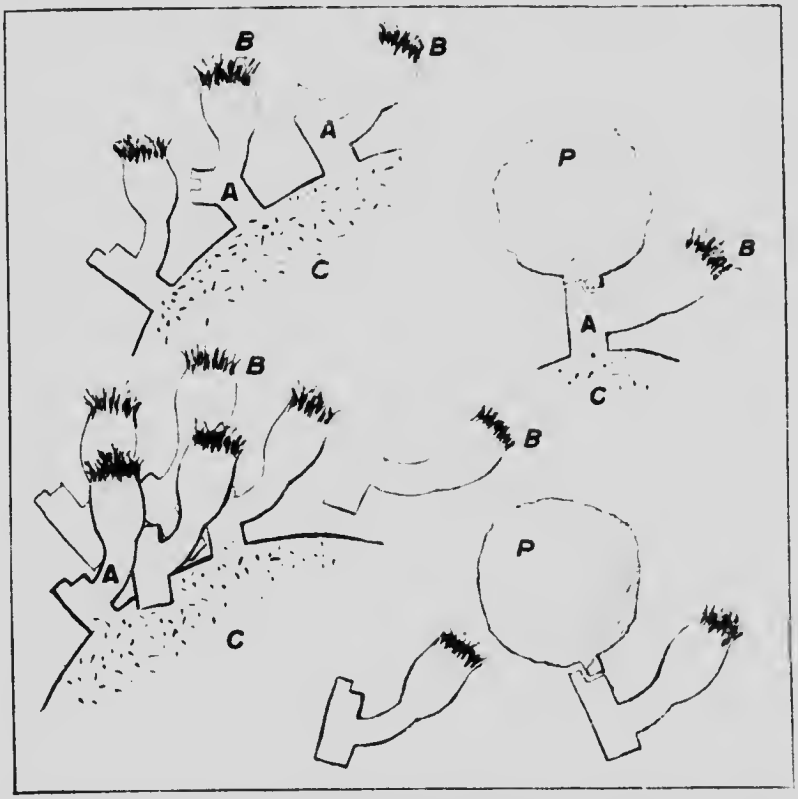


FIG. 24.—Receptors of the Second Order (Ehrlich's theory); the letters refer to the same Structures as in Fig. 22. *B* representing the Zymophoric Group of the Receptor, and *P* the Agglutinated Body.

hæmolytic power will disappear (*vide supra*), but it will still agglutinate.[1]

We are now in a position to study the various substances in which hæmolysins and agglutinins naturally occur, and for this purpose, it will be most convenient to study them according to

[1] It is possible that the same substance in different concentrations may have a different action.—(*Editor's Note.*)

the source from which they are derived. In this connection we shall consider the following:—

I.—Hæmolysins present in normal blood.
II.—Hæmolysins in the tissues of lower animals.
III.—Hæmolysins in bacterial cultures.
IV.—Hæmolysins in certain plants.

I.—Hæmolysins present in Normal Blood

In 1888 Mosso noticed that eel's blood was very poisonous when injected into other animals. It was not till 1898 that the cause of this was found to be a normally occurring hæmolysin in eel's blood, very active towards the erythrocytes of most animals. Similar hæmolysins have been discovered in the blood of various other animals; thus, to take another example, 0·5 c.c. of goat's serum can lake 5 c.c. of a 5 per cent. suspension of rabbit's erythrocytes. These natural hæmolysins appear to act in the same way as do those experimentally produced, for, as Daremberg (5) has recently shown, heating the serum to about 55° C. removes its hæmolytic power. These facts are of some therapeutic interest, for they show us that intravenous injection of foreign bloods may have a poisonous (*i.e.* hæmolytic) action; but that this can be removed by heating the serum to 55° C., a temperature which will not coagulate the proteids, but will destroy the hæmolysin. A bactericidal action of normal serum has also been described by Nuttall, Behring, and others.

Further proof that normal hæmolysins act in the same way as do those artificially produced is by no means easy to furnish, since there is, in normal blood, only a trace of amboceptors with a large excess of complement. That both amboceptor and complement do form the hæmolysin in this case has, however, been proved by the following experiments of Ehrlich and Morgenroth (9). A. The serum of a normal goat can lake guinea-pig's erythrocytes; if, however, the serum and erythrocytes be kept at 0° C., no laking will occur, for, as explained on p. 447, there is, at this temperature, no union between amboceptor and complement, although amboceptor and erythrocyte do unite. If, after two hours, the mixture be centrifugalised, the sediment will be found combined with amboceptors and the supernatant fluid will no longer hæmolyse guinea-pigs' erythrocytes (because it only contains complements); it can be activated, however, by adding

to it some inactivated goat's serum (that is, containing only amboceptors).

B. A hæmolytic normal serum is, as we have seen above, inactivated by heating it to 55° C., so that it can no longer lake the erythrocytes which it normally could. If this inactivated serum be mixed with some serum of a third animal, which in itself does not hæmolyse the erythrocytes under examination, the complement of this non-hæmolytic serum may activate the inert serum and so produce hæmolysis. This result depends on the fact that every serum contains various complements; if, then, a group of sera, in themselves non-hæmolytic towards the erythrocyte used, be tried systematically, one will be got in which one or more of the complements which it contains will fit the haptophoric group of the amboceptor, and so complete the reaction.

II.—Hæmolysins occurring in the Tissue Fluids of Animals lower than that from which the Erythrocytes are derived

The most interesting of those is *snake venom*. This is really a toxin, and its injection in gradually increasing dosage causes anti-venoms to be produced in the inoculated animal: just as toxins produce antitoxins when similarly injected. Snake venom is a complex fluid containing not one, but several cytolytic bodies (*i.e.* bodies capable of dissolving cells); one of these acts on erythrocytes, producing hæmolysis. This hæmolytic action can be prevented by mixing the venom with anti-venomous serum, and such a mixture, when inoculated into a susceptible animal, no longer kills it. The venom, therefore, becomes neutralised at the same time as the hæmolysin (Stephens and Myers). The hæmolytic action of snake venom differs somewhat in its nature from that of other hæmolytic bodies. This fact was shown by Flexner and Moguchi (5), who found snake venom to contain amboceptors alone; for when they mixed it with erythrocytes of the ox and goat, which had been very thoroughly washed free of all possible traces of adherent serum, no hæmolysis, but only an agglutination of the erythrocytes resulted. If a little normal serum were now added to this mixture of washed erythrocytes and snake venom, hæmolysis at once followed. The amboceptors of snake venom are very stable bodies, for they can stand heating to 90° C.

Kyes (6) has, however, shown that the serum-free erythrocytes of certain animals (*e.g.* guinea-pig, man, dog, &c.) do undergo hæmolysis with cobra venom alone, the complement in this case being furnished by the erythrocyte itself (*i.e.* by *endo-complement*); and further, that a solution of such erythrocytes in isotonic salt solution can furnish the necessary complement, and so produce hæmolysis when added to a mixture of snake venom and complement-free erythrocytes (*e.g.* those of ox, sheep, goat, &c.).

Various *insect poisons* (obtained from the stings) can produce hæmolysis (*e.g.* of man and dog) when added to the blood of certain animals, whereas when added to other bloods no hæmolysis follows. This is thought to be due to the fact that the serum of these latter bloods contains anti-bodies. Further work on this group of hæmolytic substances seems to be wanting.

From *toads* a very active hæmolysin, especially active towards the erythrocytes of the sheep, can be obtained by grinding the skin with sand in a mortar and extracting with isotonic salt solution. Such a solution can produce hæmolysis in a dilution of 1 in 10,000. This hæmolysin, like others, is inactivated by heating to 56° C., but it cannot be reactivated by adding the serum of higher animals. Pröscher (5) has, however, succeeded in obtaining an antitoxin, by repeated injection into rabbits.

The poisonous secretions of *spiders* also produce marked hæmolysis, especially of rabbit's erythrocytes. For example the hæmolysin of 1·4 grm. of spiders can hæmolyse 2·5 litres of rabbit's blood. The hæmolytic agent in this case is called *arachnolysin*. For purposes of examination, it can be easily obtained by extracting ground-up spiders (*Epeira diadema*) with isotonic salt solution. A very interesting fact concerning arachnolysin is that it has absolutely no action on the erythrocytes of the guinea-pig. It cannot even combine with the erythrocytes of this animal; for, if a mixture of arachnolysin and guinea-pig's erythrocytes be allowed to stand for some time and then centrifugalised, the supernatant fluid will be found to be, quantitatively, as hæmolytic towards rabbit's erythrocytes as before. Hans Sachs (5) has, however, shown that anti-bodies can be produced by inoculation of arachnolysin into a guinea-pig; as well as into a rabbit. These anti-bodies produced in the guinea-pig must therefore be derived from some tissue outside of the blood. In other words, the extra vascular tissue must possess receptors capable of fitting the haptophoric group of arachnolysin. That the arachnolysin anchors

on to tissue cells does not imply that it should poison these, but it nevertheless occasions an overproduction of biogen receptors so that anti-bodies are produced.

III.—Hæmolysins in Bacterial Cultures

Both bacteria and bacterial toxins can produce hæmolysis. Koch first noticed this for cholera vibrios, and some years later van de Velde (5) showed that a filtered culture of staphylococcus did the same thing. This so-called *staphylolysin* acts most sensitively on rabbit's erythrocytes. Many normal sera (that of a man) when added to the staphylolysin diminish its hæmolytic power; in other words, these sera contain anti-bodies for staphylolysin. Now, according to Ehrlich's theory, these latter must be haptophoric receptors which fit on to the haptophoric group of the hæmolysin amboceptor, and thereby prevent its uniting with the erythrocytes. They must have been cast off from tissue cells, where, when attached, they functionated in assimilating food. It is merely an accident that they should fit the haptophoric group of the staphylolysin amboceptors.

Hæmolysins have been also discovered in cultures of the tetanus bacillus. In these cultures, the *tetano-lysin* exists alongside of a *tetano-spasmin*, which latter is the specific toxin, producing tetanus. That these two toxins (tetano-lysin and tetano-spasmin) are distinct from one another is proved by the fact that erythrocytes take up the tetano-lysin but not the tetano-spasmin, and also by the fact that tetano-lysin is more easily destroyed by heat than is tetano-spasmin. Horse's and rabbit's erythrocytes are especially sensitive towards tetano-lysin, and specific anti-bodies against tetano-lysin become developed in animals inoculated with tetanus.

Many other cultures have been shown to contain hæmolysins, and no definite relation can be shown to exist between the virulence of a bacterium and the hæmolytic power of the medium in which it is grown; indeed, the strongest bacterial hæmolysin hitherto observed (by Todd) was found in a saprophyte (*B. Megatherium*). A hæmolysin contained in pyocyaneus cultures has been shown by Bulloch and Hunter (10) to be distinct from the pyocyaneus toxin which such cultures also contain: another proof of the complexity of toxins.

Bacterio-hæmolysins can also produce their action in the blood-vessels, and this may account for the anæmic symptoms so common

in many of the infections. Welch [11] suggests that some obscure anæmias, not of infectious origin, may be associated with the growth of certain hæmolysin-producing saphrophytes in the tissues. It is possible that anæmias produced by this cause might be prevented by injecting anti-hæmolysin; in fact, Todd has shown experimentally that a hæmoglobinuria produced in guinea-pigs by injection of *B. Megatherium* can be prevented by the simultaneous inoculation of an anti-serum.

IV.—Hæmolysins and Agglutinins of certain Plants

These are called phytalbumoses, because they give chemical reaction like albumoses; ricin and abrin are the most important members of this group. The former of these is obtained from the seeds of the *Ricinus Communis*, from which also castor-oil is prepared. It is very poisonous when inoculated into animals, and its injection in gradually increasing sub-lethal doses leads to the production in the injected animal of anti-ricin. Moreover, this anti-ricin serum when inoculated into another animal (mouse) will confer on it immunity against ricin inoculation. Ricin does not possess any hæmolytic power, but it is capable of causing agglutination of the eryt' ocytes of most animals, and an anti-body to this reaction can also be produced by progressive inoculation. The agglutinating and toxic actions seem to run parallel with one another, and Jacoby [5] has recently shown that a precipitate forms when a clear ricin solution is mixed with anti-ricin serum. Jacoby describes the ricin receptor (that is, the anti-body) as having three combining groups: one haptophoric, uniting with erythrocytes; one toxophoric, causing poisoning; and one agglutinophoric, causing agglutination. He was able to produce three modifications of ricin, one in which the agglutinating group remained active but the toxic one was saturated; another in which the reverse obtained, and a third in which both agglutinophoric and toxophoric groups were saturated. Perhaps the most interesting part of Jacoby's work, however, is that he was able to prepare ricin free from any trace of proteid. All other toxic substances which can produce antitoxins seem to be proteid in nature, and the preparation of ricin in a proteid-free state would tend to indicate that the apparent proteid nature of some at least of the other bodies is due to admixture with proteid. It should be mentioned here, however, that Osborne and Mendel [36]

have been unable to obtain proteid-free ricin: the active product which they obtained by very careful work gave an elementary composition identical with proteid; it also gave certain of the proteid reactions.

It is of interest to note in this connection that other vegetable poisons, such as alkaloids, do not produce anti-bodies. In fact, we do not know of any substance, whose chemical structure is accurately known, which does. The discovery of the chemical constitution of any of these poisons which can, when inoculated, lead to the production of anti-bodies would be of inestimable value, for then a much clearer insight could be obtained into the chemical nature of anti-bodies and of their numerous modifications.

Abrin, a phytalbumose obtained from the seeds of *Abrus precatorius*, also possesses toxic and agglutinating properties, and, by inoculation, anti-bodies can be produced against its action.

The agglutinating properties of these two vegetable toxins are very closely related to a hæmolysing action shown by certain other phytalbumoses such as phallin and crotin. The latter poison produces hæmolysis of rabbit's erythrocytes and agglutination of those of the sheep, pig, and ox. There can, therefore, be no doubt that the two processes are akin, and Ehrlich suggests that the agglutinating substance of phytalbumoses, which is the most evident, is the same thing as the hæmolysing substance; the agglutination, where it alone is exhibited, hindering hæmolysis by clumping the corpuscles together. In fact, Baumgarten has shown that vigorous shaking of erythrocytes agglutinated by means of ricin and abrin causes partial hæmolysis. It is difficult to see, however, how this view of Ehrlich can be accepted, if he considers as different, the agglutinins and hæmolysins derived from other sources.

Cytotoxins

Erythrocytes and bacteria are not the only animal cells which can, when injected into an animal, cause lysogenic substance to be formed. Any other animal cell can do the same thing. Thus, as was discovered by Metchnikoff ([13]), if the leucocytes of a rabbit (lymphatic gland or red marrow of bone) be injected every four days into a guinea-pig for several weeks, the serum of the guinea-pig will, if added to rabbit's leucocytes, quickly dissolve them. This can be observed under the microscope. The so-called

leucotoxin which is thus produced in the guinea-pig is very specific in its nature, affecting the leucocytes only of animals of the same species. If the leucotoxic serum be heated to 55° C. it loses its lysogenic power, but can be reactivated by mixing with normal serum. A large injection of leucotoxic serum will kill an animal (in the above case a rabbit), but if the injections be at first in sub-lethal doses and be repeated in gradually increasing dosage, an *anti-leucotoxin* will be developed in the injected animal's serum. In every respect, then, these bodies act like hæmolysins, and, indeed, they are produced by the same mechanism, amboceptors of the third order being liberated in the blood by the overproduction of certain receptors of the tissue cells, on account of similar ones having been rendered functionless by the injected cell uniting with them.

Spermatozoa can also produce *spermotoxins* when injected into an animal. These differ from leucotoxins, however, in that they can produce solution not only of spermatozoa, but also of the erythrocytes of the same animal. Such a serum, therefore, contains both hæmolysins and spermotoxins. The explanation of this result is that spermatozoa possess some receptors which are similar to those of the erythrocytes. In this case also, an anti-spermotoxin can be produced by the gradual injection of spermotoxic serum.

Ciliated epithelium, obtained by scraping the trachea of the ox, and suspended in isotonic salt solution, when injected into another animal also produces a so-called *anti-epithelium serum*, *i.e.* an epitheliolysin. Here, again, a hæmolysin became coincidently developed. This interesting result, discovered by von Dungern (7), led to the hope that other forms of epithelium would also produce epitheliolysins, and that one for cancerous cells might be obtained. So far, however, this hope has not been realised.

It would be superfluous here to describe in detail the other cytotoxins which have been obtained. They all behave according to the laws we have described above, and the most important of them are: *Nephrotoxin*, obtained by injecting a fresh emulsion of kidney cells; and producing albuminuria, when injected into suitable animals; *N toxin*, obtained by injecting into the abdomen of geese, emulsion of dog's brain; and producing, when injected into the cerebrum of dogs, marked convulsions and death; *Hepatotoxin*, obtained by injecting emulsion of liver cells, &c.

Præcipitins

Not only toxins and living cells, but also proteids can, when injected into an animal, lead to the production of specific bodies with which they react. In this case the reaction takes the form of a precipitation, hence these bodies are called Præcipitins. For example, the normal blood serum of a rabbit mixes with the normal blood serum of a horse without any precipitate forming; if, however, horse's serum be frequently inoculated in small dosage into a rabbit, the serum of the latter will, after some time, produce a precipitate when mixed with horse's serum (Tsistowitsch [7]). It may, in fact, be stated as a law that the repeated inoculation into an animal of a proteid foreign to its tissues leads to the production, in the blood serum, of a præcipitin acting only on the proteid used for inoculation. In such experiments with blood serum there will, of course, be produced, besides the præcipitins, various other anti-bodies (anti-complement, anti-agglutinins, &c.). The mechanism involved in the production of præcipitins is probably identical with that involved in the production of agglutinins—*i.e.* receptors of the second class are regenerated in over excess as a result of similar receptors having been fitted by the injected proteid.

A large number of præcipitins have been described, and it may be of interest to mention a few of the more typical of these. If cow's milk be injected into a rabbit, the rabbit's serum will precipitate the caseinogen of cow's milk (lactoserum); albuminuric human urine produces in the serum of an injected animal a præcipitin for the proteid of albuminuric urines; vegetable proteid, a serum precipitating vegetable proteid; egg albumin, a serum precipitating egg albumin; peptone, one precipitating peptone, and so forth. It is of importance to note here, however, that præcipitins produced by the injection of the blood serum of one animal into another react with the serum of all closely allied animals. Thus if human serum be inoculated into a rabbit, the præcipitin thereby produced in the rabbit will precipitate the proteid, not only of human serum, but also of that of the higher apes. Similarly, the serum of a rabbit, inoculated with the serum of a hen, can precipitate the proteids in the serum of a pigeon. In the same way no præcipitin is produced by injecting the blood serum of an animal into another animal of the same genus.

Thus by injecting the blood of a pigeon into a hen, or that of a rabbit into a guinea-pig, no præcipitin is developed. The præcipitin produced by injecting the milk of one animal into another of a different species is also specific in its nature. Thus if cow's milk be injected into a rabbit, the rabbit's serum will precipitate the casein of cow's milk, but not that of a goat or of human milk.

The production of præcipitins has received a *practical application* which may be of considerable medico-legal value. This applies to the detection of human blood which, when present in small amount, cannot be distinguished, with certainty, from that of any other animal, by any of the older tests. The new test we owe to A. Wassermann (7). It depends on the fact that the serum of a rabbit, subcutaneously inoculated several times with human serum, will give a precipitate in any solution containing the proteids of human blood serum, or of some of the higher apes, but not of other animals. The exact *technique of the method* is as follows: The blood stain or crust is macerated in isotonic salt solution, and filtered. The clear filtrate is divided into two parts, each of which is placed in a small test-tube. To one of these is added the præcipitating rabbit's serum; the other is left standing to serve as a control; into a third tube is placed some of the præcipitating serum alone; and into a fourth tube a mixture of the præcipitating serum and some indifferent serum (of an ox) in physiological saline. These four tubes are placed for about an hour in an incubator at body temperature. If the stain be due to human blood, a precipitate or cloudiness will develop in the first tube, but all the others will remain clear. The amount of precipitate produced by mixing the human præcipitin with the blood serum of an ape is most marked with those apes higher up in the scale of the Primates. Indeed, as Nuttall (14) and others have shown, the biological relationship of one animal to another can be very clearly defined by this reaction. It may be well to mention that, although the use of Wassermann's reaction for the detection of human blood is undoubtedly of great medico-legal value, it will require some further elaboration before it can be used as an absolute test; there being, apparently, a tendency in some cases for anti-præcipitins to be developed, and these of course mask the reaction. The method has a further practical application. If an extract of horse flesh be inoculated into a rabbit, this rabbit's serum will produce a precipitate in extracts

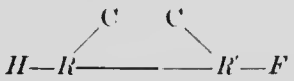
of horse flesh, but not in extracts of ox flesh. In this way horse flesh can be distinguished from ox flesh.

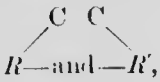
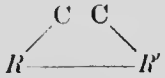
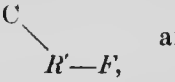
Although Ehrlich's theory shows us how anti-bodies in general may be produced, it does not explain why certain bacteria, such as those of typhoid fever and cholera, should exercise a toxic action on the tissues, when neither of these bacteria liberates any toxins into the culture fluids in which they are grown. The side-chain theory tells us that anti-bodies against bacteria are produced by a process analogous with that which produces hæmolysins, but it leaves unexplained *how these bacteria themselves poison the tissues*. Radzievsky [15] has tried to explain their action by supposing that they are destroyed in the body, and that, as they fall to pieces, the toxins which act on the tissue cells are liberated. As Welch has shown, however, such a mechanism is impossible, and to explain their toxic action this worker has suggested another hypothesis. He points out that all invading cells (bacteria, erythrocytes, epithelial cells, spermatozoa, &c.) are acted on by the tissue cells in the same way as these latter act on the bacteria, &c.; that is, by the intermediation of receptors. The bacterium, he supposes, possesses a variety of receptors, some of which are fitted by receptors of tissue cells, and are, therefore, rendered functionless to the bacterium. As a consequence of this, then, the bacterium reproduces its lost receptors in over excess, and sheds them into the plasma, where, obeying the same laws which apply to tissue receptors, they will anchor on to the tissue cells of the host, and, by bringing the complement into contact with these, will thereby poison them.

"It may perhaps aid in grasping the ideas here presented to imagine the bacterium in the capacity of the host, as a structure so large that one could inject animal cells into it. Provided the proper receptor apparatus be present, the resulting reaction on the part of the bacterium, as described, would be a process of immunisation against the animal cells, through the intermediation of specific cellulicidal substances" (*Welch*).

This reciprocal reaction of tissue cell and invading cell might be represented by a formula somewhat on the same plan as that suggested by Sollmann [16]. Let H represent the cell of the host, F the invading cell, R the amboceptor attached to H, R' that attached to F, and C the complement. We have seen that the complement C is present in normal serum, and that it is unaffected

by inoculation; we may, therefore, for the sake of argument, represent it as always present. When *F* comes in contact with *H* the following reaction will ensue—



The amboceptors *R* and *R'*—each with two valency bonds, one of which, in each case, is saturated with *C* and the others combining together—are therefore rendered functionless for the cells *H* and *F*, and are reproduced by these cells in excess, so that they come to float free in the blood plasma, in which will, therefore, be found two mutually antagonistic groups  , both incapable of anchoring on to the cell which produced them, but capable of uniting together or with receptors of the opposing cell. If these groups be in equal amount, a neutral body represented by the formula  will be formed, and no linkage of *F* to *H* will be possible. If either should be produced in excess of the other, however, damage of the opposing cell will ensue; thus, if  be in excess, it will anchor on to  and *F* will be destroyed. By such a conception, the production of anti-hæmolysins, &c., can be explained; these being represented by  the free combining group of *R'* uniting with the free group of *R*, and thereby preventing its union with the erythrocytes. (See also Arrhenius's explanation on p. 471.) Such a formula would require modification for antitoxins, since in this case no amboceptors, but only a simple receptor, unites the toxin to the cell. The formula representing the action of toxin *T* on a cell *H* would stand H – R – T, and the anti-toxin would be R, which, combining with the available affinity of *T*, would neutralise it and prevent its action. The toxin being no living cell, cannot regenerate its lost receptors.

It has been stated above that the process of hæmolysis is analogous with that of bacteriolysis, and that it is by the latter process that the tissue fluids destroy invading bacteria. Such a view has

for several years been generally accepted in place of the older one of Metchnikoff and his pupils, that invading bacteria are dealt with by the leucocytes by a process of phagocytosis.[1]

By an accurate study of hæmolysis it was hoped that a sufficient knowledge of the exact nature of the kindred process of bacteriolysis would be forthcoming, whereby a cure for these infections due to bacterial invasion could be obtained. Such a hope, however, has not been realised, and very recently a revival of the older theory of phagocytosis has occurred, a revival due in main to the discovery by Wright and Douglas ([17, 18]) of certain substances in normal serum which so act on bacteria as to render them susceptible of being taken up by the leucocytes.

Let us consider one or two of the main experiments which demonstrate this power of normal serum. To measure the phagocytic power of leucocytes, Wright and Douglas mixed a suspension of washed blood corpuscles, or other fluid containing leucocytes, with an equal bulk of a suspension of bacteria (*e.g.* B. typhosus, D. pneumonia, B. coli, streptococci, &c.), and placed the mixture in the incubator at 37° C. for about fifteen minutes; they then made a smear of the suspension and stained it in such a way as to demonstrate the bacteria which had been taken up by leucocytes.[2] In this way they were able to count the average number of bacteria taken up by each leucocyte.[3]

If leucocytes, washed free of serum by isotonic saline, be treated, as above, with a suspension of bacteria, the leucocytes do not take up any of the bacteria, but if normal blood serum be present in the mixture then each leucocyte may take up as many as 25 bacteria. Serum evidently, in some way, stimulates phagocytosis. How does it do this? It might be that the serum had stimulated the leucocytes to increased phagocytic power; or that it had not affected the leucocytes themselves, but had rendered the bacteria more susceptible to being taken up by them. The latter was shown by Wright and Douglas to be actually what occurred, for if the bacteria be first incubated with some normal serum, and then washed free of all trace of adherent serum by saline, they are readily taken up by the

[1] In this older theory it is supposed that the leucocytes, in virtue of their power of phagocytosis, catch hold of the invading bacteria, and by including them in their protoplasm render them innocuous unless the bacteria be of such virulency as to poison the leucocytes, and thus paralyse their phagocytic power.

[2] *i.e.* by Leishman's method.

[3] It is the polynuclear leucocytes which act as phagocytes. To obtain the average, the bacteria taken up by about twenty-five phagocytes should be counted.

leucocytes when a bacterial suspension is mixed with a suspension of washed leucocytes.

Something must therefore be contained in normal blood serum which renders bacteria susceptible of being absorbed by leucocytes.

This substance they have named an *opsonin* (feast preparer).

By heating the normal serum to 60° or 65° C. for fifteen minutes its opsonin is destroyed, and it is now no longer capable of sensitising bacteria towards leucocytes.[1] If, on the other hand, the bacteria be first of all incubated at 37° C. with normal serum and then heated to 60° or 65° C. they remain sensitised.

These experiments recall much of what has been described in connection with Ehrlich's side-chain theory. The opsonin probably possesses a haptophoric group whereby it unites with the bacterium, and an activating group, whereby it produces some chemical or other change in the leucocyte which stimulates the latter to absorb the bacterium. The activating group can produce this effect on the leucocytes only after the haptophoric group has become fixed to the receptor of a bacterium. The one group is like the haptophoric group of a complement, the other like the acting group, and this analogy to the complement is further sustained by the instability of opsonins to heat.

The opsonins in the blood serum of one species may sensitise bacteria towards the leucocytes of a different species.

Dead bacteria can absorb opsonins, for, if serum be digested with these it can no longer sensitise living bacteria ; the receptor group of the bacterium therefore still remains capable of joining with the haptophoric group of the opsonin after the bacterium is dead.

In some interesting experiments recorded by Bulloch and Atkin ([19]) it is shown that all healthy leucocytes possess an equal phagocytic power, provided they be brought in contact with the same bacteria sensitised by the same opsonin. If, on the other hand, different opsonins be used to sensitise the same bacteria, and these latter be brought into contact with the same leucocytes, the number of bacteria picked up by each leucocyte will be found variable. Opsonins from different individuals possess different sensitising powers. Of great significance is one result obtained by Bulloch and Atkin showing that the blood serum of a girl suffering from facial lupus and tubercular sores on the

[1] The heat destroys the amount of opsonin present in normal serum (as tested by Wright's method). A patient infected with tuberculosis has opsonin in his blood which is not all destroyed by heat in 15'. By this simple test cases of tuberculosis can be diagnosed (Wright). The injection of nuclein increases the quantity of opsonin (Bulloch).—(*Editor's Note.*)

hands contained much less active opsonin towards the tubercle bacillus than did the blood serum of a healthy individual. On the other hand, the leucocytes of the tubercular girl could take up as many tubercle bacilli as those of healthy serum when normal blood serum was employed to furnish the opsonin.[1]

In conclusion, it may be stated that although the side-chain theory is the only one which can be at present employed to explain all the intricate reactions and interactions of immune bodies, there possibly exists a much simpler relationship between them. Indeed Arrhenius (8) and Madsen have recently shown that many of the processes behave according to simple chemical laws of neutralisation and solution. For example, the neutralisation of tetanus toxin by its antitoxin behaves according to the same laws as those which govern the neutralisation of a weak base by a weak acid;[2] and the action of varying amounts of agglutinin on a suitable bacterium is analogous with the behaviour of a soluble substance in varying amounts of two different solvents.

The extreme importance of Ehrlich's theory as a means of predicting what interactions of toxins and allied bodies are possible cannot, however, be overestimated, and a thorough mastery of the theory in all its details will well repay all who possess it. It is the scaffolding from which the worker is able to build up and piece together the disconnected material presented to him. The complicated scaffolding must stand till the simpler, but more durable, superstructure is completed. Without it, no progress in the work is possible.

The opsonic value of the serum is increased specifically by the injection of minute doses of dead bacteria, *e.g.* $\frac{1}{100}$ grm. tuberculin. (A negative phase precedes the positive phase.) Staphylococcic infections (boils and acne) and tubercular lesions are being successfully treated by this method of vaccination. Auto inoculation occurs on infection, and this may be too much, leading to a persistent negative phase and death, or too little, leading to a chronic lesion. In the first case auto-inoculation is limited by rest, *i.e.* by keeping the circulation quiet in the infected part. In the second case fomenta-

[1] Barratt (20) has demonstrated that red corpuscles may be eaten by phagocytes under the influence of an opsonin after removal of amboceptor from the serum. The serum of a goat injected with sheep's red corpuscles caused phagocytosis of rabbit's, sheep's and dove's red corpuscles. This proves that opsonin and amboceptor are different substances.—(*Editor's Note.*)

[2] This conclusion is controverted by experiments of J. Craw (21), who finds he can separate a neutral mixture of a toxin and an antitoxin by means of a gelatine filter, a result which is also not in agreement with Ehrlich's theory. Craw thinks the union of antitoxin and toxin and bacteria with agglutinin resembles that of a tissue with a dye.—(*Editor's Note.*)

tions, &c., are employed to bring more blood to the part. Thus the old methods of treatment are explained by the discovery of opsonin. Vaccination is made into healthy subcutaneous tissues to excite the production of the specific opsonin there (Wright).

Neisser and Sachs have made the following striking discovery:—

(1) A rabbit is immunised against ox blood. The rabbit's serum is thus made hæmolytic to ox and to sheep blood (S). This rabbit's serum (A) on standing loses the complement.

(2) Normal guinea-pig's serum (B) can complement the rabbit's serum (A).

(3) A rabbit is immunised against man's serum. The serum (C) of this rabbit mixed with A and B has no effect in preventing hæmolysis of S.

(4) A trace of human serum added to A, B, and C prevents hæmolysis of S. One-millionth of a cubic centimetre of human serum can thus be identified! Serum dried on linen for two months gives the test. Ape's serum will only act in concentration of one-thousandth of a cubic centimetre, so the test is most specific.

The proteid contained in 1 c.c. of serum on which the reaction depends cannot weigh more than about ·05 grm., thus $\frac{1}{20,000,000}$ grm. of proteid can be detected. —(*Editor's Note.*)

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CHAPTER XV

THE RESPIRATORY EXCHANGE

Historical Introduction.—The necessity of respiration for the support of life must have been one of the earliest facts learnt by primitive man; the most obvious signs of life, when the voluntary activities of the body are suspended by sleep, are the movements of the chest, and sleep is most easily distinguished from death by the presence of breathing. The influence of this early knowledge, the association of life with breathing, is shown in the most ancient writings, and survives to the present day in the common phrases of every-day life.

Notwithstanding the importance which was rightly attached to the renewal of the air in the lungs, the true nature of breathing remained quite unknown for centuries. About the year 294 B.C. Erasistratus taught that the arteries carried air from the lungs to the various parts of the body; those vessels contained air and air only, and the blood was carried in other vessels, the veins.[1] This view Galen overthrew when he demonstrated by experiments that the arteries contained not air but blood alone. The pulmonary circulation, however, was unknown to him; he believed that the ventricular septum of the heart was perforated by small pores, and the sole function of respiration was to cool the blood. It is true that he recognised two kinds of blood, the venous and the arterial, but he thought that the veins carried blood *to* the grosser organs, such as the liver, and the arteries conveyed the purer blood, which had been refined in the left ventricle of the heart, to the more delicate organs, such as the lungs. These views were accepted for centuries after the death of Galen in 198 A.D.

The circulation of the blood through the lungs was discovered about the year 1553 by Servetus (I), an ardent theologian, whose

[1] The arteries appear to have been considered by some of the ancients to be ramifications of the wind-pipe, or arteria, called later ἡ ἀρτηρια τραχεια or ἡ τραχεια. "Sanguis per venas in omne corpus diffunditur et spiritus per arterias."—Cicero, *De Natura Deorum*, 2, 55, 138.

search after the connection between the "breath of life" and the soul led him to this important discovery and to the stake. Not only did Servetus clearly describe the passage of the blood from the right ventricle through the pulmonary artery, the lungs, and the pulmonary veins into the left side of the heart, but he pointed out that the bright colour was given to the blood by the lungs, and not, as Galen had taught, by the heart. Although the complete circulation of the blood was discovered by Harvey before the year 1628, the real meaning of the pulmonary circulation escaped him; he thought that the function of the lungs was to cool the blood. The fundamental knowledge of the circulation was completed when Malpighi, about the year 1661, discovered the alveoli of the lungs and saw the blood flowing through the capillaries of a frog's lung.

Further advance in the study of respiration required the knowledge of physics and chemistry. This was soon forthcoming. Boyle in 1666 showed by numerous experiments with the air-pump that a supply of air was essential to both animal and vegetable life, and he expressed the opinion that "the depuration of the blood was one of the ordinary and principal uses of respiration." A year or two earlier Fracassati had noticed that the lower layer of a clot of blood was much darker in colour than the upper layer, but lost its venous colour and became florid red when it was exposed to the air. A similar aeration of the blood was effected by the respiratory movements, the object of which was not, as some physicians taught, to maintain the circulation of the blood, but to ventilate the lungs with air. This important fact was demonstrated by Hook to the Fellows of the Royal Society at one of their meetings in 1667. The trachea of a dog was connected with a pair of bellows, and the ribs and diaphragm were removed; the dog was seized with convulsions and appeared to be dying, but revived when air was blown into its lungs. Small punctures were now made into various parts of the lungs, and by means of two pairs of bellows the lungs were kept fully distended with fresh air; the dog remained quiet and its heart beat regularly. The circulation continued although there was no alternate expansion and collapse of the lungs; moreover, a further experiment showed that even when the lungs were allowed to collapse the blood continued to circulate for some time.

The real function of respiration was first set forth by Mayow (2)

whose admirable work, published in the years 1668 and 1674, has been neglected, especially in his own country, and has only recently received the recognition which it deserves. He it was who showed that air was a mixture, and that one of its constituents, which he called the nitro-aerial gas, was necessary for the existence of all forms of life: this gas, which is now known as oxygen, was necessary for the support of a flame; it combined with substances such as sulphur to form acids, and with metals during calcination. Respiration and combustion were analogous, and the function of respiration was the absorption of the nitro-aerial gas and the removal of the vapours arising from the blood. The respiration of the fœtus was recognised: the placenta served as a lung from which the blood in the umbilical vessels took up the nitro-aerial gas and conveyed it to the fœtus. The embryo chick absorbed the nitro-aerial gas through the porous shell of the egg. Evidence for the absorption of the gas by the blood was found in the fact that blood exposed to a vacuum gave off minute bubbles of gas, which Mayow thought were composed of the nitro-aerial gas.

Mayow's views were too advanced for the knowledge of his time, they were not accepted, and after the early death of their author at the age of thirty-five were soon forgotten.

The next advance was the recognition of the waste products which are given off from the lungs during respiration. Stephen Hales, about the year 1726, showed that "noxious vapours" were produced by repeatedly breathing air in a bladder, and that these vapours were removed by potash, and thus the air could be breathed again. He even suggested the use of a bladder of air and such an absorbent as potash for use in the foul air of coal-mines, and thus anticipated the modern employment of oxygen and soda-lime, whereby the members of a rescue-party are enabled to penetrate a mine after an explosion.[1] His knowledge of respiration was, however, very defective, for, although he had demonstrated that animals in a closed vessel absorbed air and a similar change was effected by a burning candle, he rejected the views of Mayow, and maintained that the function of respiration was to cool the blood and remove aqueous and noxious vapours.

[1] It is only necessary to recall the fact that the poisonous nature of the air after an explosion is due to lack of oxygen and to the presence of carbon monoxide, not to excess of carbon dioxide.

The experiments of Stephen Hales led to the discovery by Black about the year 1757 that a quantity of "fixed air" was given off from the lungs during respiration, and the presence of this gas constituted the chief difference between expired and inspired air.

A still greater advance was made in 1772, when Priestley published his "Observations on Different Kinds of Air." By experiments he proved that "fixed air," which is now known as carbon dioxide, is produced during putrefaction, and by plants during the night-time; green plants growing in the sunlight restore the property of supporting animal life to air which has been vitiated by the respiration of animals or by the burning of a candle; oxygen and nitrogen are constituents of the atmosphere, and venous blood becomes arterial by exposure to oxygen, a change which occurs even when the blood is separated from the gas by a moist membrane or by the thin walls of the blood-vessels of the lungs. Such were Priestley's contributions to the knowledge of respiration; his experiments were sound, but his views on respiration were erroneous, vitiated as they were by his belief in the old theory of "phlogiston" introduced by Stahl in 1697. Phlogiston was the material and principle of fire, not fire itself, and respiration, according to Priestley, was a phlogistic process, whereby the phlogiston absorbed by animals with their food was discharged. Venous blood was phlogisticated, arterial blood dephlogisticated; a clot of blood placed in "fixed" or phlogisticated air became very dark, but regained its red colour when it was transferred to oxygen or dephlogisticated air. This old theory was overthrown a few years later by Lavoisier, who extended and explained correctly the discoveries of Mayow, Black, and Priestley; he showed that there were differences in the so-called phlogistic processes. The calcination of metals he proved, as Mayow had observed a hundred years before, to be a combination with oxygen, whereby the metals gained in weight; respiration, on the other hand, was not only an absorption of oxygen, but a production and discharge of carbon dioxide produced by the union of the oxygen with carbon. Further, in a joint research with Laplace, published in the year 1780, he showed that the heat of an animal's body might be accounted for by this process of combustion. This view he modified a few years later by including in the process of combustion the oxidation of hydrogen, for quantitative experiments showed that not

all the oxygen absorbed by an animal reappeared combined with carbon as carbon dioxide. Lavoisier maintained, in the joint memoir which he and Sequin published in the year 1790, that the seat of this combustion was in the lungs, but in earlier works he had admitted that it might be in other parts of the body. The view of Lavoisier and Sequin was contested by Lagrange, who argued that if all the heat of the body were produced in the lungs, the temperature of that organ would be raised to such a degree as to destroy its vitality. For many years this objection was held to be fatal to Lavoisier's theory, until Berthelot by a careful calculation showed that, granting all the heat to be produced in the lungs, the temperature of that organ would be raised only a minute fraction of a degree, owing to the great volume of air and blood contained therein, and the rapidity of the circulation, whereby the heat would be distributed all over the body. It is indeed in the tissues that respiration occurs. This has been proved by the researches of Spallanzani, W. F. Edwards, Paul Bert, Pflüger and his pupils, and will be considered in detail in later portions of these lectures. Although the brilliant and laborious work of these observers has shown that the real seat of respiration is in the tissues, we are still far from possessing a satisfactory knowledge of respiratory exchange. The evidence upon some essential points is so conflicting that it is difficult, or even impossible, at present to draw definite conclusions. We know that respiration is not a simple combination of oxygen with carbon or hydrogen, but we cannot, with our present knowledge, follow the sequence of events, the beginning of which is the absorption of oxygen, the end the discharge of carbon dioxide.

Definition of Respiration.—Respiration may be defined as the exchange of gases between the tissues and the surrounding media; oxygen is absorbed by the tissues and carbon dioxide is discharged. A brief consideration of comparative physiology shows that this definition embraces the process of respiration in all forms of life. The organism composed of a single cell is the seat of such a gaseous interchange, and the more complex animal built up of numerous cells breathes in a similar manner, although there may be a system of tracheæ, or air-tubes, as in insects, or internal media, the blood and lymph, as in vertebrates, to facilitate the exchange between the component cells and the external medium.

The ventilation of the lungs is merely an accessory part of respiration, and its mechanism will not be considered here.

The necessity of oxygen for the vital activity of unicellular organisms can be demonstrated by simple experiments. If an amœba or a rhizopod be observed in a moist chamber placed under the objective of a microscope, the movements of its pseudopodia will afford indication of its activity: the passage of a stream of some inert gas, such as hydrogen or nitrogen, will displace the oxygen from the chamber and liquid in which the organism is confined, and the movements will become less and less until they cease. A renewal of the supply of oxygen will restore the power of movement.

Plants breathe; they absorb oxygen and discharge carbon dioxide, and show no special method of respiration which will enable the physiologist to draw a hard-and-fast line between respiration in animal and vegetable forms of life. The phenomena of assimilation, the absorption and breaking up of the carbon dioxide of the atmosphere by the action of chlorophyll in the presence of sunlight, the building up of carbon compounds and the discharge of oxygen, are not processes of respiration. In the daytime this assimilation may mask the respiratory exchange, but at night it is easy to show that plants absorb oxygen and discharge carbon dioxide. The difficulty is absent at all times in fungi which contain no chlorophyll, and in the plasmodium of some of the myxomycetes masses of unicellular protoplasm can be obtained large enough for a direct determination of the respiratory exchange.

In the lower forms of life the exchange of gases may be a simple process of diffusion, but there is no definite knowledge upon the question, and it is possible that a ferment, an oxydase, is concerned in the exchange. The problem of their respiration would indeed appear to be the same as that of the internal respiration of the higher animals, all of which pass through an unicellular stage during their development. Increased complexity is introduced by the growth and multiplication of the cells; the outermost cells have a more direct means of gaseous exchange with the surrounding medium than the middle layer of cells; compensation is, therefore, necessary, and may take the form of a system of channels, which communicate with the exterior and ramify through the different tissues; such a system is found in the trachcæ, or air-tubes, of

insects. On the other hand, compensation may be effected by a special differentiation of one part of the body for the absorption of oxygen and the discharge of carbon dioxide; gills and lungs are such absorbing and discharging surfaces, and are able to supply by means of the blood and lymph the respiratory needs of the whole body.

After this brief consideration of the comparative physiology of respiration, the process of gaseous interchange must be discussed, and especially in relation to the higher animals, the vertebrates. In these animals respiration can be divided into (i) *external respiration*, the exchange of gases between the external medium and the blood; and (ii) *internal respiration*, the exchange between the internal media, th blood and the lymph, and the tissues.

External Respiration.—The exchange of gases between the external medium, the air, and the internal medium, the blood, can be studied in two ways. Analyses of the air passing in and out of the lungs furnish data which show that oxygen is absorbed and carbon dioxide and water are discharged by the lungs. On the other hand, comparative analyses of the gaseous contents of the venous blood passing to, and the arterial blood leaving, the lungs also prove that oxygen is absorbed and carbon dioxide discharged by the blood during its passage through the lungs. It must be remembered that these methods of observation do not show how these changes are produced. It is necessary to know by what means the oxygen is absorbed and the carbon dioxide discharged. Is it simply by diffusion that the one gas passes in and the other passes out? Is it a more complex process, a secretion by the living cells of the lung of oxygen into the blood, and an excretion of carbon dioxide from the blood? These questions are in urgent need of solution, for the gaseous interchanges are not such simple physico-chemical processes as some observers have considered them to be; the difficulties, indeed, are so great that some physiologists, especially Bohr and Haldane, have considered them to be beyond explanation by our present knowledge of physics and chemistry.

The gaseous interchange must now be discussed in detail, and in the first place must be considered the composition of the external medium, the air or water, as the case may be.

Composition of the Atmosphere.—The composition of pure

air, measured at standard temperature and pressure, 0° and 760 mm., is :—

	Volumes per Cent.
Oxygen	20·94
Carbon dioxide	0·03
Nitrogen	78·09
Argon	0·94
Helium, Krypton, Neon, Xenon, and Hydrogen	Traces

In addition, air contains aqueous vapour,[1] which varies greatly in amount in different places and at different times. The temperature of the air is important not only as regards its direct effect upon the respiratory exchange of animals, but also as one of the most important factors which determine the amount of moisture in the air. The nitrogen and argon appear to be inert as far as the higher animals are concerned, but it must be remembered that some bacteria are able to fix nitrogen, and thus enrich soil ; cultures of such bacteria have even been used upon a large scale to render soil more fertile. If a fully grown pea plant be uprooted, tubercles will be found upon its roots ; these in microscopic sections are seen to be composed of vegetable tissue with very large cells and to contain large numbers of bacteria, Bacterium radicicola. These bacteria stand in symbiotic relationship to the plant, and enable it to obtain nitrogenous compounds at the cost of the atmospheric nitrogen.

The proportion of carbon dioxide in pure country air appears to be 3 volumes per 10,000, but it is liable to a variation in summer owing to the influence of vegetation ; it may rise by night to 3·5 volumes and fall by day to 2·6, for, as is well known, vegetation under the influence of sunlight decomposes carbon dioxide. These facts are well shown in the following table, which gives the average results of exact determinations of the carbon dioxide in air, made by J. S. Haldane and E. S. Haldane in the Ochil Hills, Perthshire.

Time of Year.	Time of Day.	No. of Analyses.	CO_2 per 10,000. Maximum.	Minimum.	Average.	
April 1–September 30	Day	29	3·11	2·58	2·88	2·98
	Night	22	3·55	2·82	3·08	
December–January	Day	6	3·12	2·93	2·99	3·00
	Night	5	3·06	2·94	3·01	

[1] See p. 258.

The partial pressure, or, as it is often called, the tension, of the component gases is :—

Oxygen approximately $\frac{21}{100} \times 760 = 159{\cdot}6$ mm. of mercury or 21 per cent. of an atmosphere.

Nitrogen approximately $\frac{79}{100} \times 760 = 600{\cdot}4$ mm. of mercury or 79 per cent. of an atmosphere.

Carbon dioxide approximately $\frac{0{\cdot}03}{100} \times 760 = 0{\cdot}228$ mm. of mercury or 0·03 per cent. of an atmosphere.

Such is the composition of pure country air. Many men, however, are obliged to breathe air which is vitiated by the products of their own respiration, the combustion of gas or coal, dust produced during work, or chemical changes occurring in the soil. A knowledge, therefore, of the composition of the air of dwelling-rooms, workshops, schools, mines, and crowded cities is of the greatest practical importanee.

Air of Dwellings, &c.—The impurities present in a workroom arise from the workers, lights or fires, the floor and furniture of the room, the raw material and accidental sources, such as escapes of gas. The carbon dioxide is in practice the best indicator of the sufficieney of ventilation ; but it will be shown later that it is only one of the impurities present in overcrowded rooms. The proportion of carbon dioxide will vary according to the sources of contamination and the ventilation of the room ; it may be during the daytime as low as 3·2 volumes, or as high as 33·6 volumes per 10,000 volumes of air, and at night-time it may rise as high as 47 volumes, if many gas-lights be burning. Haldane and Osborn (3) have investigated the ventilation of a large number of workshops and factories, and recommend that the standard of ventilation should be such that the proportion of carbon dioxide does not exceed 12 volumes per 10,000 of air during daylight, and 20 volumes after dark, when gas or oil is used for lighting. During a dense and prolonged fog this proportion may be exceeded even in the open air of a large city, for Russell found as much as 10 to 14 volumes of carbon dioxide per 10,000 volumes of air in samples collected in London during very foggy weather.

Expired Air.—In the healthy man or animal the air is inspired through he nose, and only during severe exercise is prolonged buccal breathing to be considered normal ; buccal respiration

then offers less resistance to rapid breathing and also has the advantage of cooling the animal, for during muscular work the internal temperature of the body is raised. In the dog the open mouth, lolling tongue, and rapid, panting breaths are characteristic during and directly after active exercise; Richet has shown that if this polypnœa be prevented by a muzzle, the dog suffers severely from the high temperature which would be produced by severe exercise or exposure to the sun.

The advantages of nasal respiration are to be found chiefly in the warming of the inspired air, the supply of moisture, and the removal of foreign particles, such as bacteria and dust. The inspired air passes chiefly through the middle passage of the nose, in a less degree through the upper, and least through the lower passage. The direction of the stream of inspired air is such that particles of dust will be retained by the moist and highly vascular mucous surfaces of the turbinate bones, which not only act as a sieve but also as a radiator of moist heat. The effectiveness of nasal respiration in these respects is shown by the following observations. Block found that, when the external air was $-8°$, $-0.5°$ to $3·5°$, $12°$ to $16°$ and $18°$, the air was warmed to $24·5°$, $26°$, $30°$, and $31°$ respectively by the time it reached the pharynx. The air is also saturated with water to one-third of its capacity at those temperatures during its passage through the nose. The retention by the nasal mucous membrane of particles of soot is known to every one who has passed a day in one of the thick fogs for which London is so justly celebrated. Particles are not breathed out, at least in the last portions of the expired air, as Tyndall showed, and bacteria, according to Jundell, are generally absent. The bacteria in inspired and expired air have been counted by Straus; he found in one experiment that the expired air only contained forty bacteria, whereas the inspired air held over twenty thousand. Haldane observed that the air of a room contained fewer bacteria after than before occupation, for the bacteria were filtered off by the breathing of the occupants. Even in the case of phthisical patients the tubercle bacilli are retained by the mucus: the expectoration, not the expired air, is the means whereby the disease is disseminated, and simple observation shows that particles of saliva are often projected from the mouth during speaking. There is no doubt, therefore, that even the most perfect ventilation of schools will not prevent the spread of infectious diseases, and that compulsory education, which enforces the early

association of infants and young children in large numbers, has had in many respects untoward results, has often increased the infections diseases to which children are liable, has diminished the responsibility of mothers for the care and training of their children, and has imposed prematurely upon young children an artificial and unhealthy routine of life.

The mucus secreted by the nose appears to possess properties antagonistic to the growth of bacteria, and thus acts both as a chemical and mechanical defence. There are limits to the effectiveness of this nasal sieve, for the dwellers in cities and the workers in coal-mines have numerous particles of carbon in their lungs. It is true that in these cases the objection may be raised that the particles are inhaled through the mouth; it has been shown, (4) however, that in rabbits, which breathe only through the nose, the mucous surfaces of the turbinate bones act at first as an effective filter during the inhalation of air heavily laden with particles of dust. If the experiment be continued the mucous membrane becomes covered and can retain no more dust; there is a second line of defence in the pharynx and larynx, but even that is overcome if the exposure to the dense dust be prolonged. A similar condition obtains in the case of miners. Haldane, Martin, and Thomas (5) have shown that the alarming prevalence of so-called miners' phthisis among the Cornish miners is to be attributed to the permanent injury of the lungs produced by the stone-dust which is inhaled in large quantities by the miners, especially those who work with rock drills. The impairment of the respiratory functions reacts upon the general health and predisposes to tuberculosis of the lungs: thus the death-rate from diseases of the lungs among the tin miners of Cornwall is about four times as high as that for all occupied males in England and Wales.

The expired air varies in composition according to the rate and depth of respiration; this is shown by the following analyses made by Speck.

Type of Breathing.	Volume of Expired Air per Minute.	Percentage of Oxygen.	Percentage of Carbon Dioxide.
	c.c.		
Normal . .	7,527	16·29	4·21
Very shallow .	5,833	15·50	4·63
Very deep .	17,647	18·29	3·17

The rate and depth of respiration does not, as Pflüger showed, determine the rapidity of combustion in the body; the tissues set the pace, and apart from the increased muscular activity of the respiratory muscles, rapid breathing does not bring about a greater total exchange of gases than slow breathing. This is demonstrated in an interesting way by Pflüger, who combined the apparently discordant results obtained by Lossen and Berg, and showed that the mean values were practically the same.

Carbon Dioxide Discharged in Fifteen Minutes.

Observer.	Five Respirations per Minute.	Sixty Respirations per Minute.
	Grm.	Grm.
Lossen . .	7·96	6·63
Berg . .	7·712	9·106
	15·672	15·736
Mean . .	7·836	7·868

The rate and depth of breathing vary greatly in different individuals, in some respiration is deep and slow, in others shallow and frequent. As an average figure for an adult man may be given 500 c.c. for each respiration, when the frequency is 16 per minute; these data, however, are not of much value, for the range of variation in healthy men is very great. Haldane and Priestley have recently shown that when the respirations are increased in frequency they decrease in depth, and *vice versâ*; so that for the same individual the percentage of carbon dioxide in the alveolar air remains unaffected by alterations in the frequency of breathing, provided that the respirations are not forced. The following table shows these results.

Subject.	Respirations per Minute.	Percentage of Carbon Dioxide in Alveolar Air.		
		At End of Inspiration.	At End of Expiration.	Mean.
J. S. H.	9	5·59	5·87	5·73
	19	5·56	5·70	5·63
J. S. H.	9	5·33	5·47	5·40
	20	5·44	5·60	5·52
J. G. P.	10·5	5·95	6·74	6·35
	30	5·98	6·05	6·02

The expired air is saturated with moisture at a temperature between 36° and 37°, to which it has been raised during inspiration, and in this way a considerable quantity of water and heat are extracted from the body. It has been calculated that an adult man may thus lose in twenty-four hours as much as 450 grm. of water under ordinary conditions of external temperature and moisture.

There is a decrease of about 1 50 in the volume of the expired air as compared with the inspired air, when both are measured at 0° and 760 mm.; the deficit is due to the absorption of a small quantity of oxygen, which does not reappear in combination with carbon as carbon dioxide, but passes out of the body in other products of oxidation. The increased proportion of nitrogen in the expired air must be taken into account when the respiratory quotient is calculated from volumetric analyses; thus for every 100 c.c. of expired air the slightly larger volume of inspired air contained the following volume of oxygen :—

$$O_2 = \frac{20{\cdot}93 \times \text{Nitrogen of expired air}}{79{\cdot}07}.$$

The respiratory quotient, therefore, in a case in which the percentages of nitrogen, oxygen, and carbon dioxide are 81, 16·44, and 4, would be correctly calculated as follows :—

$$\text{Oxygen of inspired air} = \frac{20{\cdot}93 \times 81}{79{\cdot}07} = 21{\cdot}44.$$

$$\text{Oxygen absorbed} = 21{\cdot}44 - 16{\cdot}44 = 5{\cdot}00 \text{ cc.}$$

$$\text{atory quotient } \frac{CO_2}{O_2} = \frac{4}{5} = 0{\cdot}8.$$

There is no evidence of an active absorption or discharge of nitrogen by the lungs: the older results which supported such a process or processes appear to have been errors of experiment. It is possible that small quantities of nitrogen may be formed in the alimentary canal and may be directly discharged by the bowel or indirectly by the lungs; the quantity, however, would appear, from experiments directed to this point, to be negligible. Small quantities of hydrogen and marsh-gas are frequently found in expired air; the gases are formed during fermentation in the alimentary canal, and are absorbed by the blood and discharged by the lungs. In the case of ruminants a considerable quantity of these gases is present in expired air.

It is now necessary to discuss whether the vitiation which occurs in the air of overcrowded rooms is due to the products of normal respiration or to other substances of a poisonous and volatile nature. Upon these points the results of numerous experiments are conflicting; some observers have maintained that the expired air contains an organic impurity which can be collected in the condensed breath and will produce death when it is injected into rabbits. This Haldane and Lorrain Smith (") were unable to confirm; they were led by their research to the following conclusions: "The immediate dangers from breathing air highly vitiated by respiration arise entirely from the excess of carbonic acid and deficiency of oxygen, and not from any special poison. The hyperpnœa is due to excess of carbonic acid and is not appreciably affected by the corresponding deficiency of oxygen. The hyperpnœa begins to appear when the carbonic acid rises to from 3 to 4 per cent. At about 10 per cent. there is extreme distress. Excess of carbonic acid is likewise the cause, or at least one cause, of the frontal headache produced by highly vitiated air. Hyperpnœa from defect of oxygen begins to be appreciable when the oxygen in the air breathed has fallen to a point which seems to differ in different individuals. In one case the hyperpnœa became appreciable at about 12 per cent., and excessive at about 6 per cent." These observers, however, draw attention to the fact that the vitiation of the air in overcrowded rooms may arise from the products of disease, want of cleanliness of the occupants, or the room itself; in fact, "air, which, judged by the carbonic acid standard, is sufficiently pure, may be exceedingly impure when judged by the number of micro-organisms present in it, and *vice versâ*. The carbonic acid and micro-organisms have different sources. The amount of the former depends on the number of persons in the room as compared with the means of ventilation—that of the latter being determined chiefly by the condition of the room itself as regards cleanliness. The test of smell, or of feeling of comfort or discomfort in breathing the air of a room, may give results equally at variance with the carbonic acid test." The susceptibility to the effects of the air of overcrowded rooms varies greatly in different individuals; some are more influenced by the odour, others by the increased quantity of carbon dioxide, moisture, and temperature. It is probable that too little attention has been given to the last two conditions, and further research is greatly needed to show the effects of these factors.

So far the discussion has been confined chiefly to the quality of the expired air. The quantity of the expired air, if its composition be known, gives the measurement of the respiratory exchange of the body; a few samples, however, taken over short periods, are not sufficient for exact determinations, for the depth and frequency of respiration are easily altered, especially when a mask is placed on the face. It is unnecessary to describe here the methods used for the determination of the total respiratory exchange in man and animals, but some account must be given of the various factors which influence it.

Relation between Functional Activity and Respiratory Exchange.— The investigation of the relation between functional activity and oxidation in animals is beset with many difficulties and sources of error. The animal, not the cell, is the physiological unit. Surviving organs cannot be regarded as comparable to the organs intact within the living body of a warm-blooded animal. Such an animal on exposure to cold increases its activity and its processes of oxidation, whereas its excised organs would react in exactly the opposite manner. It is probable that much further knowledge of respiration might be gained by more extensive research in comparative physiology. Natural conditions vary in different animals, one living process may be more marked than another, or may show large variations in its range during different seasons. Thus, hibernation is a physiological condition in which the processes of combustion are reduced to the lowest ebb compatible with the life of mammals; in such a condition the relation between functional activity and oxidation can be conveniently studied. It is well known that physiological activity is closely associated with oxidation, but the study of the respiratory quotient, the ratio of the intake of oxygen to the output of carbon dioxide, shows the possibility of oxidation taking place in the body without a coincident production of carbon dioxide, and on the other hand the possibility of the production of carbon dioxide without a coincident absorption of oxygen.

Respiratory quotients as low as 0·3 have frequently been observed in torpid animals (†). During some periods of its deep winter sleep the animal may actually gain in weight, for, although it loses water and carbon dioxide, it absorbs a greater weight of oxygen. This oxygen does not reappear immediately combined with carbon as carbon dioxide, but is used, it would seem, for the partial combustion of the reserves of food material stored in the

body. Voit and Chanveau maintain that fat may form sugar, which in turn may be stored up as glycogen in the liver and muscles. This process would explain the low respiratory quotient exhibited by hibernating mammals, for it is well known that they possess a large deposit of fat which gradually disappears during the period of torpidity; moreover, it has been shown by various observers that glycogen accumulates during hibernation. If olein be taken as an example, the partial oxidation may be represented as follows:—

$$2C_3H_5(C_{18}H_{33}O_2)_3 + 64O_2 = 16C_6H_{12}O_6 + 18CO_2 + 8H_2O$$

$$\frac{CO_2}{O_2} = \frac{18}{64} = 0{\cdot}281.$$

During the germination of fatty seeds, such as those of the radish, the respiratory quotient is low, for the seeds take up a relatively large amount of oxygen; the fat undergoes partial oxidation and gives rise to carbohydrates.

The evidence, therefore, in favour of oxidation taking place in the body without an immediate and corresponding production of carbon dioxide is satisfactory, for it cannot be well maintained that the carbon dioxide is formed but not discharged. It will be shown later that in man and other animals respiratory quotients below the value for the combustion of fat have been observed by Zuntz, Lehmann, and others; such quotients may persist for hours or even days, if the conditions be favourable.

The hibernating mammals likewise yield evidence of the production of carbon dioxide without a corresponding absorption of oxygen (5). During the autumn the marmot feeds eagerly upon food consisting chiefly of carbohydrates, and rapidly deposits fat in its body as a reserve for consumption during its winter sleep. The respiratory quotient is during this deposition of fat constantly greater than unity, and may be even as high as 1·39. Such high quotients cannot be explained by a reduction in the absorption of oxygen, for compared with the condition during fasting there is a considerable increase. The probable explanation is that suggested by Hanriot; during the formation of fat from carbohydrates a considerable quantity of carbon dioxide is split off from the carbohydrate molecule. The following equation is given by Hanriot to represent the change:—

$$13C_6H_{12}O_6 = C_{55}H_{104}O_6 + 23CO_2 + 26H_2O.$$

The formula for the fat is a hypothetical one to represent the mean composition of fats, oleostearopalmitin, but a similar equation can be given for olein or stearin. According to this equation 100 grm. of glucose would during its transformation into fat yield 21·8 litres of carbon dioxide.

In the marmot the deposition of fat is extremely marked in the autumn; during the winter this store undergoes combustion during the animal's very prolonged, but perfectly natural, fast. On general biological grounds it is improbable that the processes of metabolism in this animal differ fundamentally from those in non-hibernating mammals; the difference is probably only quantitative and not qualitative. In fact, there is evidence to show that this view is correct. Respiratory quotients greater than unity have been found by various observers both in the case of men and animals after the ingestion of a meal rich in carbohydrates.

It is probable that the respiratory quotient represents the resultant of various processes of combustion, in some of which oxygen is absorbed without a corresponding output of carbon dioxide, in others carbon dioxide is discharged in excess of the oxygen absorbed, the extra quantity of oxygen contained in the carbon dioxide being derived from the intramolecular oxygen of the food. Fasting and feeding accentuate these processes respectively, and since it is probable that the liver is most actively engaged, the results obtained from determinations of the gases of the blood flowing through any particular organ may appear to be out of harmony with the character of the total respiratory exchange. The one is a local respiratory exchange, the other the resultant of the respiration of all the tissues of the body.

Stress has been laid upon these points, for there has been a tendency to deny, or at least ignore, the existence of respiratory quotients above unity; it is also necessary to enforce the fact that an analysis of one of two samples of the air expired during a short period may give an inaccurate idea of the total respiratory exchange of an animal or man.

Total Respiratory Exchange.—The following table (see p. 490) shows the value of the total respiratory exchange of man and various animals during a condition of rest.

Animal.	Weight Grm.	Oxygen Absorbed per Kilo and Hour. Grm.	Carbon Dioxide Discharged per Kilo and Hour. Grm.	$\frac{CO_2}{O_2}$	Temperature of Air.	Remarks.	Observer.
Vermes—							
Common worm .	—	0·1013 (70·8 c.c.)	0·108 (54·9 c.c.)	·77	—	—	Regnault and Reiset.
Insecta—							
Field cricket .	0·25	—	2·305 (1172 c.c.)	—	—	—	Pott.
„ „	0·05	—	2·127 (1084 c.c.)	—	17°–21°	—	„
Common cockroach	—	—	0·268	—	15°–20°	—	Butschli.
			1·045	—	30°–35°	—	„
Crustacea—							
Cray-fish .	31	0·0543 (38 c.c.)	0·0642 (32·7 c.c.)	·86	12·5°	—	Jolyet and Regnard.
Pisces—							
Tench . .	222	0·0796 (55·7 c.c.)	0·0721 (36·7 c.c.)	·66	14°	—	„
Torpedo . .	315	0·0672 (47·0 c.c.)	0·0540 (27·5 c.c.)	·58	14·5°	—	„
Amphibia—							
Edible frog	—	0·063 (44·2 c.c.)	0·060 (30·76 c.c.)	·69	15°–19°	Minimal and maximal of five experiments	Regnault and Reiset.
	—	0·105 (73·4 c.c.)	0·1134 (57·7 c.c.)	·78	—		
Common frog . .	31·64	—	0·038	—	13°	Winter frog	Pembrey.
	34·47	—	0·033	—	12·5°	„ „	Vernon.
	13·9	—	0·355	—	19°–20°	—	Pott.
Aves—							
Common hen . .	1280	1·058 (740 c.c.)	1·327 (675 c.c.)	·9.	19°	—	Regnault and Reiset.
Pigeon . .	232–380	—	3·236	—	—	Mean of 10 experiments	Corin and van Beneden.
Sparrow . .	22	9·595 (6710 c.c.)	10·492 (5334·5 c.c.)	·79	18°	—	Richet.
Mammalia—							
Ox	638,000–660,000	—	0·389–0·485	—	—	—	Henneberg.
Sheep . .	66,000	0·490 (343 c.c.)	0·671 (341 c.c.)	·99	16°	—	Reiset.
Dog . . .	6213	1·303 (911 c.c.)	1·325 (674 c.c.)	·74	15°	—	Regnault and Reiset.
Cat . . .	2464–3047	1·356 (947 c.c.)	1·397 (710 c.c.)	·75	– 3·2°	—	Carl Theodor.
	„	0·645 (450 c.c.)	0·766 (389 c.c.)	·86	29·6°	—	„
Rabbit . .	1433	1·012	1·354	·97	18°–20°	—	Pembrey and Gürber.
Guinea-pig .	444·9	1·478	1·758	·86	22°	—	Pembrey.
Rat (white)	80·5	—	3·518 (1789 c.c.)	—	7°	—	Pott.
Mouse „	25	—	8·4	—	17°	—	Pembrey.
Man	66,700	0·292	0·327	—	—	—	Magnus-Levy and Falk.
	—	—	0·283	—	—	—	Johansson.
	—	—	0·302	—	—	—	Zuntz.
	—	—	0·446	—	—	—	Tigerstedt and Sondén.

The above data for the respiratory exchange have been expressed in relation to one kilogram of body-weight, but as far as possible the weights of the animals have also been given. The physiological unit, it cannot be too often insisted, is the animal, and there is some danger of misunderstanding when the results are always expressed in terms of one kilogram of body-weight. The ratio of surface to mass is in the case of warm-blooded animals a far more important factor, for, as later data will show, it throws light upon the relatively greater respiratory exchange of the small mammals and birds as compared with the bigger members of the same genera, and the more rapid respiration of the child or young animal as compared with the adult. In addition, an examination of the above table shows that the respiratory exchange of most of the cold-blooded animals is very small, but that in the case of insects the metabolism is most active and often equals that of the larger mammals. This interesting exception to the general rule is to be associated with the high temperature and great muscular activity of insects. A comparison of the data for birds and mammals will show that as a general rule the former have a more rapid respiratory exchange, and this difference is also associated with a higher bodily temperature.

It is unnecessary here to discuss all the conditions which influence the respiratory exchange, but there are some which are of fundamental importance; such are the age and size of the animal, the influence of muscular activity, food, and external temperature.

Influence of Age upon the Respiratory Exchange.—The process of respiration in the embryo and fœtus will be discussed in a later portion of this article; here the question to be considered is the total intake of oxygen and output of carbon dioxide. In the case of mammals there are great technical difficulties in the estimation of the respiratory exchange of the fœtus, for incision of the uterus interferes with the circulation of the blood through the placenta. It is probable from the few data which exist that the respiratory exchange in the fœtus towards full term is weight for weight equal to that of an adult, for, although its muscular activity is not great and its capacity to regulate its temperature is imperfect, it is rapidly growing and is kept warm by the heat of its mother's body. In the case of birds the respiratory exchange can be studied under favourable conditions during the develop-

ment of the chick within the egg, but it is necessary to remember that the output of carbon dioxide and the intake of oxygen for the conventional terms of one kilogram of body-weight and one hour must be calculated not from the weight of the egg but from the weight of the embryo contained therein. Thus on the nineteenth day of incubation the carbon dioxide discharged by an egg (") was ·007 grm. in 15 minutes at a temperature of 40°; the weight of the egg after the shell was removed was 52 grm.; that of the embryo without its yolk-sac 27·5 grm. The discharge of carbon dioxide per kilogram of body-weight and one hour is thus almost exactly 1 grm. A first-day chick discharged 1·714 grm. per kilo and hour at a temperature of 37·75°, and 3·606 grm. at 17°, a value which much exceeds that obtained by Regnault and Reiset for a hen, namely, 1·327 grm. at 19°.

In the case of the newly born animal and infant, determinations show that the respiratory exchange is under natural conditions more energetic than in the adult; it is easily influenced by changes of external temperature, for the regulation of temperature is imperfect, and it will be shown later that in many cases cold will produce a marked fall in the respiratory exchange of the newly born. Against this effect there are natural safeguards; the breeding season generally begins in the spring, and the young are produced when the weather is mild; the mother selects a sheltered spot for her nest or bed and broods over her helpless offspring. Warmth is necessary and warmth is secured; the care of the mother is not for the relief of weakness due to parturition, for such weakness is obviously not present in birds and is also absent in woman and mammals untouched by the degenerating influence of over-civilisation and domestication; moreover, this parental care is seen in many cock-birds, which share the duties of incubation and brooding with the hen. The degenerates of modern society have degraded maternity from its position as the highest duty, and too often the woman of society either cannot suckle her offspring or has lost the imperative natural instincts of cherishing her infant with the warmth of her body and feeding it with her own milk.

Under favourable conditions the infant's respiratory exchange is most active. Thus Babák (10) found that in an infant 1½ hours old and weighing 2670 grm. the intake of oxygen and output of carbon dioxide were 552 c.c. and 368 c.c. per kilo and hour;

for the same infant, when it was eight days old and weighed 2650 grm., the respective values were 826 c.c. and 528 c.c. This may be contrasted with the values obtained by Geppert for a man at rest, 264 c.c. of oxygen absorbed and 208 c.c. of carbon dioxide discharged per kilo and hour.

Babák's results are of great practical importance, for a rapid metabolism necessitates the ready absorption of a perfect food, if the infant is to grow rapidly. The most perfect and the cheapest food is the mother's milk. Artificial feeding is without a doubt the chief cause of the wholesale sacrifice of infant lives, which is to be observed in this country.

In the child also, as would be expected, the respiratory ex-

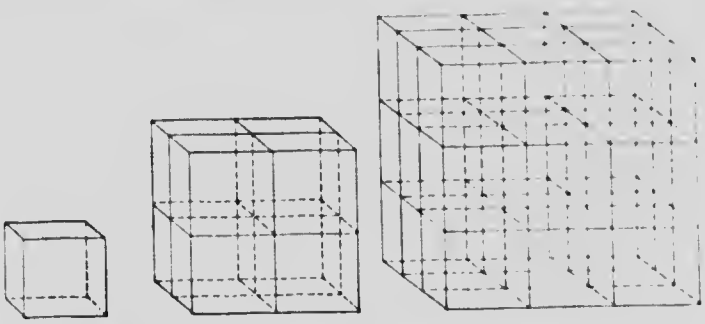


FIG. 25.—Diagram to Illustrate the Relation between Volume or Weight and Surface (*Waller.*)

The volumes are	.	1	8	27 cubic centimetres.
The weights are	.	1	8	27 grm.
The surfaces are	.	6	24	54 square centimetres.
i.e. their ratio of increase is		1	4	9

change is relatively much greater than in the adult: data upon this point will be given in the next section, for the influence of age is closely associated with that of the size of the body.

In aged subjects the respiratory exchange is relatively smaller than that of the adult; a result which could be predicted from the diminished activity and muscular tone of the aged.

Influence of the Size of the Warm-blooded Animal upon its Respiratory Exchange.—The larger an animal is, the smaller is the ratio between its surface and its mass, for surface increases as the square and weight as the cube. This important relation between the weight or mass of a body and the extent of its surface is well shown by the diagram given above (Fig. 25), and the

curves (Fig. 26). Thus it is that a small animal has a far greater surface in relation to its weight than has a large animal. The smaller mammals and birds have an internal temperature which is as high or even higher than that of the bigger animals; it therefore follows that owing to its relatively greater cutaneous surface

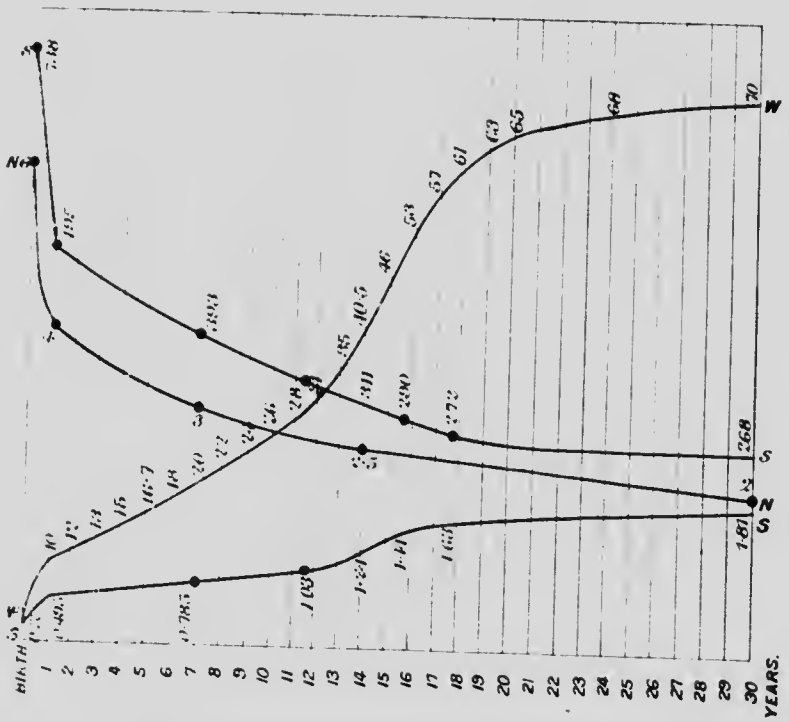


FIG. 26.—Weight and Surface of Body at Different Ages. Proteid required (*Waller.*)

WW = body-weight in kilos (1 mm. = 1 kilo).
SS body-surface in sq. metres (1 cm. = 1 sq. metre).
ss = body-surface per 1 kilo body-weight in sq. cms. (1 cm. = 1 grm.).
NN = proteid per kilo body-weight in grms. (1 cm. = 1 grm.).

the small animal must possess either a special covering of fur or feathers for the prevention of an excessive loss of heat or a more rapid production of heat than the big animal. Both means are employed, but here it is only necessary to discuss the latter. An increased production of heat necessitates increased combustion, increased absorption of oxgyen and discharge of carbon dioxide,

and a greater supply of food. An examination of the table for the respiratory exchange of different mammals and birds will show that a mouse, which weighs 25 grm. and has a temperature of 37°, discharges 8·4 grm. of carbon dioxide, and an ox, which weighs 660,000 grm. and has a temperature of 38°, discharges 0·5 grm. of carbon dioxide per kilo of body-weight and hour; a comparison between a sparrow, which weighs 22 grm., and maintains an internal temperature of 42°, and a hen, which weighs 1280 grm. and has a similar high temperature, shows that the respective figures for the output of carbon dioxide are 10·5 and 1·3 grm. For an infant and a child, for a child and an adult, a similar comparison could be made. Indeed Sondén and Tigerstedt's [11] researches upon the respiratory exchange and total metabolism of man show that over and above the greater respiratory exchange due to the relation between surface and mass, there is a greater combustion due to the age of the child; the younger the child the greater is this excess. It is interesting to note also that these observers find that the output of carbon dioxide, whether it be calculated for weight of body or for cutaneous surface, is much greater in the male than in the female child even when individuals of similar age and weight are compared. The average results show that the ratio is as 140 to 100. This sexual difference in metabolism gradually diminishes and disappears with the approach of old age; it is probably due to the greater muscular tone and restlessness of the boy, for it is not shown in the results obtained by Magnus-Levy and Falk upon children fasting and lying down. The following table (see p. 496) gives the average results obtained by Sondén and Tigerstedt; the observations were made upon individuals sitting down and a short time after a meal, two conditions which would explain the increase in the respiratory exchange above that observed by Magnus-Levy.

This question is one of great practical importance, for it shows that the dietary for children must not be calculated on the basis of their weight; they require relatively a much more liberal diet than adults. Apart from the influence of the large cutaneous surface upon the combustion of food substances in children, it is obvious that active growth cannot occur unless there be a surplus of intake over output. Serious mistakes have been made owing to neglect or ignorance of these facts. Haldane pointed

out that the diet allowed for the Boer children in the concentration camps, which were formed during the South African War, was inadequate, and there is little doubt that the infant mortality

MALES.

Average Age.	Average Weight.	Carbon Dioxide Discharged per Hour.	Carbon Dioxide Discharged per Kilo and Hour.	Carbon Dioxide Discharged per Square Metre of Cutaneous Surface and Hour.
Years.	Kilos.	Grm.	Grm.	Grm.
$9\frac{3}{4}$	28	33	1·21	29·9
$10\frac{1}{2}$	30	33	1·11	28·2
$11\frac{1}{2}$	32	34	1·06	27·5
$12\frac{1}{2}$	34	34	1·00	26·5
14	45	45	1·00	27·6
$14\frac{1}{2}$	45	44	0·96	26·7
$15\frac{1}{2}$	51	42	0·81	23·5
17	56	45	0·81	24·2
$19\frac{1}{2}$	60	43	0·72	21·8
23	65	38	0·58	18·6
25	68	38	0·57	18·5
35	68	35	0·52	16·9
45	77	37	0·48	16·3
58	85	34	0·41	14·2
		FEMALES.		
8	22	25	1·13	26·6
10	27	23	0·85	20·8
11	31	26	0·85	21·8
12	36	27	0·74	20·1
13	40	28	0·70	18·5
14	44	29	0·66	18·2
15	49	27	0·56	16·0
16	50	32	0·64	18·3
$17\frac{3}{4}$	54	27	0·50	14·8
30	54	29	0·54	16·3
45	67	37	0·55	17·9
$65\frac{3}{4}$	67	26	0·39	12·6

was inereased owing to this deficiency. Directly the mistake was reeognised the diet was ehanged by the order of the Government, and the death-rate fell. It is necessary, however, to remember that the infant mortality was never so great as that found in the

great towns of England, and this difference is to be attributed in great measure to the fact that the ideal of maternity among the Boer women is more natural, and therefore higher, than that among many of the degenerate dwellers in large cities.

Richet made an interesting series of experiments upon the output of carbon dioxide of dogs of different sizes, and found, as the following table will show, that it bore a very constant relation to the surface of the body.

Weight of Dog. Kilogrammes.	Carbon Dioxide Discharged per Kilo and Hour.	Carbon Dioxide Discharged per Square Decimetre and Hour.
	Grm.	Grm.
26	0·925	0·250
24	0·940	0·244
20	0·970	0·236
16	1·200	0·270
14	1·045	0·228
12	1·120	0·229
10	1·200	0·233
8	1·300	0·233
6	1·400	0·227
5	1·550	0·242
4	1·750	0·245

A series of similar observations made upon birds did not show this constant relation between surface and mass; this is probably explained by the great difficulty of maintaining comparable conditions as regards muscular activity.

Influence of Muscular Work upon the Respiratory Exchange.—The muscles are the most important seat of respiratory exchange, for they make up nearly 40 per cent. of the weight of the body and are subject to great activity; even during a condition of apparent rest they are in a condition of tone, and the respiratory muscles and the heart are subject to frequent contraction, alternating with relaxation, throughout life. The practical importance of the great effect of muscular exercise upon the processes of oxidation in the body cannot be overestimated in these days, when over-civilisation tends to weaken the physique and moral fibre of man. There is no other condition, whether it be physiological or pathological, which will produce such a great increase

in the absorption of oxygen and the discharge of carbon dioxide, such a widespread effect upon the exchange of material in the body. Man in a primitive condition is forced to muscular exercise in order that he may obtain food or protect himself from wild animals, or more often from his fellow-man. Civilisation cannot override in a generation or two the characteristics impressed through countless ages, and the need for exercise becomes imperative and finds satisfaction in sport, when, owing to the process of division of labour, some classes become differentiated for work necessitating but little muscular activity. An agricultural population find the requisite exercise in their daily toil, which is sufficiently diversified to exercise all the muscles; in a manufacturing community the specialisation is so great that muscular activity is in some forms of work very slight or directed into some narrow channel, and outdoor sport becomes a necessary pleasure to the fit. One of the marked characteristics of life is oxidation; but the benefits of muscular work are not to be attributed to that alone. Muscular activity is not a simple increase of oxidation; the body is not a machine from which more work can be obtained simply at the expense of more fuel and increased wear and tear. The co-ordination of all the systems of the body is necessary, and all parts are affected; the growth and vitality of the body is favoured by the work performed. In these respects muscular exercise is of the utmost importance, and one may see in the training for warfare among the highly civilised, and especially the manufacturing, nations a blessing in disguise. Should the danger of war ever be entirely removed the only safeguard against degeneration would be outdoor sport.

The effect of muscular exercise can be best shown by a contrast of the respiratory exchange during rest and exercise. The most marked rest is sleep; the respiratory exchange is then at its daily minimum. When, however, a man is awake but lying down, the relaxation of the muscles is incomplete and the respiration is greater; it is only by the exercise of great care that the subject of the experiment can more completely relax his muscles, and thus imitate the condition during sleep. Loewy and Johansson took such precautions in some of their observations, and then obtained an output of carbon dioxide and an intake of oxygen which were but little above the quantities found during sleep; from their results an average figure 0·3 grm. may be given for the discharge

of carbon dioxide per kilogram of body-weight and hour under such conditions. It is necessary to point out that this is not rest in the ordinary sense of the word; such muscular relaxation requires the constant exercise of the will in the man who is awake and the subject of observation. For this reason the data already given for the discharge of carbon dioxide by male and female children at different ages are to be considered normal: the greater muscular tone, even the restlessness, of the boy is a natural characteristic which distinguishes him from the girl.

More muscles are brought into activity in the standing than in the sitting posture, and more in the sitting posture than in the prone position. The differences are demonstrated by the increase in the respiratory exchange, the temperature of the body, and in the sensations of effort and fatigue. Although the first careful experiments upon these questions were performed in this country by E. Smith, the most exact investigations have been carried on abroad, chiefly by Zuntz and his pupils and Johansson. E. Smith found that a man discharged 161·6 c.c. of carbon dioxide per minute when he was asleep, 569·5 c.c. when he was walking at a rate of two miles (3048 metres) an hour, and 851·2 c.c. when he quickened the pace to three miles (4571·9 metres) per hour. The most marked increase, to 1581·9 c.c. per minute, was produced by work upon a treadmill.

The more recent observations have been made upon men, horses, and dogs in the condition of rest, work of different kinds, and rest immediately following work. A comparison of the effect of walking upon level ground with the ascent of an incline was made by Katzenstein, and the following results were obtained for the respiratory exchange of three individuals.

Walking on Level Ground. Per Metre and Kilo.		Ascent. Per Kilogram-Metre.	
Intake of Oxygen in c.c.	Output of Carbon Dioxide in c.c.	Intake of Oxygen in c.c.	Output of Carbon Dioxide in c.c.
0·168	0·136	1·187	0·987
0·115	0·093	1·244	1·113
0·086	0·080	1·504	1·236

Johansson found that the output of carbon dioxide increased with the load and the number of contractions in a given time, but

the sensation of fatigue bore no direct relation to the output of carbon dioxide. The effect of speed is well shown by the following observations made by Zuntz and Lehmann upon horses.

Condition.	Air Expired in Litres per minute.	Carbon Dioxide Discharged in Litres per minute.	Oxygen Absorbed in Litres per minute.	$\frac{CO_2}{O_2}$
Rest . .	44	1·178	1·601	·92
Walk .	177	4·342	4·766	·90
Trot . .	333	7·516	8·093	·93

These figures show not only the great increase produced in the ventilation of the lungs and the respiratory exchange, but also the absence of any alteration in the respiratory quotient.

A bicycle ride at the rate of 827 feet (252 metres) per minute or 9 miles (15 kilometres) per hour necessitated an absorption of 4674 c.c. of oxygen per metre of the distance traversed; this was increased to 5672 c.c. when the speed was quickened to 1165 feet (355 metres) per minute or 12½ miles (21·3 kilometres) per hour. This marked increase observed by Leo Zuntz is not to be entirely attributed to the extra rate of riding, but in part to the extra muscular effort necessary to overcome the resistance of the air, a factor well known to all.

It is a matter of general experience that practice makes the performance of work easier, fatigue renders it more difficult. It is also well known that at the commencement of exercise or muscular work there is often absent even in the trained man that smoothness in the co-ordination of the muscular contractions, respiration and circulation, which comes after a short time, and is expressed in the colloquial language of the labourer as "getting into the swing of the work." The adaptations involved are no doubt very complex, but here can be discussed only the respiratory exchange. There are indeed quantitative, if not qualitative changes, in the respiratory exchange which appear to agree with the experience of the man, and even in animals such a condition obtains. Thus Zuntz and Lehmann showed that when a horse was made to perform a moderate task of considerable duration, he worked after a short time more economically, discharged less carbon dioxide, and absorbed less oxygen than in the early stages of the work.

Observations made by Gruber and Schnyder upon man show that the output of carbon dioxide for the performance of a definite piece of work can be reduced by practice to two-thirds of the original value; this is also proved by the results obtained by Zuntz and Schumburg(1) in the case of recruits and trained soldiers. The recruit learning to march at first uses more muscles than are necessary, and works uneconomically even the ones which are necessary for the movements; during any given march he discharges a larger amount of carbon dioxide than he will do after training.

Fatigue is also accompanied by an extravagant metabolism; from this cause the output of carbon dioxide may be increased even as much as 21 per cent. An abnormal rise in the temperature of the body is produced by excessive and prolonged work under unfavourable conditions, and apparently causes a further extravagant combustion.

Soreness of feet caused by unsuitable boots will cause the sufferer to bring into action muscles other than the usual ones required for walking; this he does, even unconsciously, in order to diminish as far as possible the movement or pressure which causes pain. The abnormal use of such muscles causes an extravagant combustion and more fatigue. Soldiers in such a plight are often forced to fall out of the ranks, especially if they have been marching rapidly in unsuitable clothes, exposed to a hot and moist atmosphere and loaded with rifle, ammunition, and kit. The combined action of these unfavourable conditions will in many cases cause such an abnormal rise in the temperature of the body that "heat stroke" is the result. Work which is performed painfully by man or beast is uneconomical. The truth of these statements has been fully demonstrated by the successes and failures of forced marches. Pain is beneficent; it is a warning, a natural safeguard, an incentive to rest; the sensations of pain may be neglected, or may be deadened by drugs and work can be performed, but it is extravagant work, and the penalty has to be paid sooner or later.

An examination of the respiratory and nitrogenous exchange indicates that muscular work is performed without any actual destruction of the proteid of the muscles. The carbohydrate moiety of the complex proteid molecule appears to be split off, and to yield on combustion the energy and heat of muscular con-

traction. Numerous experiments made upon men and animals have shown that work does not increase the discharge of nitrogenous substances in the urine; even more numerous observations have proved that the respiratory exchange is immediately and markedly increased by exercise, and within limits varies as the work done. The conclusion is, therefore, drawn that muscular energy is obtained at the expense of non-nitrogenous substances. The demand for this energy can be met by food consisting largely of carbohydrates and fats; proteid, it is true, can be used and often with great advantage, for it is easily digested. The nitrogenous excretion under ordinary conditions varies with and in the same direction as the nitrogenous intake, and the balance of evidence tends to show that proteid food is entirely broken down during digestion; the non-nitrogenous products of this decomposition can be retained and formed into fat and carbohydrate, and in the latter form may be again linked to the proteid of the tissues.

It is impossible to adequately discuss here the sources of muscular energy, but it may not be amiss to point out that the great increase in the output of carbon dioxide during the performance of muscular work shows the necessity of an adequate supply of food. Men and horses work best when they are well fed, and feed best when they are well worked. Work creates a craving for food, and thus assists digestion and absorption. Here is a natural stimulus to the appetite, more potent than any drug; it is known to some, but many will not be cured of their ailments by muscular work: they seek some miracle-working drug, or the waters of a fashionable health-resort where they are unconsciously made to take exercise and lead a more natural life.

These facts are of practical importa for they show how far-reaching are the effects of exercise, and how necessary a rational training is for soldiers, athletes, and horses. Experience is indeed the result of a series of experiments made upon a large number of individuals, and its teaching cannot be safely ignored. The chief danger lies in dogmatic and rigid systems; the wide range of physiological variations is known, is even well expressed by the saying, "Ae man's meat's anither man's poison," but it is frequently forgotten. Some men have as great a craving for regulating their own and other people's food and exercise as theologians have for uniformity in belief. Vegetarians, flesh-

eaters, chewers, nitrogen economists, all bear witness to some portion of truth, but believe in the way characteristic of people with a mission that their little piece is the whole truth and nothing but the truth. The same may be said of many of the exponents of systems for muscular training.

The personal equation can only be determined by experiments carried out upon the individual himself; instinct and experience result therefrom, and are the natural and safest guides. The idea that a man should determine his diet by its chemical composition or calorie value is not only repugnant but also unscientific; likewise it is impossible to lay down hard-and-fast rules for the amount and nature of exercise to be taken. The personal equation is the primary factor.

Influence of Food upon the Respiratory Exchange. There is a daily variation in the respiratory exchange, which in its main features agrees with the variation in the temperature of the body; there is a rise during the day, the time of activity and work, and a fall during the night, the time of rest and sleep. One of the chief factors in the production of this cycle is muscular activity, which has already been discussed; the next important factor is the intake, digestion, and assimilation of food.

The general effect of food can be best demonstrated by a comparison of the respiratory exchange of a fasting animal with that of the same animal when it has been well fed. In addition to the general effect, the special influence of the different classes of food-stuffs, proteids, carbohydrates, and fats, must be considered.

The earliest work upon the subject is that of Lavoisier, who showed that in man food caused an increase in the output of carbon dioxide and the intake of oxygen. It is, however, the classical research of Regnault and Reiset, which first clearly demonstrated the influence of fasting and feeding upon the respiratory exchange. These observers found that the respiratory quotient varied from 0·62 to 1·04, according to the nature of the food consumed by the animal; during fasting the respiratory exchange of a herbivorous animal resembled that of a carnivorous animal, for it was living upon its own flesh; an animal well fed with corn often discharged, combined with carbon as carbon dioxide, a greater quantity of oxygen than that absorbed by the lungs. Lavoisier believed that the combustion in an animal's body was

a simple process of oxidation, the union of oxygen with carbon and hydrogen to form carbon dioxide and water. This view Regnault and Reiset contested, for they held that the oxidation was a more complex process, in some respects complete, in others incomplete, as shown by the discharge of such substances as urea and uric acid.

Within recent years many observers have carried out extensive investigations to determine the influence of fasting and feeding upon the respiratory exchange of men and animals, and upon many points of interest have obtained concordant results. The respiratory exchange shows a marked decrease, when no food is taken, quickly reaches a minimum, and then remains remarkably constant during a prolongation of the fast. In the case of the fasting man, Cetti, the average absorption of oxygen was found by Zuntz and Lehmann to be 4·65 c.c. per kilo body-weight and minute for the third to the sixth day, and 4·73 c.c. for the ninth to the eleventh day of this fast. Within twelve or twenty-four hours of the last meal the respiratory exchange reaches the minimum shown in the following table.

Per Kilo Body-Weight and Minute.		Observers.
Carbon Dioxide Discharged.	Oxygen Absorbed.	
c.c.	c.c.	
3·1	4·65	Zuntz and Lehmann
2·98	3·89	Magnus-Levy
2	—	Jaquet and Svenson
2·5	—	Johansson, Landergren, Klas Sondén, and Tigerstedt

The respiratory quotient falls during fasting to 0·75 or even to 0·63; the animal is living upon its proteid and fat, which contain a relatively large quantity of carbon and hydrogen and a small quantity of oxygen. The lower figure is even below the theoretical value for the combustion of fat, and is probably to be explained by the formation of glycogen and sugar from the fat and proteid of the tissues. The consideration of this question can be rendered easier by the following formulæ, which represent the oxidation of different substances:—

$$C_6H_{12}O_6 + 6O = 6CO_2 + 6H_2O$$

Dextrose.

$$\begin{matrix} CO_2 & 6 \\ O_2 & 6 \end{matrix} = 1.$$

$$C_3H_5(C_{18}H_{33}O_2)_3 + 80O_2 = 57CO_2 + 52H_2O$$

Olein.

$$\begin{matrix} CO_2 & 57 \\ O_2 & 80 \end{matrix} = 0{\cdot}71.$$

$$C_{72}H_{112}N_{18}O_{22}S + 77O_2 = 63CO_2 + 38H_2O + 9CO(NH_2)_2 + SO_3$$

Empirical formula for albumin (Lieberkuhn).

$$\begin{matrix} CO_2 & 63 \\ O_2 & 77 \end{matrix} = 0{\cdot}82.$$

$$2C_3H_5(C_{18}H_{33}O_2)_3 + 61O_2 = 16C_6H_{12}O_6 + 18CO_2 + 8H_2O$$

Olein.

$$\begin{matrix} CO_2 & 18 \\ O_2 & 61 \end{matrix} = 0{\cdot}281.$$

The last equation represents the formation of sugar from the partial oxidation of fat, and the probable origin of the low quotients which are observed in hibernating animals.

Recent researches tend to show that fat is present in small quantities in the bodies of animals which have died from starvation, and it is probable that the ferments of the tissues exert a digestic action similar to that of the juices of the alimentary canal, and by their action upon the tissues supply the starving animal with the energy which is necessary for the immediate support of life. The formation of small quantities of fat and carbohydrate may thus continue as long as life lasts.

When food is taken the respiratory exchange rises rapidly; this is due in part to the muscular and glandular activity involved in mastication, swallowing, peristalsis, and digestion, but chiefly to the combustion, complete or incomplete as the case may be, of the food ingested. Zuntz would attribute the greater intake of oxygen to the increased activity of the alimentary canal, for he found in a joint research with von Mering that food placed in the stomach of an animal increased the absorption of oxygen, but substances such as lactic acid, glycerin, sugar, and egg-albumin had no marked

effect upon the intake of oxygen when they were injected into the blood. In the case of a horse, Zuntz and Hagemann calculated that nearly half the available energy of the hay would be spent in the work of mastication and digestion. These views are supported by Löwy, who determined the respiratory exchange of fasting men before and after a dose of sodium sulphate or a draught of cold water, whereby the alimentary canal was stimulated. The respiratory exchange was increased by 10 or 20 per cent., but was unaltered when sodium chloride and sodium-bicarbonate were given instead of sodium sulphate. Löwy suggests that the therapeutic value of the waters of Carlsbad and Marienbad may be partly due to this stimulating effect of sodium sulphate. It is probable, however, that Zuntz has overestimated the part played by the activity of the alimentary canal, for experiments by Magnus-Levy, Bleibtreu, Koraen, Pembrey, and Spriggs([15]) show that the increased respiratory exchange is determined not so much by the digestibility of the food as its effect upon the metabolism of the body. This is even granted by Zuntz himself in order to explain the marked effect of maize upon the respiratory exchange of a horse.

The effect of food depends upon its quality and quantity. Fat appears to produce only a very slight increase in the respiratory exchange, less than 10 per cent.; proteid, a rise of 30 to 50 per cent. of the amount observed in the fasting animal. A meal rich in carbohydrates causes a much more marked effect upon the output of carbon dioxide than upon the intake of oxygen; the former may in the case of rats rise to 97 per cent., the latter to 35 per cent. above the values obtained in the fasting condition. The increase is well marked within an hour of the meal, and steadily increases during the next two or three hours. Although the output of carbon dioxide is 14 to 97 per cent. higher than the minimal discharge during hunger, the oxygen absorbed is increased to a less extent, and thus the respiratory quotient may rise to 1·17, and remain as high as 1·14 for a period of twenty hours. The explanation is to be found in the conversion of carbohydrates into fats, a chemical change accompanied by the liberation of carbon dioxide. These high quotients are only observed during the deposition of fat, and have already been discussed in an earlier part of this article.

The organs concerned in the assimilation of food respond most readily and efficiently to the natural stimulus, food, if the meal has been preceded by a fast. It is, therefore, interesting to note that there is generally among all classes of men an interval of twelve hours between the last meal in the evening and the first meal on the following morning; the respiratory exchange may thus fall to a very low value, or even reach the minimum observed during fasting, and the digestive organs will be in the most fit condition for the digestion and assimilation of food.

The very constant value of the minimal respiratory exchange and the rapidity with which it is attained during the first day of a fast indicate that the vital activity of an animal is associated with certain chemical changes, which must be maintained above a definite level. The processes of oxidation may be greatly increased beyond the minimum, but cannot fall below it without endangering life. To this there is an apparent exception in hibernating animals; but it is apparent only, for the great decrease in the respiratory exchange is accompanied by a reduction of vital activity to the lowest ebb.

Effect of External Temperature upon the Respiratory Exchange.— The two groups of animals, the cold-blooded and the warm-blooded, show a marked difference in their response to a change in the temperature of their surroundings; the temperature and respiratory exchange of the former vary with and in the same direction as the external temperature, whereas the temperature of the latter is fairly constant for a wide range of heat and cold, and their respiratory exchange is increased by cold and diminished by heat. The warm-blooded animal is able to maintain its constant high temperature by the control which its nervous system exerts over the production and loss of heat. The calorimeter is the only efficient method of measuring the loss of heat, but variations in the production can be studied with the respiration chamber as well as the calorimeter, for the respiratory exchange is a measure of the combustion. Thus it is that the influence of changes of external temperature upon the respiratory exchange cannot be discussed apart from the question of the regulation of temperature. In no case is this connection so close as in those animals which form a link between the cold-blooded and the cold-blooded

animals. The Darwinian theory leads to the conclusion that the former class has arisen from cold-blooded ancestors, and experiments upon the respiratory exchange and temperature of animals have fully confirmed this conclusion ([14]). The temperature of newly born pups, kittens, rabbits, and rats falls when they are deprived of the warmth of their mother's body, and steadily declines until it reaches a point a few degrees above the temperature of the air. Death generally occurs when the internal temperature falls below 20°. Newly born guinea-pigs, on the other hand, are able to maintain their temperature, if the exposure to cold be not excessive; they differ from the other young animals in their great development at birth, they are born well covered with fur, with eyes open and such control over their limbs that they can run about. The young rabbit, which may be taken as an example of the other class, is born blind, helpless, and naked. Similar differences are found among young birds; the chick is able to run about, see, peck up its food, and perform other complicated movements within an hour or two of leaving its shell, and is well covered with down; the newly hatched pigeon is blind, helpless, and naked. The chick can regulate its temperature, the pigeon cannot.

The young of warm-blooded animals can, therefore, be classified in two groups: the members of the first group respond to changes of external temperature in a similar manner to that of cold-blooded animals; those of the second group resemble warm-blooded animals, they increase their combustion when they are exposed to cold. The evolution and development of this power of regulation can be traced not only in the animal series, as shown by the observations of Sutherland and C. J. Martin ([15]) upon marsupials and monotremes, but also in the same individual at different stages of its existence. Hibernating mammals, moreover, have apparently retained even in adult life some of the characteristics of their cold-blooded ancestors; during the summer they are active and warm-blooded, during the winter they are torpid and cold-blooded.

Comparative determinations of the respiratory exchange in these different animals demonstrate clearly these facts, and some illustrative data may now be given.

An adult mouse, by reason of its large cutaneous surface in

comparison with its mass, responds very rapidly to a change of external temperature.

Change of External Temperature.	Percentage Increase or Decrease in the Output of Carbon Dioxide.	Interval within which the Increase or Decrease in the Output of Carbon Dioxide occurred.
Degrees.		Minutes.
32·5 to 11	+211	30
30 „ 10·5	+118	10
30 „ 13·75	+ 75	5
30 „ 18	+ 74	2
33·25 „ 17·5	+ 60	1
11 „ 31·5	- 46	30
9·75 „ 29	- 28	10
12·75 „ 30	- 11	5
18 „ 34·5	- 18	2
17 „ 32	- 5	1

The relationship ([16]) between muscular activity and the production of carbon dioxide and heat is well marked; in cold surroundings the mouse is very active, whereas with a warm external temperature it is quiet and often goes to sleep.

Young mice one day old show a decrease in the output of carbon dioxide when the external temperature is changed from 30° to 20°; within half-an-hour the decrease may be 50 per cent. of the original value and the temperature of their bodies falls, in some cases as much as 9°. At this early age the mice respond for higher temperatures in a manner similar to that seen in adult animals; a rise in external temperature from 30° to 40° reduces the discharge of carbon dioxide to about one-half of its previous value and raises their temperature. A chick during its development passes first through a cold-blooded stage, then through an apparently neutral stage, and when it is hatched responds as a warm-blooded animal to changes of external temperature. The marked contrast exhibited by the young pigeon during the first few days after it is hatched is shown by the following figures (see p. 510).

As in the case of the young mice, a rise in external temperature to 40° determines a decrease in the output of carbon dioxide and a rise in the temperature of the young pigeon.

1st Day Chick.			1st Day Pigeon.		
External Temperature.	Carbon Dioxide Discharged in Decimgrms.	Remarks.	External Temperature.	Carbon Dioxide Discharged in Decimgrms.	Remarks.
Degrees.			Degrees.		
37·75	165	Chick quiet	36	79	Pigeon slightly active.
37·75	138	„ „	36	70	
17	322	Chick active, chirping	36	70	Pigeon quiet.
17	309	Chick active, chirping	22	46	Pigeon active at first, then quiet.
			21·5	25	Pigeon quiet.

The chick was placed in the respiration chamber with the water-bath at 37·5°, and ventilation was continued for thirty-five minutes before the first period; the young pigeon was in the ventilated chamber with the water-bath at 38·5° for twenty-five minutes before the first period. The periods are consecutive, and each determination of the output of carbon dioxide is for fifteen minutes.

The practical interest of the evolution of the warm-blooded animal is centred in infants. Observations made by Babák upon the respiratory exchange and the production of heat in infants have shown that the power of regulating temperature is imperfectly developed in the newly born. The regulation of the loss of heat is imperfect, and its deficiency is clearly shown if the infant be insufficiently clothed, when it is exposed even to moderate cold; the regulation of the production of heat by variations in the combustion is also inadequate, and only within narrow limits can the newly born child respond to changes of external temperature in a manner similar to that exhibited by the adult. Many premature infants have been reared by proper attention to the temperature of their surroundings; the more premature and weak the more nearly do they resemble cold-blooded animals in their inability to regulate their temperature. The heat of their bodies and their respiratory exchange vary with and in the same direction as the external temperature. Cold, even moderate cold, to such premature or weakly infants is not a stimulant but a depressant, for they can regulate neither the loss nor the production of heat.

Thus it has been possible to trace the evolution of the warm-

blooded animal by a study of the response of different animals to changes in the temperature of their surroundings; the consequent variations in the respiratory exchange indicate the changes in the production of heat. Several interesting questions now arise. What are the relative advantages of the different effects produced by changes in external temperature upon the respiratory exchange of warm-blooded and cold-blooded animals during their struggle for existence? Is it possible to reduce the warm-blooded animal to the condition of a cold-blooded one?

It has already been shown that life, both animal and vegetable, is accompanied by constant chemical change, the production of heat and gaseous interchange. The living tissues require a high internal temperature for the proper manifestation of their activities; the warm-blooded animals maintain this favourable condi- an optimum temperature, by a constant struggle against - l heat; the cold-blooded animals have not acquired this . and within certain limits the activity of their tissues is . ained by the external temperature. Both of these conditions are advantageous to the respective groups of animals in the struggle for existence; the warm-blooded animals are able to live a more ardent life, unbroken by long periods of inactivity or torpor, and within wide limits are independent of the temperature of their surroundings. The constant exercise of the different organs of the body, especially during the search for food in cold weather, leads to a greater development of their nervous system and intelligence. The cold-blooded animal, on the other hand, depends for its food supply upon the external temperature; a period of torpidity with the activities of its tissues reduced to the lowest ebb consistent with the retention of life tides it over the cold winter months, when food is scarce, and prolongs its life beyond one brief summer. It has already been mentioned that even some of the mammals, hibernating animals such as the marmot, hedgehog, dormouse, and bat, have retained this ancestral characteristic for their better preservation in the struggle for existence.

The intensity of life is dependent upon a high and constant temperature, and the power to maintain this temperature is associated chiefly with the control of the nervous system over the

skeletal muscles and the muscles of the blood-vessels. During natural sleep, when nervous and muscular activity are at their lowest, the respiratory exchange and temperature fall; during muscular exercise they rise. The means whereby the power of regulating temperature can be diminished, suspended, or abolished are those which lessen or interrupt the influence of the nervous system upon the muscles, both voluntary and involuntary. The experience of Arctic explorers and Alpine climbers shows that the weary traveller, who gives way to the imperative desire to sleep upon the line of march, is doomed to death. During the deep sleep which follows intense fatigue, the sensibility of the nervous system is so greatly reduced that the response to cold ceases; the unconsciousness of sleep gradually passes into a condition of paralysis and the man dies, "frozen to death," it is said but in reality killed long before his temperature falls to zero. The activities of the tissues, once the nervous control is lost, are reduced by the cold; chemical change and the production of heat become less and less, until an internal temperature about 20° is reached, when life ceases. During such marches the desire to sleep and the effects of cold must be resisted by forced activity and increased production of heat until shelter and artificial heat can be obtained.

In deep anæsthesia produced by chloroform, ether, or other drugs the activities of the body are so profoundly depressed that the regulation of temperature is suspended; the production of heat is greatly diminished, and the animal, if it be exposed even to moderate cold, will rapidly cool. An anæsthetised mammal or bird is in many respects comparable to a cold-blooded animal. The influence of the anæsthetic is manifested especially on the central nervous system; sensations of heat and cold no longer arise, or should they arise no longer produce reflexly decreased or increased activity of the skeletal muscles and the muscles of the blood-vessels; control is therefore lost over both production and loss of heat. The temperature of anæsthetised animals will fall to 22° when they are exposed to cold, and the cold rather than the drug brings about a fatal issue. The respiratory exchange of an anæsthetised mammal is increased by a rise, diminished by a fall in external temperature. This may be illustrated by the following details of an experiment upon a mouse.

External Temperature.	Output of Carbon Dioxide in Decigrms.	Remarks.
Degrees.		
25	437	Mouse anæsthetised with ether during this period.
25	392	Mouse fully anæsthetised.
13·5	290	" " "
13·5	308	" " "
13·5	185	" " "

The mouse was in the ventilated respiration chamber, surrounded by a water-bath at 25°, for fifty minutes before the first period. The mouse was fully under the anæsthetic when it was taken out of the respiration chamber and recovered in about fifteen minutes. The periods are consecutive, and each determination of the output of carbon dioxide is for fifteen minutes.

The practical importance of the influence of anæsthetics upon the regulation of temperature is now recognised by many surgeons. An anæsthetised patient during and after a long operation should be protected from exposure even to moderate cold, and his temperature should be maintained by external sources of heat, such as warm water-bottles. Drugs which produce profound anæsthesia can never be considered harmless: it is, indeed, probable that the recovery of patients from the effects of operations is frequently retarded or prevented by the depressing influence of the anæsthetic, whereby the combustion of the body, one of the most characteristic signs of life, has been so greatly diminished, and for a time at least has been removed from the control of the nervous system. Pain is a natural safeguard, and brings about appropriate responses to sensations which are too often quenched by an anæsthetic. Animals and savages rapidly recover from serious wounds or operations, although no drug is used to deaden the pain, and civilised man does not succeed in abolishing pain even during operations without paying a daily toll in human lives.

The normal response of a warm-blooded animal to changes of external temperature can also be abolished by curare or section of the spinal cord, whereby the central control of the muscles is removed.

After the consideration of these abnormal conditions, which demonstrate so clearly that the effect of external temperature upon the respiratory exchange is bound up with the regulation of

temperature and the control of the nervous system over the muscles, it is necessary to discuss the question in special reference to man under natural conditions. Over all animals man is supreme in his power of adapting himself to his surroundings or changing his surroundings to suit his needs; he can live either in the arctic regions or in the tropics, for by means of appropriate clothing he can readily control his loss of heat. Even unclothed a man can compensate for considerable changes of external temperature, and this he effects by altering both his loss and production of heat. Upon the relative parts played by the physical and chemical methods of regulation there has been much debate. Löwy concludes from the results of fifty-five experiments upon sixteen men that the only involuntary regulation of temperature in a man exposed to moderate cold is effected by changes in the vascularity of the skin; he found that the respiratory exchange was unaltered in twenty experiments, diminished in nine, and increased in twenty-six; in the last series the increase varied from 5 to 90·8 per cent. above the normal. It is noteworthy that the greatest increase in the gaseous exchange was observed in the men who shivered or moved, when they felt cold, whereas no increase or even a decrease occurred in those who remained quiet, and by an effort of the will suppressed any inclination to move or shiver. This result has been confirmed by Johansson. It must not be thought, however, that man is an exception to the general rule that warm-blooded animals in cold surroundings increase, in warm surroundings diminish, their respiratory exchange and production of heat. The normal effect of cold is increased muscular activity; this is within the experience of every inhabitant of a temperate climate. The increased muscular activity is not necessarily brought about by a conscious effort of the will; it is to a great extent produced reflexly. A man works more energetically when the external temperature is low, and, if he be obliged to remain inactive, the reflex effect of cold may be so great as to produce shivering, forcible involuntary movements which are accompanied by a great increase in the respiratory exchange and production of heat.

Observations upon both man and animals appear to show that a very high external temperature will increase the respiratory exchange; the limits of regulation are apparently exceeded, the tissues are abnormally heated and respond with increased respiratory exchange. A condition in many respects similar is found in fever

The influence of climate upon the respiratory exchange is due not to the temperature alone, but also to other factors, such as moisture and wind. The capacity of dry air to take up heat is much less than that of moist air; hence it happens that in dry, calm air several degrees below zero the sensation of cold may be much less than that felt in moist air with a temperature a few degrees above the freezing point. On the other hand, a hot moist atmosphere, by preventing the evaporation of sweat, produces a more marked feeling of heat than dry air at the same temperature. The more stationary the cool air is, the less the loss of heat, for the body becomes surrounded with a layer of air which is warmer than that of the atmosphere. Between the clothing of a man and the fur or feathers of an animal strata of air are enclosed, the temperature of which gradually increases above the outside air, until the layer in actual contact with the greater part of the body is 24° to 30°. A cold wind quickly reduces the temperature of these different layers. The sailors of Parry's expedition to the Polar Seas found that when the air was perfectly calm they could bear better intense cold, −40°, which would freeze mercury, than a temperature of −12·2°, when there was a wind.

The depressing effects of hot moist climates are well known. European children languish in hot humid climates, but rapidly improve in health in the cooler hill-country or in England. Cold increases their respiratory exchange, general metabolism, and activity, and is favourable to a vigorous and sturdy growth. The success also of the open-air treatment of phthisical patients depends, it would seem, not so much upon any greater purity of the air, as upon free exposure: the open air and exercise increase the combustion and respiratory exchange, improve the appetite, and augment the metabolism. A slightly greater pressure of oxygen in the atmosphere is not the important factor, for in many of the health resorts it is, owing to the altitude, less than the normal, and actual experiments upon men and animals show that small changes have little or no effect: a high percentage of carbon dioxide is in itself no strict indication of an unhealthy city or dwelling, it is a sign that there is no free ventilation, and therefore a tendency for the air to become stagnant, humid, and warm. It would even appear that the number of bacteria affords no certain test of the purity of air. Evidence steadily accumulates to show that the bad effects of overcrowded rooms are due, not to diminished

oxygen, not to an increase of carbon dioxide, not to any toxin discharged from the human body, but to the absence of free ventilation, to the warm and humid atmosphere, which reduces the respiratory exchange and metabolism of the inmates and renders them less resistant to the attacks of micro-organisms. The danger to health appears to lie in the massing of numerous dwellings upon a small area, which, owing to the exigencies of water supply, is often situated in a valley; there are doubtless many other factors but temperature, moisture, and wind are the ones which have a marked effect upon respiratory exchange.

The power of adaptation must not be overlooked. It is known that men and animals become "seasoned" after living for some time in a climate very different from that in which they were bred. This has also been proved by experiments upon the respiratory exchange; the first marked change of weather is the most felt and produces the greatest effect upon the respiratory exchange. The body by practice improves its power of regulating its temperature on exposure to heat and cold, and thus the physiologic resistance to changes of temperature can be maintained by training. The idea that the danger of "colds" can be warded off by the maintenance of an equable temperature in living-rooms and bedrooms is widely prevalent, but is erroneous, and in practice leads to deterioration and defeats its own aim. Weaklings and debilitated subjects need artificial conditions; but rules of health should be directed to the survival of the fit, not of the unfit.

Exchange of Gases between the Blood and Air.—The gaseous exchange which is in constant progress between the blood and the air may be studied in two ways: the changes in the composition of either the blood or the air during its passage through the lungs may be determined. The former method involves a comparison of the gases in the venous blood of the right side of the heart with the gases of the arterial blood, the latter method comprises qualitative and quantitative analyses of the inspired and expired air. The data so obtained give the gross results, but do not yield information upon the important questions how and where the gaseous interchange occurs. The air which is in contact with the epithelium covering the pulmonary capillaries has not the composition of the inspired air, for owing to the exchange of gases it is poorer in oxygen, richer in carbon dioxide, and saturated with aqueous vapour at the temperature of the body; nor has it

composition of the expired air, for the latter contains much air from the "dead space" of the respiratory tract together with alveolar air. It is necessary to know the composition of this alveolar air, and thus ascertain the pressures of oxygen, carbon dioxide, and nitrogen, to which the blood has been exposed. Likewise in the case of the blood, both arterial and venous, it is not only necessary to determine th quality but also the pressure, or, as it is often called, the tension, of its several gases. Is the pressure of oxgyen in the venous blood less than that of the oxygen in the alveolar air? Is the pressure of carbon dioxide in the venous blood greater than that of the carbon dioxide in the alveolar air? These are important questions, which at present cannot be definitely answered. Some observers maintain that the respective pressures are such, that according to physical and chemical laws oxygen must pass into, and carbon dioxide out of, the venous blood in the lungs; others as strongly insist that the pressure of oxygen in the blood is above, and that of carbon dioxide below, the pressures of the corresponding gases in the air of the pulmonary alveoli. The former find a sufficient explanation of the gaseous exchange in ordinary physical and chemical processes; the latter regard the exchange as a special physiological process, a secretion of gas, which cannot be explained by our present knowledge of physics and chemistry. Around this subject has been and still is waged the contest between the Mechanical and Vitalistic Schools of Physiology. Before an attempt is made to discuss this difficult question, it will be necessary to consider the simple qualitative results of the analyses of the gases of venous and arterial blood.

Gases of the Blood.—The presence of gases in the blood was first demonstrated in 1636 by Boyle, who found that fresh defibrinated blood gave off bubbles of gas when it was exposed to the vacuum of an air-pump. The importance of this experiment was grasped by Mayow, who considered the gas to be nitro-aerial gas, that is, the gas now known as oxygen. The next advance was made by Priestley about the year 1776; he noticed that blood placed in an atmosphere of hydrogen or nitrogen gave off oxygen. A few years later the question, whether the blood did or did not contain gas, became the subject of many experiments and much debate; some observers, among whom may be mentioned Humphry Davy and his brother, John Davy, obtained carbon dioxide and oxygen or carbon dioxide alone; others could find no gas. The contra-

dictory nature of the results of different observers so impressed the celebrated physiologist Johannes Müller that he felt obliged to make experiments himself, before he expressed an opinion upon the question. The results of his experiments were unfortunate, for he, the leading physiologist of his time, confirmed the negative results of other observers. He found that amphibia could produce carbon dioxide in an atmosphere free from oxygen; blood shaken up with air would yield carbon dioxide, but venous blood itself contained no carbon dioxide. So great were the difficulties to explain, that Müller considered that no satisfactory theory of respiration could be advanced: the carbon dioxide might be secreted from the lungs and skin, but the puzzle remained, and was only to be solved by further experiments. Such was the position when the second edition of Müller's *Handbuch der Physiologie* was published in 1835. Two years later Magnus showed that, with improved methods for the extraction of gas, carbon dioxide, oxygen, and nitrogen could be obtained from blood.

The perfection of the gas-pump was necessary, and great advances were made when Ludwig and Setschenow, Pflüger, and Helmholtz constructed their mercurial gas-pumps, based upon the principle of the Torricellian vacuum. Such gas-pumps, or modifications of the same, are now used for the extraction of the gases of the blood; their construction and use will not be described here, only the principles involved need be mentioned. The liberation of the gases present in the blood in a state of simple solution and the dissociation of those which are in loose chemical combination can be best effected by exposure to a vacuum, by warming and shaking the blood: the liberation of the carbon dioxide can be facilitated by the addition of a weak acid. Three other methods require mention here, for the reactions involved will be shown later to be of great importance in connection with the nature of the combinations in which the gases exist in the blood. Fernet found that the gases in blood can be extracted by the passage of a stream of hydrogen and the aid of a vacuum; Claude Bernard discovered that, when blood is shaken with double its volume of carbon monoxide, the oxygen is driven out from its combination with hæmoglobin, owing to the stronger affinity and the mass influence of the carbon monoxide; and Haldane ([17]) has introduced the ferricyanide method, whereby a rapid and accurate deter-

mination of the volume of oxygen or carbon monoxide, which the haemoglobin of blood can absorb, can be made without the aid of a gas-pump; the method is based upon the reaction of ferricyanide with solutions of oxy-haemoglobin or carbon monoxide haemoglobin, the combined gas is set free and methaemoglobin is formed.

The most important analyses of the gases of blood must now be examined; in the first place, those of the venous blood which is passing to the lungs. The venous blood is liable to marked differences in its gaseous contents, according to the nature and condition of the organs of the body from which it is received; it is therefore only possible to obtain results which are directly applicable to the question of the exchange of gases in the lungs, when the analyses are performed upon samples of blood removed from the right ventricle of the heart. Such samples can be obtained by passing a catheter from the right external jugular vein through the right auricle and into the right ventricle.

The following table (see p. 520) gives the results of analyses of simultaneous samples of venous blood from the right ventricle and arterial blood. The figures represent the volumes of gas in 100 c.c. of blood, and those in brackets the maximal and minimal values of several analyses.

An examination of the table shows that there are considerable variations in the percentage volumes of gas; thus in the case of dogs the oxygen of the venous blood varies from 5·5 to 17·3, the carbon dioxide from 24·96 to 64·08, and the oxygen of the arterial blood from 15·0 to 25·6, the carbon dioxide from 30·65 to 47·33. The range is greater in the venous than in the arterial blood; this result would be expected, for the composition of the mixed venous blood of the right side of the heart is liable to variations according to the activity of the muscles and glands of the body and the different distribution of blood in the various organs. Other causes of the variations are to be sought in individual differences and in errors of experiment. It is impossible to obtain two animals in exactly the same condition, and it is very difficult, except with the most perfect gas-pumps, to avoid some dilution of the blood-gases with air. This error shows itself in the figures for the amount of nitrogen contained in the blood, and is corrected by many observers in the following way. As the basis of correction, it is assumed that blood holds in solution a similar volume

of nitrogen to that absorbed by water; any marked excess in the gas extracted by the pump is to be attributed to a leakage

Animal.	Venous Blood from Right Ventricle.			Arterial Blood.			Observers.
	Oxygen.	Carbon Dioxide.	Nitrogen.	Oxygen.	Carbon Dioxide.	Nitrogen.	
Dog	11·9 (16·6) (5·5)	44·3 (47·5) (38·8)	2·0 (4·91) (1·32)	19·2 (23·3) (15·0)	38·8 (43·1) (34·8)	2·7 (5·550) (1·462)	Schoffer.
" "	5·5	50·4	—	22·1	36·1	—	Paul Bert.
" "	—	19·0	—	19·3	38·7	—	"
" "	11·7	36·5	—	17·3	33·4	—	Ewald.
" "	9·5	31·0	—	15·4	32·4	—	"
" "	12·5	36·0	—	16·5	31·8	—	Fubini.
" "	12·5	24·96	—	16·12	30·65	—	"
" "	9·9	17·5	—	17·25	42·7	—	Mathieu and Urbain.
" "	5·19	61	—	20·75	17·33	—	"
" "	17·3	[illegible]	[illegible]	25·6	11·0	1·23	Bohr and Henriques.
" "	11·9	1	1·06	21·3	12·6	1·19	"
" "	11	30·3	1·40	20·3	45·0	1·14	"
	[illegible]	[illegible] (61·6) (18·5)	—	11·0 (16·6) (9·2)	19·4 (35·5) (38·0)	—	Zuntz and Hagemann.

of air, and a proportionate reduction is made in the volumes o nitrogen and oxygen obtained from the blood. This correctio

is not quite exact for the ex[illegible] Bohr and Henriques show that blood absorbs a little more nitrogen; about 1·2 volumes per cent. are the total amount found in both venous and arterial blood. A further important point in the consideration of the values in the carbon dioxide and oxygen is the time which elapses between the removal of the blood from the animal and the completion of the extraction of the gases. Pflüger found by comparative experiments with a large vacuum that the ordinary methods for the extraction of gases give results which are for the oxygen too low, for the carbon dioxide too high. This is due to the fact that the blood is a living tissue which consumes oxygen and produces carbon dioxide; owing to this cause blood kept from contact with the air rapidly becomes darker in colour even when no putrefactive changes have occurred.

In addition to oxygen, carbon dioxide, and nitrogen, there are in blood traces of argon, carbon monoxide, hydrogen, and marsh-gas; the source of the first and second is probably the inspired air, for small quantities of carbon monoxide are found in the air of large towns, where large consumption of coal and gas occurs; the fermentation produced by bacteria in the food of the alimentary canal is to be regarded as the source of the hydrogen and marsh-gas.

It is convenient to take the following figures as the average values of the gases of the arterial and venous blood.

Arterial blood—Oxygen, 20; carbon dioxide, 43; nitrogen, 1·2 volumes per cent.

Venous blood—Oxygen, 12; carbon dioxide, 50; nitrogen, 1·2 volumes per cent.

The respiratory quotient obtained therefrom is $\frac{CO_2}{O_2} = \frac{7 \cdot 0}{8 \cdot 0} = 0 \cdot 87$.

The nature of the connection between the blood and its gases must now be considered, for the blood is very complex, and its gases are distributed in both plasma and corpuscles. A necessary preliminary to the study of this question is a knowledge of the principles which underlie the investigation of gases, whether they be free, in solution, or in chemical combination. There are certain well-known laws. Dalton's Law:—*In a mixture of several gases which have no chemical action upon one another, the pressure of the mixture is equal to the sum of the pressures of the different gases, each being considered as alone occupying the total volume.*

Furthermore, when several gases are in contact with a liquid, each is dissolved as if it alone were present. All gases exhibit a tendency to expand, and thus exert pressure against the vessels in which they are confined. This property was investigated by Boyle and Mariotte, who independently established the law that *the pressure of a gas varies inversely as the volume, provided that the temperature remain constant.* As the density of a gas also varies inversely as the volume, the law may be expressed in other words by saying that *at the same temperature the density of a gas varies directly as the pressure.* This law is not an absolutely rigid one, for gases possess unequal compressibility. Another important law is Henry's Law of the solution of gases in liquids. *In a gaseous solution in equilibrium at a given temperature there exists a constant relation between the pressure of the gas dissolved and the pressure of the gas outside the liquid.* When the fluid possesses no chemical attraction for the gas, the amount of gas absorbed depends directly upon the pressure. On the other hand, when the liquid possesses a chemical attraction for the gas, the amount of gas absorbed depends upon the pressure, but there is no regular proportion between the volume of the gas absorbed and the pressure. This law is not absolute. As a general rule the solubility of a gas in a liquid diminishes when the temperature is raised. Gases vary in their respective solubilities in different liquids. The coefficient of absorption of a gas is the volume of gas reduced to 0° and 760 mm. which unit volume of the liquid under a pressure of 760 mm. will absorb. The following table is given by Bohr for the coefficient of absorption in water of the gases most important in physiological questions.

Temperature.	Oxygen.	Carbon Dioxide.	Carbon Monoxide.	Nitrogen.
0	0·0489	1·713	0·0354	0·0239
10	0·0380	1·194	0·0282	0·0196
20	0·0310	0·878	0·0232	0·0164
30	0·0262	0·665	0·0200	0·0138
40	0·0231	0·530	0·0178	0·0118

The following example will explain the use of the above data. A hundred cubic centimetres of water are shaken until they are saturated with atmospheric air at 30°; the amount of gas absorbed is to be calculated as follows. The percentage composition of the

air is 79 volumes of nitrogen, 20·96 of oxygen, and 0·04 of carbon dioxide: the total pressure is 760 mm. The tension of aqueous vapour at 30° is 31·5 mm., and must be deducted from the total pressure, $760 - 31.5 = 728.5$ mm. The partial pressure (p) of each gas is as follows:—

$$\text{Oxygen} = 728{\cdot}5 \times \frac{20{\cdot}96}{100} = 152{\cdot}7 \text{ mm.}$$

$$\text{Nitrogen} = 728{\cdot}5 \times \frac{79}{100} = 575{\cdot}5 \text{ mm.}$$

$$\text{Carbon dioxide} = 728{\cdot}5 \times \frac{0{\cdot}04}{100} = 0{\cdot}3 \text{ mm.}$$

The absorbed gas (q) is calculated according to the formula:—

$$q = \frac{a.h.p}{760},$$

in which $h = 100$ and a = coefficient of absorption given in the above table for 30°; thus the amount of oxygen absorbed is

$$0{\cdot}0262 \cdot \frac{100 \times 152{\cdot}7}{760} = 0{\cdot}526 \text{ c.c.}$$

The total amounts are:—

Oxygen in 100 c.c. of water . . .	0·526 c.c.
Nitrogen in 100 c.c. of water . . .	1·045 c.c.
Carbon dioxide in 100 c.c. of water . .	0·025 c.c.

The absorption coefficients of the different constituents of blood can be determined by direct experiment only in the case of those gases which form no chemical combinations with any of the constituents. The data for nitrogen in all cases and for oxygen in the plasma can be determined directly, but not for oxygen and carbon dioxide in the blood or for carbon dioxide in the plasma. In these latter cases Bohr has calculated the coefficients indirectly from the absorption of indifferent gases, such as oxygen and nitrogen in the case of the plasma and hydrogen in the case of blood. The following are the respective values given by Bohr.

	Oxygen.		Nitrogen.		Carbon Dioxide.	
	15°	38°	15°	38°	15°	38°
Blood plasma .	0·033	0·023	0·017	0·012	0·994	0·541
Blood . .	0·031	0·022	0·016	0·011	0·937	0·511
Blood corpuscles	0·025	0·019	0·014	0·010	0·825	0·450

In the case of liquids containing substances which form loose chemical combinations with gases, the relation of pressure to the gas absorbed is frequently expressed by a curve in which the abscissa represents the pressures and the ordinates the amounts of gas taken up by the fluid. Under the ordinary pressure of oxygen in the atmosphere hæmoglobin readily combines with oxygen, but, if the external pressure be lowered sufficiently, oxygen is given off, the oxy-hæmoglobin undergoing *dissociation*. The force with which the oxygen separates from the hæmoglobin under these circumstances is called the *pressure* or *tension of dissociation*, and is represented graphically by such a curve as that just described.

The blood, considered as a tissue, can be divided into two portions, the fluid matrix, the plasma, and the cellular elements, the corpuscles; the distribution of its gases will, therefore, be considered in relation to the whole blood, the plasma and corpuscles respectively. It will be shown later that the investigation of the combination of gases with pure solutions of hæmoglobin is not so important a method as it was once thought to be; indeed, many of Hüfner's results with solutions of hæmoglobin have been shown to be wrong; and even the correct ones are not directly applicable to the hæmoglobin as it exists in the red corpuscles. It is difficult to obtain pure hæmoglobin, and it is probable that the properties of the crystals are not exactly the same as those of the hæmoglobin in the red corpuscles in which it exists in close relationship with proteids and salts. Moreover, different results are obtained for the dissociation of oxy-hæmoglobin, according to the method used for the preparation of the crystals and the percentage of hæmoglobin in solution.

The Oxygen in Blood.– When blood is shaken with air, it takes up a much larger volume of oxygen than the amount which can be held in solution by an equal volume of water, and animals, as shown by Regnault and Reiset's experiments, absorb the same amount of oxygen whether they breathe oxygen or air. The last fact led Liebig to the conclusion that the gases of the blood are present in a state of loose chemical combination with some of the constituents of the blood, and Fernet found that, as regards oxygen, the constituent concerned was the red corpuscle. Further proofs of the chemical combination of oxygen were supplied by Bernard and Hoppe-Seyler, who discovered that the oxygen of

the blood could be displaced by an equal volume of carbon monoxide. Hoppe-Seyler then succeeded in crystallising hæmoglobin, and proved that it combined with oxygen, but on exposure to a vacuum again yielded up the gas. A year or two later, in 1864, Stokes discovered that reducing agents abstracted the oxygen from hæmoglobin, and thereby altered its colour and spectrum.

The blood can be divided by means of the centrifugal machine into plasma and corpuscles, and a similar separation can be effected by cooling horse's blood and allowing the corpuscles to subside. These two portions show a marked difference in their relation to oxygen. The plasma contains but a small amount, and this is dissolved in proportion to the pressure, according to Henry's law; thus the quantity of oxgyen in plasma which has been shaken with air at the ordinary temperature and pressure is about 0·65 volumes per cent., a value not very different from that obtained with water. The whole blood, when treated in a similar manner, absorbs 18·5 to 24 volumes per cent.; the first figure is the average for human blood according to Haldane's experiments, the latter the result obtained by Bohr for dog's blood. These figures, therefore, represent the *oxygen capacity of the blood* of man and dog respectively. There is thus no doubt that oxygen is present in blood in the states of simple solution and chemical combination; the latter is the predominant state and requires further consideration.

According to Bunge's determinations, 100 parts of red corpuscles contain 26 parts of hæmoglobin and 63 parts of water; this would correspond to a 41 per cent. solution, but in making this statement it must be remembered that the hæmoglobin is probably not present in simple solution in the red corpuscles. The blood considered as a whole contains about 14 per cent. of hæmoglobin, which is present only in the red corpuscles. It is with the blood that the gaseous exchange takes place; the red corpuscles are the oxygen-carriers, they yield up oxygen to the plasma, which in turn supplies the tissues according to their needs. The most important question to be considered later is the pressure of the oxygen in the red corpuscles, whereby the pressure in the plasma is maintained during its course through the systemic circulation.

It is necessary to determine the amounts of oxygen taken up under different pressures and at a constant temperature. The last factor is important, especially when low pressures of oxygen

are concerned, and the temperature which should be selected is 38°, for this is the internal temperature of warm-blooded animals. Loewy and Zuntz have recently published the results of an important investigation upon the dissociation of oxy-hæmoglobin in normal blood, laked blood, and crystallised hæmoglobin. The method, which had previously been most often employed, was to shake gas-free solutions of hæmoglobin with oxygen under different pressures and then to determine the oxygen absorbed by measuring the remaining gas. This procedure they rejected, for owing to oxidation at the temperature of the body varying quantities of oxygen disappear, a difficulty which Hüfner had attempted to avoid by using carbon monoxide instead of oxygen, and thus indirectly estimating the dissociation of oxy-hæmoglobin. The method which Loewy and Zuntz employed was similar to that used by Paul Bert and Bohr. Different portions of the blood were shaken with appropriately graduated mixtures of oxygen and nitrogen until there was an equality of pressure in and outside the blood at 38° and atmospheric pressure; a sample of this blood was then placed in Pflüger's pump, and its gases were extracted and analysed; a sample of the gas with which the blood had been shaken was also analysed. Some of the results may be given here, others will be considered later in relation to the causes of the gaseous exchange between the blood and the alveolar air.

Percentage of Oxygen in Gas Mixture used.	Oxygen contained in Blood in Volumes per Cent.		Percentage Saturation of Blood with Oxygen.	
	Unlaked.	Laked.	Unlaked.	Laked.
3·34	9·42	—	53·7	—
3·48	—	10·54		69·2
4·05	—	11·62	—	76·25
4·65	—	13·91	—	91·3
5·40	11·36	—	64·8	
20·06	17·54	—	100·0	—
20·57	—	15·27	—	100·0

The above table shows that the laked blood took up a smaller quantity of oxygen, but with low partial pressures had a relatively higher saturation than that of the unlaked blood. The figures below give the results obtained from similar experiments with different solutions made with hæmoglobin obtained with and without alcohol.

Percentage of Oxygen in Gas Mixture used.	Oxygen contained in Hæmoglobin Solution in Volumes per Cent.		Percentage Saturation of Hæmoglobin Solution with Oxygen.	
	Hæmoglobin prepared without Alcohol.	Hæmoglobin prepared with Alcohol.	Hæmoglobin prepared without Alcohol.	Hæmoglobin prepared with Alcohol.
18·95	20·33		100	100
18·74	—	21·33		
2·41	22·37	—	85	89·5
2·31	—	19·08		—
1·45	11·07		42	80
1·40	—	17·04		—

The following figure shows graphically the results obtained by Loewy, Hüfner, and others in a comparison of various bloods.

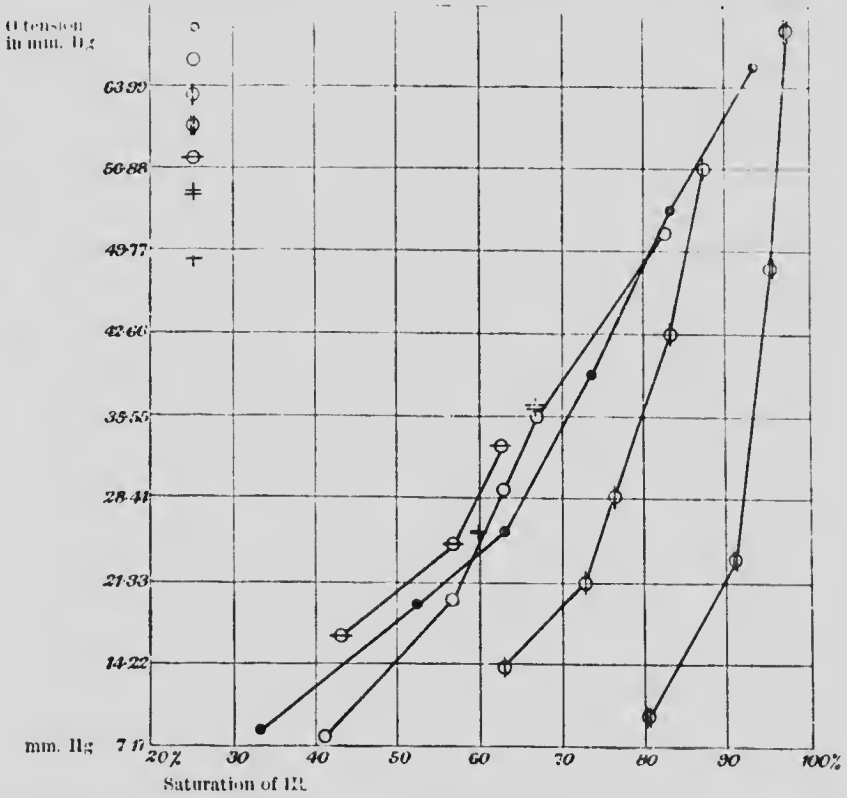


FIG. 27—Comparison of Dissociation Curves of OHb. ◡ Loewy (men); ○ Paul Bert; Φ Hüfner (new); ♯ Hüfner (old); ÷ Loewy and Zuntz (dog); ǂ Loewy (O_2 sat. of venous blood of men); + Strassburg-Wolffberg (venous blood of dogs).

The next figure (Fig. 28) gives the dissociation curve of OH obtained by Loewy in the case of human blood.

Recent experiments by Bohr, Hasselbalch, and Krogh show that the absorption of oxygen by the blood is influenced by the presence of carbon dioxide; when the pressure of oxygen is low

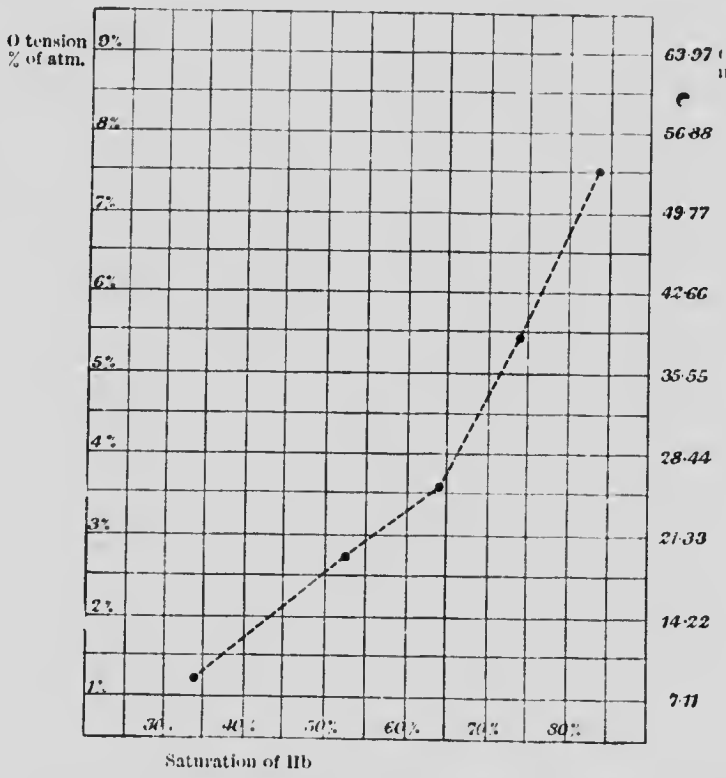


FIG. 28.—Average Dissociation Curve of OHb in Human Blood (*Loewy*).

and that of carbon dioxide is high the capacity of the blood to take up oxygen is diminished. This factor must, therefore, be taken into account. In Krogh's experiments with horse's blood the pressure of carbon dioxide was maintained at a constant level; his results are given in the following table of the quantities of oxygen absorbed by the blood at different pressures and at a temperature of 38°.

Pressure of Oxygen in mm.	In 100 c.c. of Blood.		Oxygen Absorbed	
	Oxygen in Chemical Combination.	Oxygen in Solution in Plasma.	Percentage in Chemical Combination.	In Solution in 100 c.c. of Plasma.
10	6·0	0·020	30·0	0·030
20	12·9	0·041	64·7	0·061
30	16·3	0·061	81·6	0·091
40	18·1	0·081	90·4	0·121
50	19·1	0·101	95·4	0·152
60	19·5	0·121	97·6	0·182
70	19·8	0·141	98·8	0·212
80	19·9	0·162	99·5	0·243
90	19·95	0·182	99·8	0·273
150	20·0	0·303	100	0·455

The above figures show clearly the difference in the amounts of oxygen in simple solution and in chemical combination under varying pressures of oxygen; thus a rise of pressure from 70 to 150 mm. increases the former by over 100 per cent., the latter

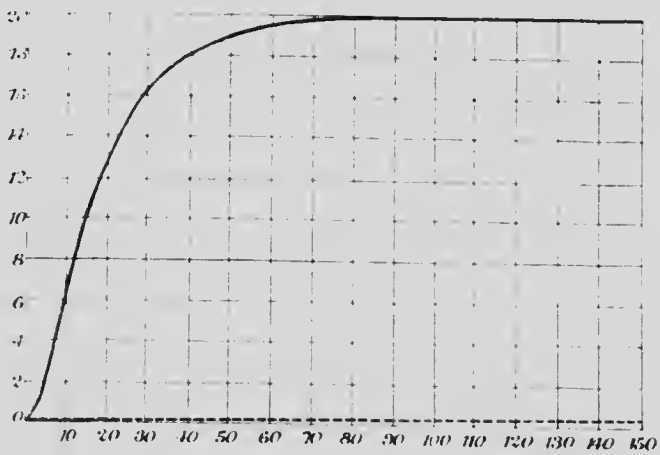


FIG. 29. Curve of the Partial Pressure of Oxygen in Horse's Blood at 38 (*Bohr*).

by less than 2 per cent.; in other words, chemical combination is by far the most effective method of absorption at low pressures.

The preceding figure is a graphic representation of the above results; the abscissa stands for the pressure and the ordinates for the quantities of gas absorbed: the curve represents the gas in chemical combination, the dotted line the oxygen in simple solution in the plasma.

Comparative determinations of the oxygen capacity of bloo have been made by Haldane with the ferricyanide method and th blood-pump; the results for the two methods are practically th same, as shown by the following table.

	Volumes of Oxygen per 100 Volumes of Blood.	
	By Blood-pump.	By Ferricyanide Method.
Defibrinated blood of ox .	24·38	24·43 24·35
Oxalated blood of ox . .	20·36	20·47 20·57
Oxalated blood of ox . .	22·40	22·20 22·33
Average . .	22·38	22·39

There is very little doubt that, apart from the small quantity of oxygen in simple solution, the whole of the oxygen which can be extracted from blood is in combination with the hæmoglobin. It is, therefore, of interest to determine whether the oxygen capacity of the blood varies with the colouring power. Haldane and Lorrain Smith ([1]) have produced satisfactory evidence that this is the case for different samples of blood taken not only from the same individual, but also from different animals. The oxygen capacity of any blood can thus be determined by comparing it colorimetrically with a sample of blood, the oxygen capacity of which has been determined by the ferricyanide method or the gas-pump. In this way Haldane has been able to give an exact value to clinical estimations of hæmoglobin; a 1 per cent. solution of ox or sheep's blood of 18·5 per cent. oxygen capacity is used as a standard solution for the hæmoglobinometer. The following (see p. 531) are the results of some of his observations upon the blood of healthy men, women, and children.

The average oxygen capacity of the blood in healthy adult men was 18·5, in women 16·5, and in children 16·1 per cent.

It is necessary to know not only the percentage but also the total oxygen capacity of the blood. Haldane and Lorrain Smith determined these in their research on the mass and oxygen capacity of the blood in man. The mass of the blood in man

Age.	Sex.	Oxygen Capacity per litre c. of Blood.	Percentage by Gowers-Haldane Haemoglobinometer Scale.
Years.		c.c.	
22	Male	189	102
19	,,	185	100
31	,,	204	110
22	,,	170	92
62	,,	185	100
22	Female	167	90
35	,,	164	89
38	,,	150	81
72	,,	165	89
8½	Male	168	91
5½	,,	157	85
12	,,	157	85
4½	Female	156	84·5
7	,,	170	92
12	,,	163	88

is about 1·9 per cent. ($\frac{1}{20.5}$) of the body-weight and varied in the fourteen persons examined between 3·34 per cent. ($\frac{1}{30}$) and 6·27 per cent. ($\frac{1}{16}$). The total oxygen capacity of the blood in litres is about 0·85 per cent. of the body-weight in kilograms, and varied between 0·57 per cent. and 0·95 per cent. The total oxygen capacity is, relatively to the body-weight, more constant than the mass of the blood.

Sufficient data have now been given to show that the oxygen capacity of the blood depends upon the colouring matter of the red corpuscles, but it remains to discuss why solutions of crystallised haemoglobin do not behave in the same manner as blood, why they have different oxygen capacities. There seems to be little doubt that the pigment of the red corpuscles is not the same as crystallised haemoglobin; the corpuscles contain about 40 per cent. of haemoglobin, but it is impossible to make such a strong solution with crystals of haemoglobin. Bohr maintains that there are at least four different kinds of haemoglobin, which combine with different amounts of oxygen and have different "specific oxygen capacities," by which term he designates the ratio between the number of grm. of iron and the number of cubic centimetres of oxygen present in a given volume of blood, blood-corpuscles, or solutions of haemoglobin saturated with air at ordinary pressure and temperature. The red corpuscles are said

to contain a mixture of different hæmoglobins, which vary not on in different animals, but even in the arterial and venous blood the same individual.

There remains considerable doubt about the validity of thes views. Hüfner maintains that in ox-blood there is only o kind of hæmoglobin, 1 grm. of which absorbs about 1·34 c.c. carbon monoxide or oxygen measured at 0° and 760 mm. Th contention is supported by the following considerations. Th hæmoglobin of ox-blood contains 0·336 per cent. of iron, and i molecular weight, on the assumption that one molecule contai one atom of iron, would be 16,669; its capacity to combine wit oxygen appears to depend upon the iron, one atom of which hold two atoms of oxygen, and calculated from the percentage of iro this value should be 1·34 c.c. of oxygen absorbed by 1 grm of hæmoglobin. Direct experiments by Hüfner gave an averag of 1·26 c.c. of carbon monoxide per grm. of hæmoglobin, figure which became 1·34 c.c. when it was corrected for incom plete saturation and for the coefficient of absorption of carbo monoxide. This figure has been generally accepted by physio logists, but recently Haldane has raised doubts about its correct ness; he points out that the results of Hüfner's individua experiments vary by as much as 10 per cent. from one another and the corrections applied are hypothetical. Further experi ments must decide the question. The general percentage of iron in the blood of different warm-blooded animals is 0·335 per cent Further, the amount of hæmoglobin in human blood is abou 14 per cent., and if 1 grm. can absorb 1·34 c.c. of oxygen, it follows that the amount of oxygen combined in arterial blood should b about 19 volumes per cent., a figure which agrees well with the results of actual experiments.

The foregoing remarks show that it is possible to give another interpretation to the question of "specific oxygen capacities." They may depend not upon different kinds of hæmoglobin, but upon mixtures of pure and partly decomposed hæmoglobin, for it has been shown by various observers that the use of alcohol in the preparation of the crystals will affect their absorptive power for oxygen; it may be that methæmoglobin, hæmatin, and hæmochromogen are present as impurities.

The Carbon Dioxide in Blood.—The conditions under which carbon dioxide is present in blood are even more complex than

those of oxygen, for it is present in simple solution and in different forms of chemical combination in both the plasma and corpuscles, and is not combined with only one definite substance, as the oxygen is with hæmoglobin. Data have already been given to show that the average amounts of carbon dioxide which can be extracted from arterial and venous blood are 43 and 50 volumes per cent. Of these amounts the larger quantity can be extracted from the plasma, and the distribution may be taken as two-thirds in the plasma and one-third in the corpuscles.

The quantity of carbon dioxide in simple solution is about 2 volumes per cent., or 5 per cent. of the total carbon dioxide. The greater part of the gas in the plasma or serum is in loose chemical combination and can be extracted by the blood-pump; the smaller part is in firm chemical combination, and can only be set free in the pump by the addition of a weak acid. In this respect an important contrast is observed between blood and serum; all the carbon dioxide can be extracted from the former by exposure to the vacuum alone, the hæmoglobin of the red corpuscles acting apparently as an acid.

The carbon dioxide absorbed by the blood in simple solution follows Henry's law, but the quantity held in chemical combination shows no such simple relation to the pressure. The following data from experiments by Jaquet and Bohr will explain this relationship.

Absorption of Carbon Dioxide by Blood at 38°.

According to Jaquet.		According to Bohr.	
Pressure in mm.	Carbon Dioxide in 100 c.c. Blood of Ox.	Pressure in mm.	Carbon Dioxide in 100 c.c. Blood of Dog.
19·4	31·7	0·6	7·1
35·0	45·7	2·3	13·7
48·2	49·2	5·1	19·5
57·7	50·1	8·2	21·7
72·1	52·1	10·6	27·0
125·1	57·7	28·3	38·1
		54·3	46·7
		82·0	55·7

The next curve (Fig. 30) has been constructed by Bohr from the data just given.

It will be necessary to consider this question of the pressure of carbon dioxide in the blood in a later chapter, when the cause of the gaseous exchange are discussed: the nature of the chemical combinations into which carbon dioxide enters must now be con-

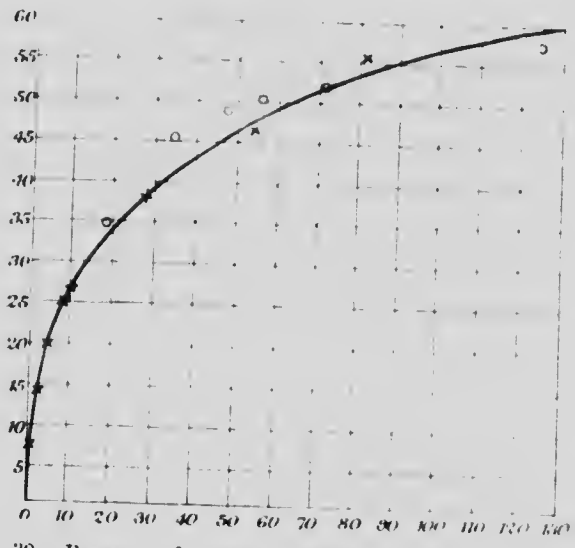


FIG. 30.—Pressure of Carbon Dioxide in Blood at 38° (*Bohr*). Jaquet's data are marked by o, Bohr's data by x.

sidered, and in the first place those found in the plasma. For many years it was held by physiologists that one of the essential factors in the combination of carbon dioxide was disodium hydrogen phosphate, Na_2HPO_4, which can combine with carbon dioxide and form sodium bicarbonate and dihydrogen sodium phosphate.

$$Na_2HPO_4 + CO_2 + H_2O \quad NaHCO_3 + NaH_2PO_4.$$

Experiments, however, have shown that the quantity of phosphoric acid in the ash of serum is so small that, if allowance be made for that derived from lecithin and nucleo-albumins, the amount is quite insufficient to play any important part in the absorption of carbon dioxide. There are, however, present in blood greater amounts of other alkalies. Thus, according to Bunge, the ash of 1000 grm. of dog's serum contains 4·341 grm. of sodium, of which 3·463 grm. are sufficient to combine with the chlorine; the remaining 0·878 grm. of sodium could combine with 0·623 grm. of carbon dioxide (316 c.c. at 0° and 760 mm.)

to form sodium carbonate, or with double that amount to form sodium bicarbonate. This determination must be considered as only approximate, for it will be shown later that the amount of sodium carbonate in serum cannot be accurately determined, owing to the combination of some of the alkali with proteids. An examination of the dissociation of a solution of sodium bicarbonate of the same strength as that of serum (0·1 to 0·2 per cent.) shows that it behaves quite differently to serum. Thus Bohr found that at a temperature of 37° and a pressure of only 0·2 mm. three-fifths of the total gas capable of dissociation were firmly combined; the solution, moreover, is completely saturated at a very low pressure of carbon dioxide, whereas serum, in Jaquet's experiments, contained only 4·5 volumes per cent. of that gas in chemical combination at a pressure of 14·8 mm. of carbon dioxide. It is, therefore, maintained by Bohr that sodium carbonate and sodium bicarbonate play a very small part in the direct gaseous exchange.

Evidence has accumulated to show that the carriers of carbon dioxide must be sought in the proteids of the plasma and corpuscles. The globulins can act as weak acids and form combinations with the alkalies in blood, but from these combinations they are displaced when the pressure of carbon dioxide is high. Loewy and Zuntz have shown that the quantity of a diffusible alkali in serum can be increased by the passage of a stream of carbon dioxide; carbonates are thus formed at the expense of the indiffusible combinations of proteids with alkali. The combinations will vary according to the mass influence of the proteids of the blood and the pressure of carbon dioxide; when the latter is high the globulin will be deprived of its alkali, but will regain it when the pressure of carbon dioxide falls. In this way the alkalies of the blood will be brought into play by the acid properties of the serum albumin and serum globulin of the blood.

It is now necessary to inquire whether the proteids of the blood-corpuscles may not also play a part in the absorption of carbon dioxide. The red corpuscles contain about one-third of the total carbon dioxide in the blood, thus the corpuscles of 100 c.c. of arterial blood contain about 13 c.c. of that gas. Less than 1 c.c. of this amount is in simple solution at the temperature of the body. The hæmoglobin acts as an acid, and in the vacuum of a gas-pump will drive carbon dioxide out of carbonates; it

is present in the red corpuscles in combination with alkali and yields up some of this alkali, if the pressure of carbon dioxide be high; in this respect it resembles the proteids of the plasma.

Bohr finds that hæmoglobin can also absorb carbon dioxide; the combination is with the globulin portion, and thus is not affected by the simultaneous combination of oxygen with the iron portion of the molecule. The amount of carbon dioxide so absorbed at a temperature of 38° and a pressure of 30 mm. is about 8 c.c. for the 14 or 15 grm. of hæmoglobin which are contained in 100 c.c. of blood. The remainder, apart from the small quantity in solution, is probably combined with alkali as bicarbonate.

These results must not be considered as rigidly representing the condition in the blood circulating through the body, for the blood acts as a whole, and according to the experiments of Zuntz and Hamburger there is a constant exchange of salts between the plasma and corpuscles, owing especially to the variations which are occurring in the pressure of carbon dioxide. It is probable that during coagulation the changes in the proteids of the blood may alter the gaseous contents of the corpuscles and of the fluid, which is no longer plasma but serum.

The Nitrogen and Argon in Blood.—The nitrogen in the blood is about 1·2 volumes per cent., is chiefly in simple solution, and thus follows Henry's law. Experiments by various observers show that blood contains about 0·6 volumes per cent. more nitrogen than water under similar conditions of temperature and pressure, notwithstanding the fact that its coefficient of absorption is smaller. This excess, according to Bohr, is due to some unknown combination, which is formed in the presence of hæmoglobin and oxygen; it does not depend upon the integrity of the red corpuscles, for a similar excess is present in solutions of hæmoglobin crystals.

As regards **Argon**, it is said that the venous blood received directly from the living body contains 0·042 volumes per cent. of this gas, a larger quantity than could be present in simple solution. Blood which has been exposed to the air, and red corpuscles in physiological saline, do not show any such excess.

Further experiments upon these questions are needed. According to the balance of evidence at the present time, the nitrogen and argon in the normal blood are considered as inert and of no special physiological significance. It is necessary, however, to

mention that the nitrogen in solution in the blood becomes of the utmost importance in cases of caisson disease; the gas, which is set free in the blood-vessels and tissues of a man who too quickly passes from the high pressure of the caisson to the ordinary pressure of the atmosphere, is almost entirely composed of nitrogen; the gas cannot be utilised by the tissues, and thus will cause gas-embolism, and, it may be, paralysis and death. This interesting subject is fully discussed in another part of this work.[1]

Carbon Monoxide in Blood.—Traces of carbon monoxide have been found by various observers in the blood of healthy animals; this was probably due to the absorption of the gas during the breathing of the air of large cities where small quantities of carbon monoxide are present, or it might have been due to the leakage of small quantities of coal-gas into the rooms in which the animals lived. Although the gas is not to be regarded as a normal constituent of blood, it is of the greatest interest to the physiologist and the physician; its property of displacing oxygen and forming a combination with hæmoglobin throws great light upon the processes of respiration, and is the cause of its very poisonous nature. Carbon monoxide is formed by the incomplete combustion of coal-dust, and is thus responsible for numerous deaths among miners after an explosion in a mine; (b) it is present as an impurity in coal-gas, and in this form is the cause of many deaths, some accidental, others suicidal.

Carbon monoxide combines with blood in the same proportion as oxygen, but the compound, carboxy-hæmoglobin, cannot be decomposed by ordinary reducing agents. The gas can be extracted by the vacuum of a gas-pump, or by the passage of a stream of oxygen or an indifferent gas, for the combination depends upon the partial pressure of the gas. It is attached to the iron portion of the molecule of hæmoglobin, and thus is explained its poisonous property, for it displaces an equal volume of oxygen and causes death from lack of oxygen; the red corpuscles are put out of action as oxygen-carriers. Haldane (c) has shown that the poisonous action of carbon monoxide diminishes as the pressure of oxygen increases, and is abolished in the case of mice, when the pressure of oxygen is raised to two atmospheres; the mice can then dispense with the oxygen-carrying function of the haemo-

[1] See article by Leonard Hill, p. 233.

globin, for they obtain the oxygen they need from the gas which is in solution in the plasma.

The amount of carbon monoxide absorbed by the blood depends upon the relative affinities of oxygen and carbon monoxide for haemoglobin and the relative pressures of the two gases in the arterial blood. The affinity of the gas is about 140 times that of oxygen. When an animal or man no longer inhales the gas, it slowly disappears from the blood. This is due not to oxidation, but to dissociation of the carboxy-hæmoglobin by the mass influence of the oxygen in the pulmonary capillaries.

It will be necessary later to consider the relation of pressure to the absorption of carbon monoxide by the blood, for the principle of Haldane's method for the determination of the pressure of oxygen in the arterial blood of man or animal is the calculation of the pressure of oxygen from the pressure of carbon monoxide in samples of the blood and the final saturation of the hæmoglobin with carbon monoxide. The subject of the experiment breathes a known very small percentage of carbon monoxide until the percentage saturation of the hæmoglobin with the gas is found to be constant. Here it need only be stated that there is considerable want of agreement between the results obtained by Hüfner, Haldane and Lorrain Smith, and Bock for the relation between the pressure of the gas and its absorption by the blood.

From the resemblances between the combinations formed with hæmoglobin by carbon monoxide and oxygen, it would be expected that differences should be found in the absorption of carbon monoxide by blood and by solutions of hæmoglobin, and there should be different forms of hæmoglobin with specific carbon monoxide capacities resembling those for oxygen. Such results have been obtained by Bohr and others.

The Alveolar Air of the Lungs.—In the preceding pages the gaseous composition of the mixed venous blood in the right ventricle and the arterial blood have been compared in order to show the effect of the circulation of the blood through the lungs, where separated only by thin walls the blood flowing through the extensive capillary network is exposed to the air of the lungs. This air is not the inspired air, it is not even the air about to be expired; it is the air of the alveoli, and, owing to its more stationary character, contains less oxygen and more carbon dioxide than the expired air. The alveoli of the lungs are exceedingly

numerous, as many as 404 millions are present, according to Aeby's estimate, in the lungs of a man; their individual size varies considerably, and as an average the following dimensions may be given, 0·37, 0·22, and 0·16 mm., for the undistended alveolus. Owing to the elasticity of their walls the capacity of the alveoli can be doubled by distension with air during inspiration. Calculated from such figures, the total surface of the alveoli will be about 50 square metres at the end of a deep expiration and 130 square metres after a very deep inspiration. From other data Zuntz calculates that there are 725 millions of alveoli, with a total surface of about 90 square metres.

The capillary network contains numerous vessels placed very close together and covered by an extremely thin layer of the membranous wall of the alveoli, and the layer of the alveolar epithelium. The thickness of this double layer which separates the capillaries from the alveolar air is barely 0·001 mm. thick.

The average composition of the air in the alveoli must now be considered. From the numerous analyses of the expired air it is possible to form only a rough estimate of the alveolar air, for the expired air is mixed with some of the air from the "dead space" of the respiratory tract, which lies between the nose and the alveoli. Loewy measured the volume of casts of the respiratory passages and estimated the "dead space" at about 140 c.c.;[1] from the composition of the expired air, its probable dilution with air from this "dead space," and with 6 per cent. of aqueous vapour, for the air of the alveoli is saturated with moisture at the temperature of the body, he calculated that the alveolar air contained 12·6 to 13·5 per cent. of oxygen at the atmospheric pressure.

In an ordinary expiration the first portion of the air which leaves the nose or mouth is from the "dead space," then mixed air, and finally air from the alveoli. This is on the supposition that the lobes of the lungs contract simultaneously, and is probably correct, although some observers maintain that in some cases all portions of the lungs are not in the same phase at the same time. At the end of a normal expiration there will be a

[1] Haldane and Priestley calculated the value of the "dead space" in their own cases, and found for the mean results 189 c.c. and 142 c.c., or about 30 per cent. of the volume of a normal respiration during rest.

minimum of oxygen and a maximum of carbon dioxide in the alveolar air; the converse will be the case at the end of a normal inspiration. Upon these principles Haldane has based a simple but efficient method of collecting samples of the alveolar air of man, and in conjunction with Priestley has carried out an important series of experiments upon the carbon dioxide in the alveolar air under different conditions and its relation to the regulation of breathing.

The following figure (Fig. 31) shows the construction of the apparatus used for obtaining the alveolar air. The subject of the experiment expires at the end of a normal inspiration quickly and deeply through the mouthpiece and closes it with his tongue,

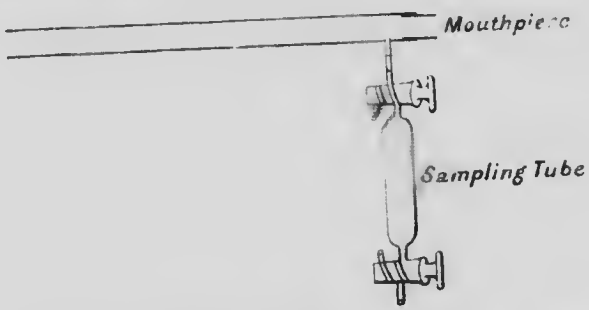


FIG. 31. Apparatus for Collection of a Sample of Alveolar Air (*Haldane and Priestley*).

The tube is a piece of stiff rubber tube about 4 feet long and about 1 inch diameter. The sampling tube is completely exhausted by a gas-pump before the experiment.

until a sample of the air has been taken by the opening and closing of the previously exhausted sampling tube. A second sample is taken, when the subject expires deeply at the end of a normal expiration. The mean of the analyses of the two samples represents the average composition of the alveolar air.

Haldane and Priestley[21] found that during rest and under normal atmospheric pressure the percentage of carbon dioxide in the alveolar air is almost constant for the same individual; in their own cases the average percentages were 5·62 and 6·28, the maximal variations being 5·40 and 5·87 in the former and 5·985 and 6·845 in the latter. Within the limits of atmospheric pressure, 646-1260 mm., which were investigated, the percentage of carbon

dioxide in the alveolar air varied inversely as the atmospheric pressure, so that the pressure of the alveolar carbon dioxide was constant. The pressure of oxygen, on the other hand, showed great variations. The following table gives the mean results obtained for the alveolar air at different pressures: the percentages of oxygen were calculated on the assumption that the respiratory quotient was 0·85.

	Barometric Pressure in mm. of Hg.	Percentage of Carbon Dioxide in Dry Alveolar Air.	Percentage of Oxygen in Dry Alveolar Air.	Pressure of Carbon Dioxide in Alveolar Air (moist) in Percentages of One Atmosphere.	Pressure of Oxygen in Alveolar Air (moist) in Percentages of One Atmosphere.
Top of Ben Nevis	446·5	6·615	13·19	5·23	10·41
Oxford	755	5·95	13·97	5·53	13·06
Bottom of Padcoathe Mine	832	5·29	14·74	5·48	15·25
Brompton Hospital compressed air-chamber	1260	3·52	16·79	5·61	26·84

During muscular work there is a rise of about 0·285 per cent. in the alveolar carbon dioxide: this will be discussed in connection with the question how the respiratory movements are regulated in depth and frequency.

The limits within which the pressure of carbon dioxide varies in the alveolar air of different individuals have been investigated by FitzGerald and Haldane ([22]). The following table (see p. 542) shows the mean and the range of the pressures of carbon dioxide.

Direct determinations of the air of an occluded portion of the lungs were made on animals by Pflüger and his pupils. For the collection of this air a special form of catheter, the lung-catheter, was used. It consists of a fine elastic catheter, surrounded except at its extremities, by a tube with a rubber enlargement towards the free end of the catheter. It can be introduced through the trachea into a bronchus of a dog without preventing the free passage of air in and out of the other portions of the lungs. When the catheter is in position, the rubber enlargement is inflated, and thus shuts off a portion of the lungs from which the alveolar air can be withdrawn

		Pressure of Carbon Dioxide in Alveolar Air Saturated at 37°.		Pressure of Carbon Dioxide in Dry Alveolar Air.
		In mm. of Hg.	In per Cent. of One Atmosphere.	
Men	Maximum	44·5	5·86	6·25
	Minimum	32·6	1·29	4·58
	Mean	39·2	5·16	5·54
Women	Maximum	41·0	5·40	5·76
	Minimum	30·4	3·99	4·26
	Mean	36·3	4·78	5·10
Boys	Maximum	42·4	5·55	5·92
	Minimum	30·6	4·03	4·30
	Mean	37·2	4·89	5·21
Girls	Maximum	40·4	5·27	5·62
	Minimum	31·2	4·10	4·37
	Mean	35·2	4·63	4·94

through the elastic catheter. The results obtained in this way by Wolffberg and Nussbaum show that such samples of alveolar air in dogs contain about 3·5 per cent. of carbon dioxide and 15-16 per cent. of oxygen at atmospheric pressure.

Loewy and Schrötter have adapted this instrument for use with the bronchoscope upon man.

The samples of air obtained with the lung-catheter are not true samples of alveolar air, but of the air in an occluded portion of the lungs. If the process of exchange between the blood and the air be a question of pressure only, the diffusion will proceed until the pressures of the gases in the alveoli of the occluded portion of the lung become equal to the mean pressures of the corresponding gases in the blood which flows through the capillaries in the walls of the alveoli. Thus there is considerable value to be attached to such samples, for, on the theory that the gaseous exchange in the lungs is a process of diffusion, the pressure of their constituent gases should be a measure of the respective gases in the venous blood. It is uncertain, however whether the gaseous exchange is of such a simple nature; according to Bohr, Haldane and Lorrain Smith the pressure of oxygen in the arterial blood is higher than the pressure of that gas in the alveolar air.

The Causes of the Gaseous Exchange between the Blood and the Alveolar Air.—It is impossible to give a satisfactory account of

the causes of the gaseous exchange between the blood and the alveolar air, for the experimental results obtained by different observers are at variance. In a case of such difficulty and importance it is best to state the arguments on each side, sum up the evidence, and indicate as far as possible what verdict should be given upon the balance of evidence. The verdict may or may not be true, for the evidence appeals differently to each observer, and it is difficult to separate from the consideration of this subject the personal attitude of each man towards the Materialistic and Vitalistic Schools of Physiology. It may be that a verdict of "not proven" will be more in agreement with the nature of the evidence.

The two cases, stated briefly, are as follows: the gaseous exchange between the blood and the alveolar air is due to the relative partial pressures of the gases in the blood and alveolar air, and can be explained according to physical and chemical laws; the gaseous exchange takes place in opposition to the known physical and chemical laws, is of a special nature, a vital process akin to the secretion and excretion of glands.

The solution of the question depends upon a comparison of the partial pressures of the gases in the arterial and venous blood with those of the corresponding gases in the alveolar air. It has already been shown how samples of alveolar air may be obtained under ordinary atmospheric pressure, and yield on analysis the partial pressures of their constituent gases: the methods used for the determination of the pressures of the gases in the blood have not yet been described. For the determination of these pressures Pflüger introduced an instrument known as the aerotonometer, the principle of which is as follows: blood in contact with a mixture of oxygen, carbon dioxide, and nitrogen absorbs or gives up gas, according as the pressures of its respective gases are less or greater than those of the corresponding gases in the mixture. If sufficient time be allowed, this interchange will result in equilibrium; the partial pressures of the gases in and outside the liquid will be the same, and thus from an analysis of the mixture at known pressure it is possible to determine the partial pressures of the gases in the blood.

In the aerotonometer the blood passes in a very shallow stream through a glass tube which contains a mixture of gases of known quantities and pressure, and is surrounded by a water-jacket with

a temperature of 39°. In different experiments it is arranged that the pressures of the gases in the tube shall in one case be greater, in another case less than the anticipated pressures of the corresponding gases in the blood. The gases in the tubes after the blood has passed through them are analysed, and from the alteration in the proportions the partial pressures of the gases in the blood are calculated.

With this instrument Pflüger's pupils, Strassburg, Wolffberg, and Nussbaum, made a series of experiments upon dogs, and found the pressure of carbon dioxide in the arterial blood to be 2·2 to 3·8 per cent., with a mean of 2·8 per cent. of an atmosphere, and that of the venous blood from the right side of the heart 3·81 to 5·4 per cent. Simultaneous determinations of the pressures of the gases in the air removed by the lung-catheter were not made in each case, but those results, in which that condition was fulfilled, have been tabulated by Bohr, and are here reproduced.

Wolffberg's Results. Pressure of Carbon Dioxide in Percentage of an Atmosphere.		Nussbaum's Results. Pressure of Carbon Dioxide in Percentage of an Atmosphere.	
Air of Occluded Portion of Lung.	Venous Blood from Right Side of Heart.	Air of Occluded Portion of Lung.	Venous Blood from Right Side of Heart.
2·5	4·1	4·2	4·1
3·6	2·4	4·8	3·9
4·6	4·9	2·8	3·3
		4·4	4·3

The general result ist hat the pressure of carbon dioxide in the venous blood is about the same as that in the sample of air obtained with the lung-catheter, and this could be explained by diffusion alone. In two cases, however, the pressure in the alveolar air was 1 per cent. higher than in the venous blood. The experiments upon the pressure of oxygen were unsatisfactory. Herter, who also made determinations with the aerotonometer, found low values for the pressure of oxygen in arterial blood, the highest value being 10·44 per cent. of an atmosphere.

One of the chief criticisms of these results is directed against the method of obtaining a sample of the alveolar air from the portion of the lung blocked up by the balloon of the lung-catheter.

It has already been shown that it is not true alveolar air. In pathological cases of collapse of the lung, and in experiments upon the production of artificial collapse, all the air in the portion of the lung involved is absorbed in a few hours; the lung-catheter may also produce conditions too abnormal for experiments to decide the question.

Bohr constructed an improved aerotonometer, the hæmatærometer, through which a constant and rapid stream of arterial blood could be maintained. Dogs were used for the experiments, the coagulation of the blood was prevented by the injection of leech-extract or peptone, and the blood from an artery, after passing through the hæmatærometer, passed back to the animal's body through the peripheral end of the artery or the central end of a vein. The composition of the expired air, as it passed the bifurcation of the trachea, was determined by qualitative and quantitative analyses of the expired air and direct measurement after the death of the animal of the volume of the "dead space," which in this case was the trachea and tracheal cannula. Bohr obtained for the pressure of oxygen in arterial blood results as high as 101 to 144 mm. of mercury, and in nearly every case the pressure was higher than that of the expired air at the bifurcation of the trachea, and therefore higher still than that of the true alveolar air. The absorption of oxygen in these cases could not be explained by diffusion. For the pressure of carbon dioxide the results vary greatly from 0·9 to 29·7 mm.; in some cases, as shown in the following table, they are much below the pressure in the air at the bifurcation of the trachea, in other cases higher.

Pressure of Oxygen.		Pressure of Carbon Dioxide.		Nature of Inspired Air.
Expired Air at Bifurcation of Trachea.	Arterial Blood.	Expired Air at Bifurcation of Trachea.	Arterial Blood.	
Mm. Hg.	Mm. Hg.	Mm. Hg.	Mm. Hg.	
127	144	16·6	10·1	Atmospheric air
131	105	14·3	16·7	" "
95	101	34·6	17·4	" "
110	122	14·8	27·6	" "
116	106	40·0	29·7	4·9 CO_2, 18·8 O_2
130	144	28·5	0·9	3·2 CO_2, 20·0 O_2

From these results Bohr concludes that the absorption of oxygen and the excretion of carbon dioxide are active secretory

processes. Hüfner and Frederieq have contested the accuracy of Bohr's experiments, and suggest that the irregularities in the results are evidence of a want of equilibrium between the pressures of the gases in the blood and in the hæmataerometer. It is to be noted that the respiratory quotients in Bohr's experiments vary from 0·54 to 1·01, owing, it may be, to imperfect and irregular ventilation of the lungs. It is probable also that the leech-extract added to the blood may alter the absorption of gases, and it is certain that the operative procedure renders the animal's condition abnormal.

Frederieq has made experiments with an aerotonometer constructed upon the same principles as Pflüger's instrument; the arterial blood is prevented from coagulation by a previous injection of peptone, and after passing through the apparatus flows back through a vein to the animal's body. The results yielded a pressure of 12 to 14 per cent. for the oxygen, and 2·7 to 2·4 per cent. of an atmosphere for the carbon dioxide. When the dog breathed a mixture of about 85 per cent. of oxygen, the pressure of the oxygen in the arterial blood was raised to 60 per cent. of an atmosphere.

So great is the want of agreement and the irregularity of the results obtained by different observers with various forms of tonometer that the suspicion arises that there are sources of fallacy in the methods. Appeal must, therefore, be made to other experiments, especially to those which can be performed upon the living animal without subjecting it to any serious operation. This latter condition is fulfilled by Haldane's method, the principle of which is as follows:—"The subject of the experiment continues to breathe air containing an exactly known very small percentage of carbonic oxide until a point is reached at which the percentage saturation of his hæmoglobin with carbonic oxide becomes constant. Now the final saturation with carbonic oxide of hæmoglobin solution brought into contact with gas containing carbonic oxide and oxygen depends on the relative tensions of the carbonic oxide and oxygen in the liquid, so that if the tension of carbonic oxide, and the final saturation of the hæmoglobin be known, the oxygen tension can be inferred. Hence if in the living subject both the carbonic oxide tension in the blood leaving the lungs, and the final saturation of the hæmoglobin with carbonic oxide, are known, the oxygen tension of the blood leaving the lungs can be calculated. But the carbonic oxide tension of the blood leaving the lungs will (after absorption has ceased) be that

of the inspired air, after allowance has been made for dilution of the latter by aqueous vapour. Therefore from a knowledge of the percentage of carbonic oxide breathed by the subject of the experiment, and of the final saturation of his blood with carbonic oxide, the oxygen tension of his arterial blood may be calculated." (Haldane and Lorrain Smith.)

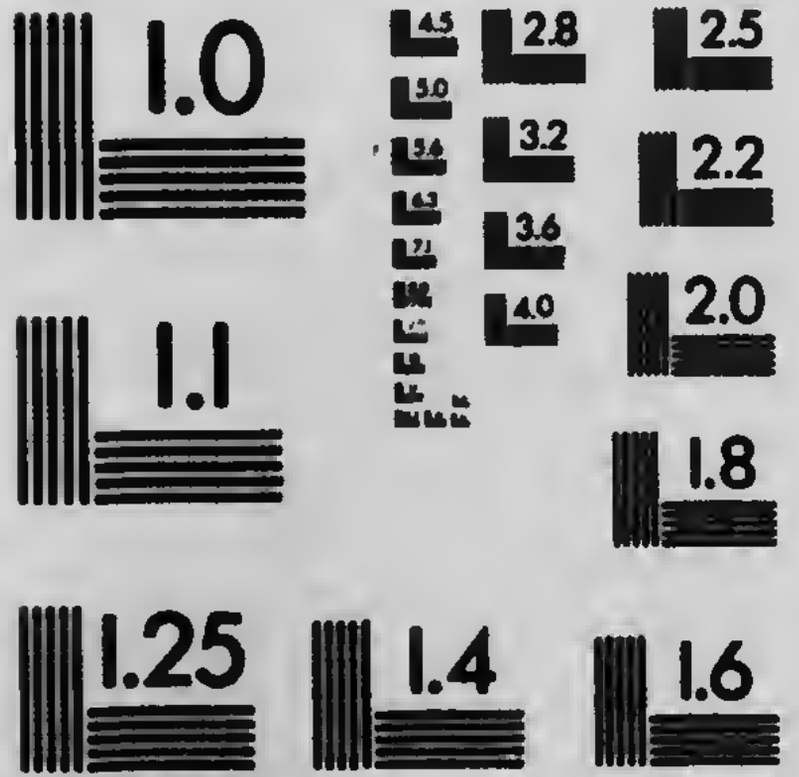
With this method numerous experiments have been made by Haldane and Lorrain Smith ([23]) upon the partial pressure of oxygen in the arterial blood of man and animals. The average results for the normal condition are shown in the following table.

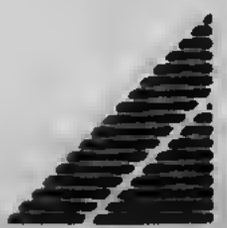
Animal.	Number of Determinations.	Pressure of Oxygen in Arterial Blood.	
		In Percentage of an Atmosphere.	In Millimetres of Mercury.
Mouse . .	20	22·6	172
Man . .	9	38·5	293
Dog . .	2	21·0	160
Cat . .	1	35·3	268
Rabbit . .	4	27·6	210
Birds . .	4	14·6	330
Frog . .	1	18·4	140

If the normal pressure of oxygen in the alveolar air of these animals be assumed to be 13 to 14 per cent. of an atmosphere, diffusion alone cannot explain the absorption of oxygen, for the pressure of the gas in the arterial blood is higher in every case. The influence of various conditions upon the absorption was also determined. A fall in the internal temperature of the body by exposure to cold reduced the pressure of oxygen in the arterial blood of a mouse from 22·6 to 15·1 per cent. of an atmosphere. An increase in the percentage of oxygen in the alveolar air, when the animal breathed mixtures rich in oxygen, caused an almost proportional increase in the pressure of oxygen in the arterial blood; on the other hand, want of oxygen, whether produced by poisoning with carbon monoxide, diminution in the percentage of oxygen in the inspired air, or diminution in atmospheric pressure, caused a fall in the pressure of the oxygen in the arterial blood, but a marked increase in the *relative* excess of the pressure of oxygen in the blood over that in the alveolar air. Thus want of oxygen acted as a stimulus to the absorption of the gas.

MICROCOPY RESOLUTION TEST CHART

(ANSI and ISO TEST CHART No. 2)





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These experiments are entirely in favour of the secretory theory, and, if there should be no sources of fallacy in the method, would decide the question as regards the absorption of oxygen in the lungs. The criticisms have been chiefly supplied by the observers themselves, and have been by them subjected to the test of experiment. It might be that the carbon monoxide was not absorbed by diffusion alone, and thus the method would be nullified; it appears, however, that the lungs exert no selective action in this case, for the absorption ceases when the relative saturation of the blood corresponds to the partial pressure of the gas inspired. Experiments by Haldane show that carbon monoxide is not oxidised in the body. The exactness of the data for the absorption of carbon monoxide under different pressures may be questioned, for the results obtained by Haldane and Lorrain Smith are exactly double the values found by Hüfner, and are also higher than those obtained by Bock. In the latter case the lack of agreement may be due to the difference in the strength of the solution of hæmoglobin, but this will not hold in the case of Hüfner's experiments, and in any case raises the question whether solutions resemble the normal blood in their power of absorption. Haldane's colorimetric method for the estimation of carboxy-hæmoglobin has been shown by Haldane and Lorrain Smith to be liable to error, for bright day-light has a most marked action in diminishing the stability of the combination. Another important point is whether an animal with 40 or 50 per cent. of its hæmoglobin saturated with carbon monoxide can be considered normal. Bohr, while he hails these results as some of the strongest evidence in favour of the secretory theory, yet maintains that the high pressures of oxygen in the arterial blood are due to an abnormal increase in the activity of the pulmonary epithelium, and the experiments of Haldane and Lorrain Smith themselves show that want of oxygen acts as a stimulus to the further absorption of oxygen. Be this as it may, the results would still show that diffusion does not explain the phenomena, and that the animals can under the conditions of the experiment secrete oxygen.

Further evidence in favour of the secretory theory can be found in the presence in the swimming-bladder of fishes of almost pure oxygen under pressures so high as to be beyond explanation by the ordinary laws of diffusion. This interesting subject is discussed by Leonard Hill in another part of this work.

According to the experiments of Krogh, the pulmonary respiration of frogs is an active secretory process, but the cutaneous respiration is due to diffusion alone; it had been previously shown by Waymouth Reid and Hambly that the transpiration of carbon dioxide through the skin of the frog occurred equally well in both directions.

From the analogy of the secretory processes in glands, it would be expected that the secretory activity of the lungs should be influenced by the nervous system. There is evidence that such is the case. Maar found that in the tortoise the gaseous exchange depended upon the tonic influence of the vagus nerves. Section of the vagus produced an increase in the absorption of oxygen by the lung of the same side, and a decrease in the case of the opposite lung; the excretion of carbon dioxide showed a similar but less marked relationship. Stimulation of the vagus produced the opposite effects. These changes did not appear to be due to vasomotor changes, whereby the volume of blood flowing through the lungs would be altered. The results of similar experiments upon warm-blooded animals are much more uncertain, for it is difficult to separate the effects produced by stimulation or section of the vagus nerves and to decide whether the changes observed in the respiratory exchange are due to changes in the activity of the lungs or alterations in the flow of blood. The operative procedure for the collection of separate samples from the right and left lung may easily introduce serious sources of error. According to the researches of Maar and Henriques, the vagus does influence directly the secretory activity of the lungs of warm-blooded animals.

The influence of the nervous system upon the secretion of oxygen by the swimming-bladder of the fish has been clearly shown, and analogy is in favour of a similar action in the lungs.

A still further piece of evidence in favour of the secretory theory is the great capacity of the pulmonary tissue to reduce alizarin-blue when, as in Ehrlich's experiments, it is injected into the living body, and air is still passing in and out of the lungs.

The body of evidence has thus been steadily increasing in favour of the secretory theory, especially as regards the absorption of oxygen. In the case of the excretion of carbon dioxide the evidence is much more imperfect, and here there is even a want of agreement among the supporters of the view that oxygen is secreted. The witnesses on the same side give conflicting evidence.

Bohr maintains that the excretion of carbon dioxide is, in part at least, an active process; Haldane and Priestley's results for the regulation of the ventilation of the lungs support, it would seem strongly, the theory of diffusion; "the respiratory centre," they state, "is exquisitely sensitive to any rise in the alveolar CO_2 pressure; a rise of 0·2 per cent. of an atmosphere in the alveolar CO_2 pressure being, for instance, sufficient to double the amount of alveolar ventilation during rest." Why, it may be asked, should the excretory activity of the lungs not be effective against this 0·2 per cent. of carbon dioxide; why should the animal respond by increased muscular work to double the alveolar ventilation if it were not to reduce by diffusion the pressure of carbon dioxide?

On the other side, research and the collection of evidence have not been wanting. Loewy and Zuntz ([21]) have recently published a long series of experiments upon the respiratory exchange of man at high altitudes and the conditions of the absorption of oxygen by the blood, and the diffusion of gases through the lung of the frog. An account of some of these experiments has been given in other parts of this work, and it is only necessary here to state their conclusion:—the conditions for the diffusion of oxygen from the alveolar air into the blood and from the blood into the tissues are so favourable that a sufficiency of oxygen is maintained thereby even in the most rarefied atmospheres which can be tolerated by man.

Although so much space has been given to this question, justice has not been done to its fundamental importance. Further experiments, further discussions are needed, for the only conclusion which appears to be justified by the conflicting evidence is that there is truth on both sides, that the gaseous exchange in the lungs is due both to diffusion and secretion. Such a verdict may be unsatisfactory to those who hold extreme views on either side, by both it may be considered as a confession of weakness, but it is consonant with the evidence and with the wider views of those who do not limit vitalism to the phenomena of living things alone.

Internal Respiration.—It has already been mentioned that the real seat of respiration is the cell. This is proved by the condition in the simplest forms of life, in which the single cell is the organism, and even the most complex organisms, man included,

are unicellular in the earliest stages of their development. The amœba breathes, but possesses, as far as the structure can be determined by the microscope, no part especially differentiated for respiration; gaseous exchange appears to be a fundamental property of the protoplasm, and appears to take place directly or by the aid of ferments; oxygen is absorbed and carbon dioxide is discharged at the surface. The fertilised ovum, which will ultimately develop into a man, appears to breathe in a similar manner. The living cell possesses a great affinity for oxygen, and absorbs it from the surrounding fluid which contains oxygen in solution; by its chemical activity it produces carbon dioxide, which passes into the surrounding fluid, in which the pressure of the gas is less. Such appears to be the process in the unicellular organism. Does this obtain in the more complex organisms, or do they acquire with the differentiation of structure special methods of respiration? Are the blood and lymph to be considered as simple respiratory media, or are they the special seats of the oxidation of the waste products of the tissues? Are the lungs only mechanisms for the ventilation of the internal medium, the blood, by exposure to the influence of the external medium, the inspired air, or do they by some special form of activity oxidise waste products carried to them by the blood? These are questions which need careful consideration. In the first place, the question of oxidation in the blood demands attention, for Lavoisier and many of the earliest investigators of the chemistry of respiration held that the oxygen breathed into the lungs combined with the carbon contained in the venous blood of the lungs to form the carbon dioxide which was expired. This view was supported for a time by the failures of many observers to extract any gas from arterial or venous blood, results which have already been shown to be due to defective methods. Blood removed from the body and kept from contact with the air becomes darker in colour, poorer in oxygen, and richer in carbon dioxide. These changes occur at the ordinary temperature of the air, but are delayed by cold and quickened by a temperature equal to that of the body; the oxidation of waste products removed from the tissues might, as some thought, be the explanation, but more modern work points to other causes. Blood is a tissue, and as such is the seat of oxidation; plasma and serum do not show the same oxidation. Putrefaction is the most important cause; it quickly occurs in

the shed blood, and the bacteria rapidly consume oxygen and produce carbon dioxide.

Experiments show that the living cells of the tissues, not the blood, are the seats of oxidation. A frog will live a day or two in an atmosphere of oxygen, after its blood has been washed out and replaced by normal saline solution; it absorbs oxygen which is in solution in the fluid and produces carbon dioxide. The respiratory exchange of rabbits deprived by bleeding of one-half of their hæmoglobin is equal to that of the same animals before the loss of blood ([25]). A mouse can live in two atmospheres of oxygen when the oxygen-carrying powers of its red corpuscles have been thrown out of action by carbon monoxide; it obtains the oxygen which it needs from the gas which is in solution in the plasma.

Tissues removed from a recently killed animal survive for a time, take up oxygen and discharge carbon dioxide. This gaseous exchange varies in different tissues, is increased by heat and diminished by cold; of all tissues muscle appears to be the most active seat of such respiration. The criticism applied to these experiments is that putrefactive changes occur and vitiate the results. This no doubt holds for many of the earlier experiments, but more recent work by Tissot and Fletcher ([26]) shows that it is possible to eliminate the influence of bacteria and to investigate that portion of the respiratory exchange which is to be considered as the survival respiration of the tissues. The latter observer has made numerous experiments upon the conditions which influence this survival respiration in frog's muscle. The discharge of carbon dioxide from an excised muscle is increased during contraction, if there be present an abundant supply of oxygen, the increase being roughly proportional to the number and strength of contractions. If, however, the muscle be made to contract in air or in nitrogen, the additional yield of carbon dioxide is incomplete or absent. Oxygen possesses great power of delaying the progressive loss of irritability, which is seen in an excised muscle, and postpones almost indefinitely the onset of *rigor mortis*.

The power of tissues to act as reducing agents is well known. Ehrlich found that alizarin-blue was decolourised by the living tissues, but regained its colour when the tissues were exposed to air. Tissues placed in a solution of oxy-hæmoglobin in normal saline quickly reduce it, but show different rates of action; thus Bernstein found muscle to be the most effective, and, if its value

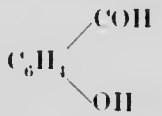
be expressed as 100, the relative powers of other tissues would be, liver 81·47, involuntary muscle 72·4, and mucous membrane of the stomach 57·05. The lungs had a very feeble power of reduction, and in this respect Bernstein's results show an interesting difference from those of Ehrlich.[1] If a frog's heart be supplied with solutions of fresh blood, the reduction of the oxy-haemoglobin can be determined with the spectroscope; working with this method, Yeo found that the heart during contraction reduces the solution about ten times as quickly as when it is at rest.

There is, however, one criticism which is effective against all such experiments upon surviving tissues; there is no circulation of blood to carry oxygen to, and remove carbon dioxide from, the cells which lie in the deeper parts of the organ; diffusion will only occur readily at the surfaces of exposure. This abnormal condition, especially the lack of oxygen, will result in disordered metabolism This difficulty is to a certain extent overcome by the method introduced by Ludwig; an artificial circulation of blood is maintained, and the gaseous or other changes in the blood are determined. The results so obtained, however interesting they may be, cannot be considered normal, for the organ has been removed from the control of the central nervous system and from the influence of that interaction of organs which is so well demonstrated by the "internal secretions": the organ may be said to be in a living condition, or with more truth, perhaps, in a living death; but the chemical changes exhibited cannot be considered to represent truly the condition in the intact organ within the body of a living animal. The blood used for the artificial circulation must be deprived of its power of coagulation, and many of the means employed for this purpose are known to alter the nature of the blood as a respiratory medium. The respiratory exchange of an excised mass of muscles, such as those of a limb, rises and falls with the external temperature; a similar group of muscles in an intact mammal would be influenced by the nervous regulation of temperature, when the animal was exposed to changes of temperature, and would show respiratory changes in an opposite direction.

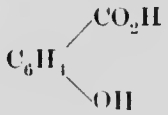
Notwithstanding these limitations, which must be borne in mind, the method of perfusion has yielded important results; it has been shown thereby that tissues have the power of taking up

[1] See page 549.

oxygen and oxidising various substances. Schmiedeberg found that blood alone produces no appreciable oxidation of benzyl alcohol ($H_6C_5CH_2OH$) and aldehyde of salicylic acid



but if the blood containing one or other of these substances is made to circulate through a freshly excised kidney, considerable quantities of benzoic acid ($C_6H_5CO_2H$), or of salicylic acid



as the case may be, are produced.

A better method than Ludwig's is the examination of samples of the arterial blood entering and of the venous blood leaving an organ in the living animal; here the conditions approach more nearly the normal, and the respiratory exchange of the organ can be investigated under different general or local conditions. In this way the effect of the contraction of a group of muscles upon the gaseous contents of the blood which flows through them has been investigated by various observers: the percentage of oxygen in the venous blood is much diminished and the carbon dioxide is increased, notwithstanding the increase in the volume and velocity of the blood which circulates through the active muscles. The following table gives the results of such experiments made by Sczelkow, Chauveau and Kaufmann, Hill and Nabarro.[27]

Gas.	Difference between the Venous and Arterial Blood Volumes per Cent.						Observer.
	Rest.		Activity.				
Carbon Dioxide	+6·71		+10·79	× 3 =	+32·37		Sczelkow.
Oxygen . .	−9		−12·26		−36·78		
Carbon Dioxide	+8·7		+10·20	× 3 =	+30·60		Chauveau and Kaufmann.
Oxygen . .	−11·4		−13·65		−40·95		
	Tonic.		Clonic.				
Carbon Dioxide	8·76	+13·90	× 3	+41·70	+19·33	× 3 = +57·99	Hill and Nabarro.
Oxygen . .	−12·92	−13·75		−41·25	−12·63	= −37·89	

The differences in the gaseous exchange are multiplied by three in order that allowance may be made for the increase in the rate of the flow of blood through the active muscles.

Hill and Nabarro also investigated the respiratory exchange of the brain by a comparison of samples of blood taken from the carotid artery and the torcula Herophili. The following table gives the average differences between the gases of the blood in the deep femoral vein and the carotid artery, and between those in the torcular Herophili and the carotid artery, of dogs in the condition of morphia anæsthesia, and during the tonic and clonic stages of epileptic fits evoked by the intravenous injection of essential oil of absinthe.

		Morphia Anæsthesia.	Tonic Stage of Fit.	Clonic Stage of Fit.
Carbon dioxide	Torcula . .	+3·87	+4·06	+2·39
	Femoral vein .	+8·76	+13·90	+19·33
Oxygen .	Torcula . .	−3·42	−4·95	−1·31
	Femoral vein .	−12·92	−13·75	−12·63

The figures show that the respiratory exchange of the brain is very low, and is but little increased during the stages of an epileptic fit. The contrast with the muscles is most marked.

The respiratory exchange of glands may be illustrated by the experiments made by Barcroft ([28]) upon the submaxillary gland; he compared the quantities of oxygen and carbon dioxide in samples of venous blood from the resting gland, venous blood from the active gland, and arterial blood from the femoral artery. The quantity of oxygen taken up from the blood is about 0·25 c.c. per minute in the condition of rest, and 0·86 c.c. per minute when the activity of the gland has been evoked by stimulation of the chorda tympani nerve; the corresponding values for the discharge of carbon dioxide are 0·27 c.c. and 0·97 c.c. After an injection of sufficient atropin to paralyse the secretory fibres of the chorda tympani, the intake of oxygen by the gland is not increased by stimulation of the nerve; the output of carbon dioxide, on the other hand, is greatly increased, at least for a time.

Barcroft's experiments show how complicated are the phenomena which have to be investigated. The venous blood which

flows from the submaxillary gland during stimulation of the chorda tympani nerve contains a smaller percentage of water than the arterial blood; after the first minute of stimulation the water lost from the blood exceeds in volume the saliva secreted. The circulation of the blood through the gland during activity is about six times as fast as during rest. Anæsthetics and leech-extract alter the gaseous contents of the blood. All these factors have to be taken into consideration.

Experiments made by Barcroft and Brodie ([29]) have shown that the production of a diuresis is accompanied by a marked increase in the absorption of oxygen, but no change in the discharge of carbon dioxide. Barcroft and Starling ([30]) have found that pancreatic secretion is accompanied by an increased absorption of oxygen, and this takes place, although the rate of the flow of blood through the organ may be sometimes greater and sometimes less than in the condition of rest. There is no doubt that by similar experiments much will be learnt about the respiratory exchange of the different organs of the body. It is known that the gaseous exchange is more active in some organs than in others, and even in the same organ varies at different times according to the condition of activity. A study of the respiratory quotient has shown that during hibernation oxygen may be absorbed without a corresponding discharge of carbon dioxide; during fattening the great increase in the output of carbon dioxide is unaccompanied by an equivalent absorption of oxygen; in the former case there is a great reduction in the muscular and glandular activity of the body, in the latter the glandular activity is especially augmented. The respiratory quotient of the normal animal must, therefore, be considered as the resultant of the respiratory quotient of the component organs, and it is probable that these differ in the various organs, and even in the same organ during rest and activity. It may be that some organs oxidise the waste products which are carried to them by the blood which has passed through other parts. Such a function Bohr and Henriques would ascribe to the lungs, for they find that the absorption of oxygen and the production of carbon dioxide by the lungs is about one-third the total respiratory exchange of the body; their results are, however, very variable, ranging from 0 to 66 per cent., and as by their method they failed to obtain the average rate of metabolism during the whole experiment, their conclusions cannot be accepted.

It remains to touch briefly upon the question whether the respiratory exchange is effected by the living cell through the agency of ferments. The work of Schmiedeberg has already been mentioned, and further research along the same lines has in recent years demonstrated that different intracellular ferments with the power of oxidising special substances are widely distributed in animal and vegetable tissues. These ferments, which are called oxydases, are present in different amounts in different tissues, and Jacquet has found that extracts of organs free from cells can still oxidise aldehydes. The chemical constitution of the oxydases is unknown; it is not apparently that of proteids or nucleo-proteids. Future work must show whether respiratory exchange is a complex process due to oxidising and reducing ferments.

The Causes of the Gaseous Exchange between the Blood, Lymph, and Tissues.—The lymph acts as the means of transport between the blood and the tissues; it carries oxygen to, and carbon dioxide from, the tissues. On the theory that this gaseous exchange is a process of diffusion, it would be expected that the partial pressure of oxygen should show a descending scale, that of carbon dioxide an ascending scale, through the blood, lymph, and tissues. This it is difficult to prove experimentally, for it is impossible to analyse the lymph lying between the capillary blood-vessels and the tissues, where the actual transference of gases occurs. The lymph for analysis must be collected from some large lymphatic vessel, such as the thoracic duct, and during its sluggish flow from the lymph-spaces to such a large vessel the lymph will have been exposed to the action of the blood circulating in the surrounding vessels, and thus some gaseous interchange may occur.

Lymph contains only traces of oxygen, but a large quantity of carbon dioxide, according to Hammarsten's analyses 0·1 volume per cent. of oxygen, 37·5 of carbon dioxide, and 1·6 of nitrogen. The transference of gas depends not upon the quantity but the partial pressure of the gas. Strassburg found that the partial pressure of the carbon dioxide in the lymph was intermediate between the values obtained for the arterial and venous blood; Gaule, on the other hand, obtained values higher than that for the venous blood.

In the case of oxygen there is a descending series from the high pressure of the gas in the arterial blood down to the tissues where the partial pressure on account of the great reducing activity

of the tissues is exceedingly small or even absent altogether. The tissues are constantly producing carbon dioxide, and here the pressure of the gas appears to be the highest in the body. If pure air be injected into a ligatured portion of the intestine of a living animal, and then after a short time the gas be removed and analysed, the partial pressure of carbon dioxide will be found to have risen from zero to 7·7 per cent. of an atmosphere. This was the result obtained by Strassburg, and it has been recently confirmed by Boycott,(31) who found that if successive observations be made, the pressure of carbon dioxide soon reaches a level

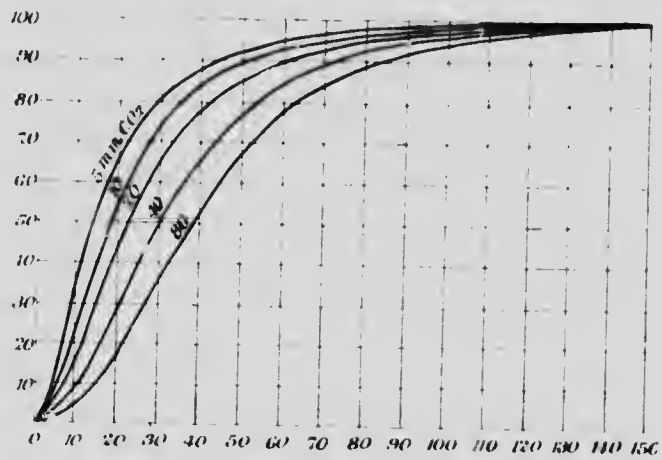


FIG. 32.—Pressure of Oxygen in Dog's Blood at 38° with different Pressures of Carbon Dioxide (*Bohr*).

which is maintained, at any rate for some hours. The balance appears to result from the continual production of the gas by the tissues, for it is greater if the blood-vessels, which supply the intestine, have been previously ligatured. Such a value for the partial pressure of carbon dioxide in the tissues is supported by the experiments made by Strassburg and by Ewald upon the partial pressure of the gas in urine, bile, and various pathological exudations; pressures varying from 7 to 12 per cent. of an atmosphere were obtained.

Other factors may come into play. It has already been mentioned that Bohr, Hasselbalch, and Krogh found that the pressure of carbon dioxide alters the dissociation curve of oxy-hæmoglobin; this would have an important influence upon the transference of

gas during internal respiration, for the increasing carbon dioxide in the capillary blood-vessels would increase the partial pressure of oxygen and maintain it, when the quantity of the gas was diminishing. The production of lactic acid during muscular work, when the supply of oxygen is insufficient, would act indirectly in a similar manner, for it would increase the pressure of carbon dioxide. The breathing of carbon dioxide may thus be useful in cases where the supply of oxygen to the tissues is insufficient, for the pressure of oxygen would be raised, and thus more would be rendered available for absorption by the tissues. The concentration of the blood during its passage through active organs would also raise the pressure of the oxygen in the blood of the capillaries, and Bohr maintains that the alteration in the "specific oxygen capacity" of the blood during its flow through the capillaries will also act in a similar favourable manner.

The part which is played by such factors and by the intracellular ferments in the processes of internal respiration must be determined by future research. The little that is known at present is exceedingly interesting and suggestive in connection with many physiological and pathological problems. Internal respiration is probably a far more complex process than it appeared to be only a few years ago.

The Regulation of Respiration in Health and Disease.—It might appear that the oxidation of the body is determined by the amount of combustible material and the supply of oxygen, for the activity and vascularity of an organ are so closely associated that under ordinary conditions they are inseparable. The numerous experiments upon internal respiration show, however, that the condition of the cells, not the supply of oxygen, determines the process of respiration both as regards the absorption of oxygen and the discharge of carbon dioxide; the animal is able to vary the supply of oxygen and the removal of carbon dioxide according to its needs.

In the preceding chapters ample evidence has been given of the variations in the respiratory exchange of the whole body and of its several parts under different physiological conditions, but so far the regulation of the ventilation of the lungs according to the needs of the organism has not been considered. This is a subject of the greatest practical importance to the physician, for he is forced by the exigencies of his practice to gauge the respira-

tory exchange of his patient indirectly; he observes the rate and depth of the respiratory movements and the colour of the face and extremities, and hence draws conclusions concerning the intake of oxygen and the output of carbon dioxide.

It will, therefore, not be amiss to consider here in some detail the means whereby the ventilation of the lungs is regulated. In the first place arises the problem of the first breath, which has long exercised the minds of physicians, lawyers, and theologians, and is still incompletely solved. What are the causes of the first respiration at birth? The fœtus has breathed for months by means of the gaseous exchange which occurs in the placenta[1] between its blood and the maternal blood; at birth the infant is obliged to ventilate its lungs in order that it may breathe. The most natural stimulus would, therefore, appear to be want of oxygen. Is lack of oxygen or accumulation of carbon dioxide the stimulus which sets in rhythmic activity the respiratory muscles, which from a condition of apparent inactivity during intrauterine life enter upon a course of rhythmic activity which will cease only with life itself? Does the stimulus only become effective at birth, or has it been present in a weak form during fœtal life? Have the respiratory muscles been exercised during the last months of intrauterine life, and thereby prepared for the activity they assume at birth, or has the condition of the fœtus been one of continual apnœa?

A cause other than the venosity of the blood has been strongly advocated by some observers. During intrauterine life the fœtus lies in the warm amniotic fluid, which shields both it and the mother from injury; at birth the infant is exposed and is cooled even in a warm room by the evaporation of the liquid covering its body; this cooling is said to be the stimulus which starts the first breath. An undue importance has been given to this supposed cause. An infant born in the tropics draws its first breath as readily as one born in a temperate climate or in the arctic regions. Numerous cases, moreover, are on record which show that breathing by the lungs may commence when only the head is born, or even when the head is in the genital canal. Infants and young animals have been born and have drawn their first breaths inside the intact fœtal membranes. It is equally certain

[1] See page 569.

that in cases of very protracted labour the full-term fœtus has given respiratory movements in the uterus itself, for after death amniotic fluid, fœtal hair or lanugo, and meconium have been found in the trachea and bronchi. Newly born infants and animals often do not draw their first breath for several seconds, it may be two minutes, after birth, notwithstanding the exposure of the body and the absence of any injury. Ahlfeld ([32]) has shown that infants delivered into a bath at the temperature of the mother's body do not delay their first breath.

Preyer ([33]) maintained that the true cause of the first respiration was cutaneous stimulation in some form or other, that venosity of the blood in itself was no stimulus to the respiratory centre in the medulla oblongata, although to a certain extent it raised the excitability of the centre for the cutaneous stimuli. The evidence against this view has already been given so far as sensations of cold are concerned; as regards mechanical stimulation it is well known that if the placental circulation be intact, the full-term fœtus may be subjected to much manipulation in cases of complicated labour or false presentations without any danger of causing it to breathe prematurely within the cavity of the uterus. Cohnstein and Zuntz removed a fœtal sheep from the uterus without damage to the placental circulation; stimulation of the skin, even blowing air into the nostrils, did not cause it to draw a breath, but only evoked general reflex movements. The premature lamb sucked the experimenter's finger when it was placed in its mouth, and from time to time spontaneously moved its body, but it did not draw a single breath until the umbilical cord was tied, when it forthwith commenced to breathe.

Cutaneous stimulation may be, and probably is, an accessory cause of the first breath, but it is obvious that others must be the effective ones. An increase in the carbon dioxide or a decrease in the oxygen of the blood will act as a stimulus to the respiratory centre. Such a condition will occur when the infant is born and the umbilical cord is severed. During the last stages of labour the placental circulation is disturbed but does not cease, for the amniotic fluid behind the body of the fœtus will prevent the obliteration of the cavity of the uterus and the complete separation of the placenta. If an increase of carbon dioxide and a decrease of oxygen in the blood be the stimuli for respiration, one would expect them to be sometimes effective during the later

stages of fœtal life, and feeble respiratory movements might occur within the uterus. The sudden assumption by the respiratory centre of the capacity to send out rhythmic impulses to which the respiratory muscles adequately respond has appeared a great difficulty to some observers, for it was one of those leaps by which Nature is said never to proceed. Against this might be brought the sucking power of the newly born infant, were it not for the fact that there is evidence that the fœtus sucks, or at least frequently swallows amniotic fluid during its intrauterine life. May it not be that the respiratory centre as well as the respiratory muscles gradually assume the power of rhythmic activity: that feeble respiratory movements, sufficient only to suck in and drive out a little amniotic fluid or mucus from the naso-pharynx, are present as a response to any considerable changes in the pressure of oxygen and carbon dioxide during the last months of fœtal life? Even if a little amniotic fluid were sucked into the trachea it would be quickly absorbed; it is sterile, is not toxic, and would cause no mechanical disturbance, for the fœtus is breathing by means of the placenta. It is possible also that reflex inhibition may occur, if the fluid penetrates too far into the naso-pharynx. Ahlfeld has described certain intrauterine movements of the fœtus which appear to be due to the contraction of the respiratory muscles; they are rhythmic but irregular movements, with periods of greater or less activity, and vary from 38 to 76 per minute; they can be felt under favourable circumstances in the region of the mother's navel. In women in labour with their first child it frequently happens that after the child's head is born, some seconds, or even a minute or two, elapse before the body is expelled by another forcible contraction of the uterus and muscles of the abdominal wall. During this short interval respiratory movements may occur; in the nostrils may be seen bubbles of mucus which are alternately blown out and sucked in. These respiratory movements are of a very superficial nature, and may be easily overlooked, so that the medical attendant may regard as the first respiration that deep breath which is taken after the body is expelled, and is so often accompanied by a general movement of the whole body and followed by a cry. Ahlfeld maintains that the superficial respirations are similar to those intrauterine movements which he and his pupils have investigated; the so-called first breath is not the first respiration, but the first deep breath

which draws air into the lungs. The respiratory movements of the newly born infant are irregular in frequency and depth, and are often interrupted by periods during which no breath is taken. The average number of respirations cannot, therefore, be stated with exactitude; it appears to be about 58 per minute, and varies from 40 to 80. Periods of apnœa and of waxing and waning respiration, as in Cheyne-Stokes's breathing, are of normal occurrence in the healthy young infant, especially during sleep; the regular sequence of respiration is not acquired until infancy is past. The excitability of the respiratory centre appears to be low in both the fœtus and the infant. Cohnstein and Zuntz found that the addition of carbon dioxide to the inspired air produced a much less marked increase in the amplitude of the respiration of the newly born animal than in a young animal a few days old. The condition of the fœtal blood and its relation to fœtal apnœa will be considered in the next chapter.

What are the factors which maintain the rhythm of respiration when once it has started? Are the same factors effective for its maintenance and regulation as for its commencement, or is some other influence at work? To these questions no decisive answer can be given, for the opinions of physiologists have been as much divided upon this question as upon the cause of the first breath; here, again, the two rival views have been stimulation of the respiratory centre by chemical changes in the blood and excitation by nervous impulses received from the periphery. The older view, that the respiratory movements are regulated by the gaseous composition of the blood flowing through the medulla oblongata, is well founded and logical, but, since the publication of the important researches of Hering, Breuer, and Head upon the influence of the vagus upon the rhythm of respiration, it has lost favour and has been abandoned by many physiologists. it was replaced by the theory that the respiratory movements are self-regulating reflex actions; each respiration by a stimulation of the afferent fibres of the vagus nerves causes the centre in the medulla oblongata to send out expiratory impulses, and each expiration in a similar manner causes the following inspiration. The vagus nerve contains two classes of afferent fibres, stimulation of the one kind inhibits inspiration and produces expiration, stimulation of the other inhibits expiration and produces inspiration. By "positive" ventilation of the lungs of a rabbit a con-

dition of expiratory apnœa may be produced, even if hydrogen be used to distend the lungs; by "negative" ventilation the diaphragm is thrown into a condition of continued contraction. In this way it is maintained that the activity of the respiratory centre under normal conditions is regulated, not by the venosity of the blood, but reflexly by impulses passing up the vagi.

There is no doubt that the wave of opinion has gone too far; new work and new evidence often produce a greater effect than is rightly their due, and this at the expense of older work. The balance can only be restored by yet newer work. This has been supplied by the important research of Haldane and Priestley upon the regulation of the ventilation of the lungs in man. Their experiments upon the composition of the alveolar air have already been described, but it may be again mentioned that they found that under ordinary atmospheric pressure the alveolar air of each individual contains a practically constant percentage of carbon dioxide. The smallest increase in the percentage of carbon dioxide in the inspired air is accompanied by an increase in the ventilation of the lung, sufficient to keep constant the percentage of carbon dioxide in the alveoli. During muscular work a similar compensation is present; the alveolar ventilation is increased in proportion to the extra production of carbon dioxide, and thus the hyperpnœa seen during muscular exercise can be explained as a response of the respiratory centre to the rise in the pressure of carbon dioxide in the arterial blood. Other experiments show that apnœa depends upon a fall in the pressure of carbon dioxide below the amount which will stimulate the respiratory centre; thus rapid respiration of air containing sufficient carbon dioxide to prevent the pressure of the gas in the alveoli from falling below this threshold value will not produce even a short apnœa. It is unnecessary to assume the existence of a true vagus apnœa, for the apnœa produced by distension of the lungs can be explained by the lowering of the pressure of carbon dioxide.

There is much independent evidence in support of these views. Fredericq proved by his well-known experiment with a crossed circulation that the condition of apnœa produced in the one animal by artificial respiration performed on the other is not due to a rise in the pressure of oxygen in the blood, but to a fall in the pressure of carbon dioxide. Mosso has shown that in man a short apnœa can be produced by a deep inspiration of hydrogen,

but not of carbon dioxide. The long periods of apnœa which occur in hibernating mammals are replaced by continuous breathing, when they are made to inspire air containing carbon dioxide, and similar treatment will abolish in man the apnœic phase of Cheyne-Stokes's respiration.

The respiratory movements appear from a review of the present evidence to be regulated by the gaseous composition of the arterial blood which supplies the respiratory centre in the medulla oblongata. The necessary impulses are not carried by the vagus or sympathetic nerves, for it is well known that after section of both vagi the respirations, although slow and deep, are yet maintained rhythmically, it may be for months, and hyperpnœa can still be produced by muscular activity or by inhalation of carbon dioxide. The view that the regulation of the rate of alveolar ventilation depends under normal conditions upon the pressure of carbon dioxide in the respiratory centre offers a satisfactory explanation of the normal rhythm, hyperpnœa, and apnœa.

It is necessary, however, to point out that there are probably other factors concerned in the regulation of respiration; some of these may be effective indirectly by altering the pressure of carbon dioxide, others may act directly upon the excitability of the respiratory centre. There is little doubt that a rise in the temperature of the body will increase the rate of respiration; this is seen especially in the dog when he is exercised or exposed to the heat of the sun, and forms an important means of regulating the temperature of the body. In man, horses, and other animals muscular work produces a rise in the internal temperature of the body, and thus another factor besides carbon dioxide may be concerned in hyperpnœa.

Nervous impulses carried by the vagi either from the lungs or the heart probably play an important part in the co-ordination of the action of the respiratory muscles and the heart, so that an adequate supply of blood may be maintained through the lungs for the rapid absorption of oxygen and the removal of carbon dioxide. The value of training appears to be largely due to the exercise of the heart and lungs in accommodation, and economy of work. The effect of section of both vagi requires further investigation from these standpoints, for Haldane and Lorrain Smith found that arrest of inspiration by dilatation of the lungs is produced in an animal with its nerves intact, even

during the onset of violent hyperpnœa due to air rich in carbon dioxide.

Abnormal breathing is one of the conditions which most frequently demands the attention of the physician. It may occur in various forms, hyperpnœa, dyspnœa, apnœa, or that alternation of hyperpnœa and apnœa which is known as Cheyne-Stokes's respiration. The abnormal condition may be shown in alterations in the rhythm, in the rate or depth of breathing, or in any combination of these. Exact observations do not exist for an adequate discussion of these pathological questions, even if it were within the scope of this work and the power of the writer. It may be of interest, however, to consider briefly some of the pathological conditions which throw light upon the normal regulation of respiration.

In addition to the hyperpnœa produced by breathing carbon dioxide and by muscular work, there is another form of hyperpnœa due to want of oxygen; this occurs at very high altitudes, where the pressure of oxygen is less than 13 per cent. of an atmosphere,[1] in carbon monoxide poisoning, and sometimes in mines. The rapid ventilation of the lungs in these cases lowers the pressure of carbon dioxide in the arterial blood below the value necessary for excitation of the respiratory centre; the frequency and depth of respiration are regulated by the pressure of oxygen in the blood. In patients in whom the circulation is inadequate, owing to heart-disease or other causes, this form of hyperpnœa is very common. The cyanosis is evidence of the want of oxygen; the increased ventilation of the lungs is inadequate, owing to the feeble circulation, to supply the necessary oxygen to the medulla oblongata, but can reduce the pressure of carbon dioxide below the stimulating value.

A very marked disturbance of respiration is seen in cases of diabetic coma; the deep ventilation of the lungs, the so-called "air-hunger," is very typical; there is no obstruction to the free entry and exit of the air, but the rapid and deep breathing persists, it may be for hours. Many observers maintain that in these cases the blood is unable, owing to its diminished alkalinity, to take up the normal amount of carbon dioxide, and thus the tissues are overcharged with the gas, and the respiratory centre is stimu-

[1] See chapter on "Mountain Sickness" by L. Hill, page 215.

lated. The composition of the alveolar air in cases of diabetic coma examined by Beddard, Pembrey, and Spriggs ([34]) was about 2·2 volumes per cent. of carbon dioxide, and 17·5 volumes of oxygen; the venous blood contained only about 20 volumes per cent. of carbon dioxide; the results suggest that the hyperpnœa is a cause, not an effect, of the reduction of the carbon dioxide in the blood, and the respiratory centre would appear to be stimulated in some other way than by excessive carbon dioxide or want of oxygen, it may be by some of the products of the abnormal metabolism. Rapid ventilation of the lungs would lower the percentage of carbon dioxide in the alveolar air, and would thus wash out carbon dioxide from the blood. Further observations, however, are needed upon these points

In certain cases of heart-disease a well-marked alternation of apnœa and hyperpnœa was observed and described by Cheyne and Stokes. The phenomena have been, since the publication of their observations, the subject of many experiments, observations, and debates, and various theories have been brought forward to explain the typical breathing; a historical account and original observations are given by Gibson ([35]) in his monograph upon the subject. Recently Pembrey and Allen ([36]) have determined the composition of the alveolar air at different stages of the waxing and waning respiration of a patient who showed well-marked Cheyne-Stokes's respiration. The following are some of the results of the analyses:—

Early Period of Waxing Respiration.			Period of Dyspnœa.			Late Period of Waning Respiration.		
	CO_2 Vol. per Cent.	O_2 Vol. per Cent.		CO_2 Vol. per Cent.	O_2 Vol. per Cent.		CO_2 Vol. per Cent.	O_2 Vol. per Cent.
3rd expiration	3·90	12·19	5th expiration	3·26	15·48	18th expiration	2·00	18·86
,, ,,	3·61	12·84	8th ,,	3·22	15·67	21th ,,	2·63	18·5
2nd ,,	3·56	13·61	10th ,,	2·76	17·60		2·93	17·49
3rd ,,	3·63	13·66	12th ,,	2·74	16·75			

In the case of the early period of waxing respiration and of the late period of waning respiration the true values of the carbon dioxide are probably higher, and of oxygen probably lower, owing to the shallow nature of the respirations. When the patient was

better, and the respirations were continuous, the percentage composition of his alveolar air was 4·44 volumes of carbon dioxide and 16·34 volumes of oxygen. Observations showed that a diminished as well as an increased supply of oxygen would abolish the period of apnœa, and by the inhalation of carbon dioxide in percentages varying from 0·76 to 11·33 all gradations from feeble respirations in the place of apnœa to continuous breathing of almost regular type could be obtained. Doses of carbon dioxide above 1 per cent. readily abolished apnœa, and large doses could be tolerated more readily by the patient than by normal subjects. A suitable mixture of carbon dioxide and oxygen produced a regular and easy respiration.

It would appear that the periodicity of Cheyne-Stokes's respira-

FIG. 33.—Cheyne-Stokes's Respiration in Man (*Pembrey and Allen*).
The curve reads from left to right, and the time is marked in seconds.

tion is due to a diminished excitability of the nervous system and defective circulation; the carbon dioxide accumulates and the oxygen diminishes, until at last the nerve-cells of the respiratory centre are stimulated, the waxing respirations begin and culminate in marked dyspnœa, whereby the carbon dioxide is washed out and a large quantity of oxygen is taken in; apnœa follows, due apparently to the absence of sufficient carbon dioxide to stimulate the nerve-cells. The alteration in the excitability of the nerve-cells may or may not be necessary in addition to defective circulation, indeed it may be caused by the latter. The occurrence of Cheyne-Stokes's respiration as a normal event in the respiration of hibernating mammals, in infants, and in some healthy adults during sleep, and as a characteristic of some cases of poisoning, is a subject of further investigation from these points of view.

Respiration in the Embryo and Fœtus.—The processes of respiration in the embryo have been the subject of numerous investigations upon the eggs of birds, reptiles, amphibians and fishes. As early as 1674 Mayow had recognised that the nitro-aerial gas, that is oxygen, was absorbed through the porous shell of a hen's egg during incubation. Blumenbach observed that the blood in the allantoid veins was brighter in colour than that in the allantoid arteries, and Paris found that the air in the air-chamber of the egg contained carbon dioxide during the last days of incubation.

The necessity of air for all stages of the development of the embryo has been proved by varnishing the egg or covering it with oil to prevent the passage of gases; development does not occur, or if the experiment be performed at a later stage of incubation death is rapidly caused. In a few experiments development has proceeded in the early stages, but these cases are due to defective varnish, development before the experiment commenced, or with the aid of the oxygen already present in the egg. It is interesting to note that Hasselbach maintains that a small quantity of oxygen may be set free by chemical changes occurring in the substance of the egg at the commencement of incubation. Eggs cannot be developed by incubation in an atmosphere of pure hydrogen or nitrogen.

The air-chamber at the blunt end of the hen's egg is present whether the egg be fertile or not, and is enlarged during the period of incubation by the evaporation of water. It serves as a reservoir of air for the embryo and even for the chick during incubation, for it is well known that the chick may, after pushing its beak through the membranes, breathe the air in this space by its lungs for some hours before it breaks the shell. The chick often draws its first breath and even chirps before it is hatched.

The total respiratory exchange can be easily determined in the developing chick. There is an absorption of oxygen and a production of carbon dioxide during the first hours of incubation, and this gaseous exchange steadily increases with the process of development; relatively it is equal to, or even greater than, that of the adult hen. It has already been mentioned that the respiratory exchange of the embryo chick is affected by changes of temperature in a similar manner to that seen in cold-blooded animals, a fall of temperature causes a decrease, a rise of tempera-

ture an increase, in its respiratory exchange. For a short time before hatching there is an intermediate condition during which a change of temperature has no marked effect and at last when the chick is hatched it responds as a warm-blooded animal to changes of external temperature.

The subject of fœtal respiration affords one of the most interesting examples of the way in which correct explanations well founded upon observation may after the lapse of years be either forgotten or replaced by erroneous theories until they are rediscovered after a further lapse of years. Mayow maintained, in his account of the fœtal respiration published in 1674, that the placenta is to be regarded as a lung, by which the umbilical vessels take up nitro-aerial gas, or oxygen, and convey it to the fœtus; he compared the condition of the fœtus to that of apnœa, and also described the process of absorption of oxygen by the blood in the gills of fishes and in the embryo chick. This view of the fœtal respiration was adopted and extended by Hulse and by Ray, who makes the following clear statement in the twelfth edition of his work. "The Wisdom of God in the Creation," published in 1759: "The maternal blood which flows to the cotyledons and encircles the papillæ communicates by them to the blood of the fœtus the air wherewith itself is impregnate; as the water flowing about the carneous radii of the fish's gills doth the air that is lodged therein to them."

Scheel, Jeffray, Bostock, and others observed that the blood in the umbilical vein was brighter than that in the umbilical artery, and thus supplied further evidence of the respiratory function of the placenta. Confusing evidence, however, based upon faulty observations, had arisen in the meantime. Johannes Müller, who is often called, and rightly so, "The Father of Modern Physiology," held that plasma or lymph passed from the mother to the fœtus, and so supplied the place of respiration; there was no difference, he maintained, in the colour of the blood in the umbilical artery and umbilical vein, and he even considered it necessary to test by experiment the view that the fœtus breathed by absorbing oxygen from the amniotic fluid by means of its skin or lungs. Such was the condition of knowledge about a century and a half after the death of Mayow; his brilliant work had been forgotten or neglected, and the correctness of his view that the

placenta acted as a lung was not fully recognised until Zweifel in 1876 showed that the spectrum of oxy-haemoglobin could be clearly seen in the umbilical cord before the child breathed by its lungs. Zweifel also demonstrated that the blood in the umbilical vein of a fœtal rabbit was brighter than that in the arteries, if the precaution were taken to open the uterus of the mother in warm normal saline solution and thus prevent vigorous contractions of the uterus: the difference in the colour of the vessels disappeared during asphyxia but reappeared when artificial respiration was performed upon the mother.

The mechanism of the fœtal respiration was more fully explained by the experiments of Zuntz, who showed that during asphyxia of the mother the blood of the umbilical vein became darker than that of the umbilical arteries, the fœtal blood yielding up oxygen to the blood of the dying mother; the umbilical vein became as dark as the arteries when the maternal blood-vessels supplying the placenta were compressed; the blood of a fœtus respiring air by its lungs lost oxygen in the placenta, which was attached to an excised piece of the uterus; the umbilical vein coming from the intact placenta contained blood as bright in colour as the arterial blood of the uterus during the normal respiration of the mother, and fœtal movements made the blood of the umbilical arteries darker in colour.

Zuntz in conjunction with Cohnstein also analysed the blood of the umbilical artery of a fœtal sheep about three weeks before full term; the total gas was 54·211 volumes per cent., of which 6·669 were oxygen, 46·542 carbon dioxide, and 1 nitrogen. Comparative analysis showed that the blood in the umbilical artery contained 4·67 volumes per cent. less oxygen and 4·72 more carbon dioxide than the blood in the umbilical vein. These observers calculated that the fœtal respiratory exchange was relatively much less than that of the adult; but there are great difficulties to overcome before exact data can be obtained for such an estimation, which must be based upon comparative analysis and determination of the rate of flow of the blood through the placenta; operative procedures quickly disturb the circulation. From these experiments it is concluded that the fœtus respires by processes of diffusion in the placenta; under normal conditions the pressure of oxygen in the maternal blood, which supplies the uterus, is higher than

that of the oxygen in the blood of the umbilical artery; during asphyxia the reverse is the case, and the mother's blood absorbs oxygen from the fœtal blood. The transference of carbon dioxide is probably effected by diffusion. Further experiments, however, are needed to show whether the placenta possesses any power of secreting gases.

Bohr (*) has estimated the respiratory exchange of the fœtus in another way: he determined the absorption of oxygen and the discharge of carbon dioxide in a pregnant guinea-pig before and after the umbilical cords of the fœtuses had been compressed. His results show that weight for weight the respiratory exchange is a little greater than that of the mother. The fœtus, although it is shielded from loss of heat and shows no marked muscular activity, is rapidly growing and the chemical changes involve a rapid respiratory exchange.

In conclusion, what is the nature of the fœtal apnœa; is it produced by a low pressure of carbon dioxide and a high pressure of oxygen, or are other factors present? This question requires further investigation before it can be answered. It is true that the blood in the umbilical vein contains about 6·3 volumes per cent. of oxygen and 40·5 of carbon dioxide; but it is not this blood but mixed blood which supplies the respiratory centre. The blood in the umbilical artery contains about 2·3 volumes per cent. of oxygen and 47 of carbon dioxide; but the pressures of the gases, not their quantities determine excitation, and they are unknown. The respiratory centre responds to compression of the umbilical cord, and that is practically all that is known beyond the fact that its excitability appears to be lower and less easily destroyed than that of the adult animal.

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CHAPTER XVI

INTERNAL SECRETION—THYROID AND SUPRARENAL GLANDS

THE progress of physiology in the last few years has shown that it is impossible to assign any one special function to any organ. The different organs of the body have a complex interaction and work together for the good of the whole organism, which is the physiological unit. Nowhere is this interaction better shown than in the case of the thyroid, suprarenal, pancreas and the generative glands. The ductless glands produce a secretion, "the internal secretion," which is absorbed by the blood-vessels or lymphatics; glands with ducts and an obvious secretion produce also other substances of the nature of an internal secretion which is necessary for the health of the animal; the thyroid and suprarenal glands will be taken as types of the former class, the pancreas belongs to the latter class, and its functions are discussed in another part of this work.

The recent investigations upon these glands have shown the futility of attacking such problems of life from an anatomical or morphological point of view. Purely anatomical reasoning in such cases leads astray. Some of the glands in question are so small that it was considered improbable that they were essential for the welfare of the animal; others were shown by the study of their morphology to represent organs more fully developed in lower animals, and it was therefore concluded that in the higher animals they were present only as rudiments, more or less useless relics. Whether we believe in Design or in Natural Selection during the struggle for existence, it is a useful guide to believe that every part of the organism is of some use; life is conducted on economical lines.

Thyroid Gland.—The function of this gland must now be considered in detail. Most of our knowledge has arisen within the last thirty or forty years. Previously to that time many fanciful

theories were prevalent; it was supposed by some that the gland by its relationship to the larynx supported that structure and strengthened the voice; others held that the thyroid body regulated the supply of blood to the brain. These are examples of the effect of anatomical reasoning. Valuable facts, on the other hand, have been learnt by the study of the changes produced in man and animals by disease or removal of the gland.

Cretinism is a peculiar disease, in most cases of congenital origin and confined to certain districts of a country. The patient presents markedly abnormal features. His stature is stunted; a man of twenty years may be less than three feet high; the body is deformed; the head is broad from ear to ear; the nose is flattened, the bridge being absent; the mouth is large and open; the skin dry, wrinkled, and of an earthy colour; the hair is coarse, sparse, and dry. The neck and shoulders are swollen out by large pads of fatty tissue; the chest is narrow, the abdomen large and pendulous. The limbs are short and misshapen. The mental condition is worse than that of an ordinary idiot, the creature being frequently unable to speak, and uttering only a strange cry or a peculiar chuckle.

Such is the spectacle presented by a cretin, and we must now see how these changes are related to the thyroid gland. In many of these cretins the thyroid gland is absent; in others it is enlarged by disease. Moreover, it is found that these subjects are in many cases born of parents suffering from disease of the thyroid gland. Men, and especially women living in certain districts, such as some valleys in Switzerland, suffer from goître, an enlargement of the thyroid gland due to causes as yet unknown. The symptoms of this disease are chiefly due to the pressure exerted by the enlarged gland upon the trachea, œsophagus, blood-vessels and nerves of the neck; there thus arise difficulty of breathing and swallowing, disturbances in the circulation and the beating of the heart. Such symptoms would probably be removed by extirpation of the gland. Kocher of Berne accordingly excised the gland of patients suffering from goitre, and the immediate effect was most satisfactory. The symptoms of the disease disappeared. Later, however, the patients passed into a condition of cachexia, now known as operative myxœdema or cachexia strumipriva; the condition resembled cretinism, but there were naturally many modifications, for the growth of the body having

ceased and the mind having developed, there could not be those great deformities which are found in the body and mind of cretins. It was observed—and this is an important fact—that if a portion of the gland were left no evil results followed the operation for goître.

A further link in the chain of evidence was discovered by Gull in 1873. He noticed a cretinoid condition developing in adults, who had previously been healthy and had not suffered from goître. The patient showed a great loss in bodily and mental energy; the features became deformed and apparently œdematous. This latter change, however, was not due to a true œdema, but to a collection of mucin. The disease was accordingly named "myxœdema" by Ord.

Recent observations have shown that in all cases of myxœdema, cachexia strumipriva, and cretinism, the respiratory exchange is diminished to one-half the normal value. The administration of preparations of the thyroid gland to these patients produced a marked rise in the respiratory exchange, which reached a certain value at which it remained only during the continuation of the treatment.

The evidence of Medicine upon the importance of the thyroid gland for the wellbeing of the organism is conclusive and cannot be neglected. The testimony of Physiology is the complement, for it is impossible to divorce Physiology and Medicine: diseases can be considered as natural vivisections performed upon man —unfortunate they may be for the individual, but of benefit to the race, for thereby Natural Selection and Knowledge are advanced.

It is now necessary to consider the contribution of Experimental Physiology to the knowledge of the thyroid gland. It is satisfactory to note that the results obtained agreed with and amplified the clinical knowledge. Schiff, as long ago as 1856, showed that complete removal of the gland was soon followed by death in the case of dogs; his results, however, were neglected until Kocher and Reverdin in 1883 described the cachectic condition produced in man by the extirpation of the gland in cases of goître. The effects of removal of the gland in cats and dogs are most marked; death generally occurs within a week. At first there is a twitching of the muscles which occurs when the animal is disturbed; the contractions become more frequent, and

later develop into severe spasms which affect the whole body and resemble epileptiform convulsions. During the progress of these symptoms there is a marked loss of appetite and wasting. In exceptional cases the acute stage is less marked or even absent, and the animal passes into a chronic condition of cachexia; its fur becomes dry and unkempt; its nutrition is impaired; its intelligence is dimmed, and its movements lack precision. The nervous control of the temperature of the body is imperfect: this is shown by the subnormal temperature. The disease in many features resembles myxœdema, and generally ends fatally within one or two months.

In herbivorous animals the loss of the gland is followed by a slow development of the cachexia. No disturbance whatever may be observed in some cases; this has been explained by the discovery of accessory thyroid glands, which are frequently present in rabbits.

In consequence of the many evil effects which arose from removal or disease of the gland, attempts were made to ingraft the gland. The operation produced good but not permanent results, for the gland in most cases failed to grow and was absorbed. Extracts of the thyroid gland were, therefore, given subcutaneously by Murray of Newcastle-on-Tyne, and this method of treatment was highly successful in cases of myxœdema. Patients were then fed on fresh thyroid glands from sheep, and even better results followed. How great is the improvement produced in cases of myxœdema and cretinism is shown by the numerous records and photographs of patients before and after treatment with extracts of the thyroid gland. It is clear from these cases that the effects are far-reaching, and influence both the bodily and mental development. The fact that the administration of the gland must be continued throughout life indicates that the nutrition of the body demands a constant supply of some substance yielded by the thyroid gland.

What, then, is the specific substance produced by the thyroid gland? An answer to this question must be sought in the structure and chemical composition of the gland. The thyroid body is a ductless gland, and the so-called "colloid," which fills the acini, is its "internal secretion." The colloid is apparently derived from the granules which can be seen in the cells of the acini, and is discharged into the acini, where it accumulates until

the pressure of the secretion causes a separation or rupture of the cell-wall, and the colloid is discharged into the surrounding lymphatics; these vessels, by means of the thoracic duct, convey their contents into the blood. According to some observers the colloid substance is absorbed also by the capillary blood-vessels.

The nervous supply of the gland is derived from the laryngeal nerves; no structural changes, however, have been observed in the cells as the result of stimulation or section of these nerves or of the vago-sympathetic nerve. Vaso-constrictor nerves to the thyroid are found in the cervical sympathetic nerve of the monkey and in the anterior roots of the third thoracic nerve of the rabbit, cat, and dog. According to His, the blind tube which passes from the region of the foramen cæcum of the tongue towards the hyoid bone is to be regarded as a rudiment of the duct of the thyroid gland—the ductus thyreoglossus. The thyroid is developed as a median evagination of the floor of the pharynx between the first and second branchial arches. The parathyroids arise as thickenings of the epithelium in the dorsal aspect of the third and fourth visceral clefts. The structure of the thyroid gland is essentially the same in all classes of the vertebrates, although its size and position may show great variations: the invariable presence of the colloid substance in closed acini surrounded by simple glandular epithelium is an interesting fact.

The natural stimulus to the gland is unknown, but appears to be the absence or presence of some substance in the blood. The removal of five-sixths of the gland leads to structural changes in the remaining portion of the gland which indicate increased activity.

Chemical [illegible]nation of the gland shows that it contains two proteids, a nucleo-albumin, and the colloid substance, which may be regarded as a compound proteid possessing iodine in considerable amount; the percentage of iodine varies, but on an average is 0·3 per cent. of the dried substance. The colloid substance is not a nucleo-proteid, for it yields no nuclein on gastric digestion. When the colloid matter is subjected to digestion, only those products which contain iodine possess active properties. The active substance is named iodothyrin and its effects upon the metabolism of the body appear to be similar to those exerted by the gland substance itself. Iodothyrin is a brown amorphous substance, almost insoluble in water, but readily soluble in weak

alkalies. It contains phosphorus and about 10 per cent. of iodine, but it gives no proteid reactions.

The effects produced by iodothyrin are similar to those caused by extracts of the whole gland. Patients suffering from cretinism or myxœdema are greatly improved by this method of treatment. The abnorm. conditions disappear; the metabolism, which in these diseases is abnormally low, is raised to the level of health; the absorption and assimilation of food and the secretion of urine are increased. It is interesting to note that toxic symptoms have been observed after the administration of large doses of thyroid extract to men and animals not suffering from disease or removal of the gland. Cardiac weakness, wasting, and the discharge of albumin and sugar in the urine may be produced. The intravenous injection of thyroid extract produces a fall of blood-pressure, but this may not be a specific effect.

The foregoing data show the great importance of the thyroid gland for the maintenance of the healthy metabolism of the body, but they do not show how it acts. Upon this latter point there are two chief theories. The more probable one is that the gland produces a specific substance which is necessary for the nutrition of the body, especially of the central nervous system; not only do the symptoms during life show that that system is especially affected by the loss of the thyroid, but after death degenerative changes are found in the nerve-cells of the brain and spinal cord. The rival theory maintains that the thyroid gland produces a substance which neutralises or antagonises poisonous products produced by the metabolism of the body. Our present knowledge, however, of the chemical changes which occur in the living body is so incomplete that it is impossible to dogmatise upon this question.

So far nothing has been said about the **Parathyroid Glands**, small glands which lie on each side of the neck, and often in close relationship to, or even embedded in, the thyroid gland. In minute structure they do not resemble the thyroid gland, for they consist of cells arranged more or less in columns and contain no vesicles and no colloid. The parathyroids were described by Sandstroem in 1880 as embryonic remnants of the thyroid gland; their functions were first investigated by Gley a few years later. Since that time numerous experiments have been made by various observers, and a different view has arisen as to the functions of the thyroid and parathyroid glands. It is maintained that re-

moval of all the parathyroids proves fatal within a short time, even if the thyroid gland be left intact; the animal dies from "tetany"; excision of the thyroid gland, on the other hand, causes "cachexia strumipriva" or "post-operative myxœdema"; previous experimenters had removed both glands, and hence the results were of a mixed character. Cretinism and myxœdema are due to insufficiency of the thyroid gland, and exophthalmic goitre may be accompanied by disease of the parathyroid glands.

The results, however, are very discordant, and do not bear out the view that the thyroid gland is less important than the parathyroid; the glands are often so closely associated that it is extremely difficult or even impossible to remove the one without serious damage to the other. Further research, especially upon the comparative physiology of the glands, must decide their relative importance.

The Suprarenal Capsule

The suprarenal capsule is a ductless gland, the functions of which were entirely unknown until fifty years ago, when Addison published his account of the peculiar disease which is now known by his name. This clinical work was quickly followed by the researches of Brown-Séquard upon the effects of removal of the glands, and his results showed that the suprarenal bodies, far from being the fœtal relics which they were once thought to be, were absolutely essential for life. The idea that the capsules were mere fœtal relics appears to have arisen from a comparison of the relative size of the gland in the fœtus and the adult. At the third month of intrauterine life the kidneys and suprarenal capsules are of the same size; at the sixth month the kidneys are twice the size of the capsules; the glands continue to grow after birth, and in the adult man each suprarenal body weighs about 4 grm., each kidney about 156 grm.

Accessory suprarenal bodies are often found in the connective tissue in the neighbourhood of the kidneys, and this fact offers a sufficient explanation of the negative results obtained by some observers, who have removed the suprarenal capsules. The development of the gland shows that it has a twofold origin, corresponding to the cortex and the medulla; the former part appears to arise from the epithelium of the cœlom in the Wolffian ridge,

the latter from ganglion cells of the sympathetic system. Comparative anatomy likewise indicates this separate origin of the two parts of the gland ; in sharks and other members of the Elasmobranche the cortical part forms the inter-renal body, an unpaired organ, separated from the medullary portions which are situated above the kidneys and form the suprarenal bodies. Physiological experiments show that extracts of the former organ produce no characteristic effects, but the latter organ contains an active substance similar to that found in the medulla of the mammalian suprarenal gland.

The microscopic structure of the mammalian suprarenal body shows that the gland, especially the medullary portion, is extremely vascular, and the suprarenal vein is surrounded in the medulla by a large bundle of involuntary muscle fibres, the function of which may be to regulate the circulation through the organ. In addition nerve-cells and branched cells containing granules of pigment are characteristic of the medulla.

The physiological evidence relating to the function of the glands can be divided into the effects of partial or complete removal of the capsules and the effects produced by injections of adrenal extracts. Brown-Séquard found that the removal of the suprarenal glands caused death in guinea-pigs, rabbits, dogs, cats, rats, mice, and pigeons ; the mean duration of life after the operation was in rabbits nine hours, in dogs and cats fourteen hours ; the symptoms which were generally observed were loss of appetite and of muscular power, cardiac weakness and convulsions.

Experiments by other observers have confirmed and extended Brown-Séquard's work. Removal of one gland does not cause death, and the remaining gland undergoes hypertrophy, and thus the animal appears to compensate for the loss of the one gland. Death generally occurs within one or two days after the removal of the second gland. The symptoms described by most observers are almost identical with those of Addison's disease, muscular weakness, loss of appetite, loss of tone in the vascular system, and paralysis of the respiratory muscles. Pigmentation has been observed in animals which have lived for a longer time after the excision of the glands.

The blood of animals, which have died after removal of the suprarenal capsules, is said to possess toxic properties, and to produce, when it is injected into healthy animals, symptoms

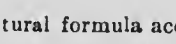
resembling those seen after removal of the glands. The question thus arises whether the animals die from the loss of a specific secretion or from the accumulation of a toxic substance which would normally be removed by the gland. The toxic effects of the blood do not appear to be due to auto-intoxication with the substances which are found in the normal gland, for the urine and tissues of animals deprived of their capsules were examined by Moore and Purinton for the suprarenal chromogen, and were tested physiologically for the presence of the active substance with negative results. Other observers have demonstrated the origin of the active substance in the living gland; the blood collected from the adrenal vein gives a reaction similar to that of the glandular substance itself, while blood from other vessels does not possess this property. Oliver and Schäfer found that extracts made from the diseased glands of patients who had died from Addison's disease did not possess the active principle of the healthy gland.

The first important work upon the physiological effects produced by extracts of the suprarenal capsules is due to Oliver and Schäfer, who showed that extract of the gland prepared with water, alcohol, or glycerine contained a specific substance which produced a marked contraction of the arterioles; a rise of blood pressure is thereby rapidly produced, and may mount up to two or even five times the normal height; the rise lasts about two or three minutes. The action is a peripheral one, the contraction of the arterioles occurs equally well after section of the spinal cord, and after division of the nerves which supply the limb. The active principle is contained only in the medulla of the gland, and it is not destroyed by boiling or gastric digestion. The injection of large doses produced death in the case of rabbits, but in dogs and cats only a slight transitory disturbance was seen. Schäfer showed that the extract of the gland was a most powerful styptic, which could be safely used to check hæmorrhage from small blood-vessels in man.

Various attempts were made by Abel and von Fürth to isolate the active principle of the gland, but this was first accomplished by Takamine, who named the substance adrenalin. It is a white, light, crystalline substance, with a slightly bitter taste, sparingly soluble in water and perfectly stable in a dry form. In alkaline and neutral solutions adrenalin is a powerful reducing agent and absorbs oyxgen from the air. All aqueous solutions on standing

turn from a rose colour to red and eventually brown. It forms salts, and an aqueous solution of the chloride having a strength of 1 in 10,000 will blanch the normal conjunctiva within a minute, while an injection of 0·000,001 grm. per kilogram of body-weight will raise the blood pressure of an animal by 14 mm. of mercury. The chemical structure of adrenalin has been worked out by von Fürth, Jowett, and Pauly; it appears to be a secondary alcohol of the formula $C_6H_3(OH)_2 . CH(OH)CH_2 . NH . CH_3$.[1] The ketone called adrenalone $C_6H_3(OH)_2CO . CH_2NH . CH_3$ is about one-tenth as effective as adrenalin in the production of glycosuria.

Since the preparation of adrenalin and its salts in the pure state numerous experiments have been made upon its physiological action. The most important results are those of Langley, who has been able to give a wider significance to the action of adrenalin; *the effects produced by adrenalin upon any tissue are such as follow excitation of the sympathetic nerve which supplies the tissue.* This generalisation has been confirmed by Brodie and Dixon and by Elliott; the former observers found that adrenalin cannot constrict the pulmonary vessels, a striking difference in behaviour as compared with the marked constriction of the systemic arterioles, and one which receives a simple explanation if the adrenalin acts only upon sympathetic nerve-endings. The muscles of the pulmonary blood-vessels are not supplied with constrictor fibres, and in default of sympathetic nerve-supply plain muscle does not react to adrenalin. Adrenalin may, therefore, be used as a test for the existence of sympathetic nerves in any organ; it stimulates the substance at the junction of the muscle and nerve, not the sympathetic nerve-cell, nerve-fibre, or muscle-fibre. Its effects may in one organ be shown by contraction, in another by inhibition; thus it causes contraction of the spleen and inhibition of the movements of the stomach, but in each case it resembles in its effect the excitation of the sympathetic supply of those organs. It has already been pointed out that adrenalin is only formed in the medulla of the gland which is developed from the sympathetic system.

[1] The structural formula according to Dakin is  $CH(OH) . CH_2NHCH_3$. Catechol, the aromatic nucleus, and not the side chain oxyethylmethylamine, is the active part. Dakin has prepared a substance with the same action, and very closely related if not the same as adrenalin, by heating catechol with chloracetyl chloride, and then acting on the chloracetyl catechol with methylamine.—(*Editor's Note.*)

In 1901 Blum discovered that the subcutaneous administration of extracts of the suprarenal capsules produces glycosuria; but no such effect was caused when the drug was given by the mouth. These results have been confirmed and extended by Noel Paton and others, but the subject of diabetes has been so fully discussed in another part[1] of this work that no further consideration of the subject is needed here.

The clinical evidence of the function of the suprarenal capsules will ever be associated with the name of Addison, who published his monograph on "The Constitutional and Local Effect of Diseases of the Suprarenal Capsules" in 1855. The characteristic symptoms of the disease are anæmia, general debility, feebleness of the heart's action, gastric disturbances, and pigmentation of the skin and mucous membranes. The suprarenal glands are generally found after death to be affected by tubercular disease, atrophy, or malignant growth. It is interesting to note that in some cases no lesion has been found in the suprarenal bodies themselves, but in the semilunar ganglia. The two chief theories which have been advanced to explain the symptoms of the disease are (1) that the condition is due to the loss of the internal secretion of the suprarenal gland, and (2) that it is caused by a pathological condition of the abdominal sympathetic system. The unfavourable results which have attended the treatment of the disease by the administration of extracts of suprarenal gland or of adrenalin do not support the theory of internal secretion, and the pathology of the disease requires further consideration in the light of Langley's work upon the relationship between adrenalin and the sympathetic system.

In adrenalin the physician and the surgeon find their most valued styptic. The drug is now extensively used in dentistry, ophthalmic surgery, midwifery and gynæcology, as well as in ordinary medical and surgical practice. It is interesting to note that it was discovered in a purely scientific research which involved the vivisection of animals, and that its limitations have been studied by similar work. Its administration should be guided by the fact that the effects produced upon any tissue are such as follow excitation of the sympathetic nerve which supplies the tissue. It is contra-indicated, as Brodie and Dixon show, in cases of hæmorrhage from the lungs. Although the progress of discovery has been so

[1] Macleod, p. 364.

rapid since the publication of Oliver and Schäfer's work, yet much remains to be explained. How does the adrenalin act, and what are the chemical conditions which underlie its property of stimulating muscle fibres and glands which are, or have been, supplied by the sympathetic nervous system? This is a question of fundamental importance, and time alone can show whether its solution is beyond the combined efforts of physiologists and chemists.

Note.—There is probably a connection between the suprarenal glands and the sexual system. In the frog there are seasonal variations in the structure of the gland corresponding to the period of pairing (Stilling). In the case of rabbits the suprarenal capsules undergo changes in volume during pregnancy, the outer zone of the cortex becoming twice the normal thickness at the expense of the medulla and inner cortex (Gottschom). Several cases have been recorded of extraordinary precocity of sexual development in children associated with hypertrophy of the suprarenal capsules. Atrophy is associated with non-development of the pubic hair and genital organs. The cortex is, therefore, probably connected with the growth of the body and the development of puberty and sexual maturity.

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CHAPTER XVII

THE PRODUCTION OF LYMPH

Between the capillaries and the tissue elements is a system of intercommunicating spaces filled with tissue fluid. These interstitial spaces are in direct communication with a system of overflow vessels, the lymphatics, which in turn empty themselves into the otherwise closed vascular system.[1]

The tissue fluid is ultimately derived and replenished from the blood in the capillaries. From it the tissue elements take up the materials necessary for their various anabolic processes, and to it they return the products of their katabolism.

The problem to be discussed is how this interchange of material between the blood and the tissues is carried on across the fluid in the tissue space.

We shall first consider the experimental evidence, showing the means by which material may be made to pass to and from the tissue fluid, processes which are called briefly lymph formation and absorption. Having thus ascertained the possible factors at work, we shall consider how the nutrition of the tissues is normally subserved by the tissue fluid—in other words, how the tissue fluid is regulated both in quantity and composition according to the needs of the tissue cells.

It is obvious that tissue fluid might have a second function to perform. It might give up and take from the blood material, and so aid the excretory glands in regulating the volume and composition of the circulating blood. This function will be dealt with incidentally.

The elucidation of these problems is from the outset fraught with the fundamental difficulty that we cannot collect tissue

[1] It is assumed for simplicity throughout this article that the lymphatics form an open and not a closed system of vessels. The point is at present one of uncertainty. If the system is closed, tissue fluid in order to reach a lymphatic vessel would have to pass through the endothelium of the lymphatic.

fluid for examination. Consequently we have no direct means of knowing the exact composition of the materials interchanged between a tissue cell and tissue fluid on the one hand, and the tissue fluid and blood capillary on the other.

The nearest approach to tissue fluid which is available for examination is the lymph which can be collected from one of the main lymphatic trunks of the body. The flow along the thoracic duct and the main trunk of the neck is continuous, but in the case of a limb it is necessary to perform passive movements of the limb in order to obtain a continuous flow.

The lymph in a large lymphatic vessel represents the overflow from any or all the interstitial spaces which the vessel drains. Since, as we shall see, material passes back from the tissue fluid direct into the blood capillary, it follows that lymph in a lymphatic is not the same as tissue fluid. In composition it must be different from that of any tissue fluid, and it will vary according to the different combinations of tissues from which it is being derived. In quantity, while it is conceivable that an active interchange might go on between the tissue fluid, the tissues and the blood without any overflow from the tissue spaces, an increased overflow into the lymphatic vessels must have been preceded by an increased formation of tissue fluid.

In spite of these necessary differences between the fluid in the tissue spaces and that in the lymphatic vessels, both may be referred to generally as lymph.

The subject of lymph formation and absorption is one not merely of physiological interest, but also of great pathological and medical importance. In many conditions of circulatory disturbance and of inflammation fluid accumulates in the subcutaneous tissues and the serous cavities. Dropsy is also liable to occur in certain forms of kidney disease, and a localised œdema of the skin may take place in certain individuals after eating shell-fish, strawberries, &c., and in other less well-defined conditions. We cannot hope to understand these pathological œdemas until we have arrived at an accurate knowledge of the process of normal lymph production and the conditions which regulate it.

Experiments dealing with Lymph Formation

The method of investigating normal lymph formation is necessarily experimental. Although we classify all such experiments as physiological, it must be remembered that many of them are just as pathological as those which occur in the body as the result of disease. It is clear that tissue fluid is always being formed and absorbed in the body, and we wish to find out how and in response to what this process is carried out. In order to do this with certainty, our experiments should, if possible, reproduce conditions which are normal to the healthy body. Such experiments are truly physiological. The same, however, cannot be said of many of the earlier experiments dealing with this subject. They brought to light a number of factors which can alter lymph formation, but under experimental conditions which were highly abnormal. For instance, the injection of leech extract into a dog increases the lymph flow along the thoracic duct. This is a wholly abnormal condition, and it does not follow that the factors involved play any part in normal lymph formation. The first thing to be decided is, under what physiological conditions in the body is the formation of lymph altered. And then to discuss by what process or factors this alteration is brought about.

The answer to the first question has been given by the work chiefly of Asher and his pupils.

Asher's view was first published in 1897. He maintains that lymph in a lymphatic vessel is a product of the activity of tissue cells and independent of blood pressure. The immediate cause of increased lymph flow is to be looked for in an increase of the metabolism or specific activity of cells, and according to the intensity of this activity the quantity and concentration of the lymph will alter.

Asher draws a sharp distinction between tissue fluid and lymph in a lymphatic vessel. Tissue fluid is derived from the blood and carries nutriment to the tissues: it also receives katabolic products of cell activity. Of these products some are non-poisonous and pass back direct into the capillary; others, however, are poisonous, are removed from the tissue spaces in solution as lymph, and in their passage through lymph

glands are rendered non-toxic before passing into the general circulation.

The experimental evidence brought forward by Asher to show the toxicity of lymph consisted in injecting into the carotid artery of a dog 20 c.c. of its own defibrinated lymph derived from the cervical lymph trunk. He observed alterations in the blood pressure curve and in the respiratory and cardiac rhythms, which were absent when similar quantities of normal salt solution or of the animal's own defibrinated blood were injected. In the cat, Brodie has shown that intravenous injection of the animal's own blood serum or defibrinated hydrocele fluid causes arrest of respiration, inhibition of the heart, and general vaso-dilatation; that the active substance is a proteid, produced when the blood clots and connected with the presence of blood-corpuscles. He was unable to obtain similar typical effects in dogs. Considering the smallness of the effects observed by Asher, it may well be doubted whether they were due to poisonous substances produced by tissue metabolism, and not to substances liberated into the lymph during defibrination. Asher has not shown that lymph after leaving lymph glands is less toxic than before it has reached them.

Asher's most important experiments in proof of the proposition that increased lymph flow always accompanies increased cell activity are the following:—

(1) Activity of the salivary glands brought about reflexly from the mouth increases the flow along the cervical lymph trunk. But if the glandular activity is prevented by atropin, then, as Cohnheim showed, stimulation of the chorda tympani leads to no increase in lymph flow in spite of the vaso-dilatation. Bainbridge has confirmed this result.

(2) Intravenous injections of bile or the liberation of hæmoglobin within the circulation are known to increase the activity of the liver. Correspondingly they increase the flow of a more concentrated lymph along the thoracic duct. Bainbridge has confirmed these results, using isotonic solutions of sodium taurocholate and hæmoglobin. He has further shown that the pressures in the aorta, inferior vena cava, and portal vein are unaltered; that the lymph does come from the liver, but is not so concentrated as that obtained by injection of peptone; and that the results of their injection and their mode of action is different from either class of Heidenhain's lymphagogues.

(3) A meal of pure proteid causes an increased flow of more concentrated lymph along the thoracic duct. The curve of this increased flow runs parallel to the curve of nitrogen excreted in the urine, showing maxima at the second and sixth hours after the meal. Asher did not investigate the vascular changes during digestion. He also showed that by injecting the solution of an assimilable proteid, like casein, into the portal vein, he produced an increased flow along the thoracic duct, presumably by stimulating the liver cells.

(4) He attempted to show that injections of solutions of ammonium carbonate or tartarate into the portal vein increased the flow of a more concentrated lymph along the thoracic duct by stimulating the liver cells to produce urea. Bainbridge has repeated these experiments, but was unable to confirm Asher's conclusions. He found that all ammonium salts were highly toxic, producing intravascular clotting and cardiac failure, or muscular twitching and fall in arterial pressure or extreme dyspnœa; that an increased lymph flow only occurred when toxic symptoms were present and could be amply explained by them: further, that in fasting dogs ammonium chloride is not converted into urea, and yet it causes an increased lymph flow.

(5) He further attempted to show that the formation of glycogen by the liver following an extremely slow injection of dextrose into the portal vein was accompanied by an increased flow of lymph of unaltered concentration. The experiments are not conclusive, no data are given to show that glycogen was formed, and the injection of a crystalloid raises other possible explanations.

(6) Bainbridge has shown that the secretion of pancreatic juice caused by the injection of secretin is accompanied by an increased flow of lymph along the thoracic duct. Secretin also causes vaso-dilatation of the pancreas, lasting as long as the juice is being secreted, but, as in the case of the salivary glands and liver, it is probable that the increased lymph flow is independent of the vaso-dilatation, although this has not been proved for the pancreas.

Asher and others have thus demonstrated that in certain cases increased tissue activity is associated with increased lymph flow, and, as no instance is known of increased cell metabolism unaccompanied by increased lymph formation, it is fair to assume that this association is constant.

But is increased cell metabolism the only physiological condition associated with increased lymph flow? Asher considers that it is. So far as this association is concerned we have still to discuss the way in which lymph formation is altered during tissue activity. We shall deal with this point later.

Previous observers, Ludwig, Heidenhain, Hamburger, Starling, &c., had demonstrated a number of experimental means by which lymph flow could be increased. It is true that in many cases it would be hard to maintain that the experiments reproduced conditions which are known to occur in the normal body. Nevertheless these experiments are of great importance in the history of the subject. Besides detailing these older experiments and considering the views held on their mode of action, it is necessary to consider their relation to the results obtained by Asher. There are three possible relations between the factors concerned in lymph formation as demonstrated by these experiments and tissue activity: they might alter lymph flow by affecting tissue activity, or they might be the means by which tissue activity brings about an altered lymph flow, or they might constitute an independent experimental means of altering lymph flow which might or might not play a part in lymph formation in the normal body.

Before proceeding to the actual experiments it is necessary to consider briefly two factors, filtration and diffusion, which play a considerable part in these views and experiments.

By filtration is meant the passage of water and dissolved substances through a membrane owing to differences of hydrostatic pressure on its two sides, which difference constit the filtering force.

The filtrate obtained from any given fluid varies both in quantity and composition with changes in the filtering force and the permeability of the membrane.

A dead animal membrane is of such a nature that while it lets through all crystalloids in solution, it keeps back all solid undissolved substances and a proportion of dissolved colloids.

If such a fluid as blood serum is filtered through an animal membrane it is found that the total quantity of filtrate varies directly as the filtering force, and for different membranes directly as the permeability of the membrane.

As regards the composition of the filtrate, a distinction has to

be drawn between the crystalloids and colloids in solution. In the filtration of crystalloids the concentration of the filtrate is approximately that of the original solution. In the filtration of colloids the concentration of the filtrate is always less than that of the original solution, and varies directly as the permeability of the membrane. The relation of the filtering force to the concentration of colloid in the filtrate is different. With a given membrane, when the filtering force is increased the absolute quantity of colloid passing through is also increased: but its concentration is decreased because the rate of filtration of the solvent rises even more rapidly.

By diffusion (dialysis) is meant the interchange of fluid or substance in solution between two fluids separated by a permeable membrane and under the same hydrostatic pressure.

The rate of diffusion depends upon the relation between the permeability of the membrane and the size of the molecules of the substance in solution. The molecules of blood proteids are so large that their diffusibility through dead animal membranes is insignificant and may be disregarded.

Neither experimental filtration nor diffusion alone represents the conditions in the body. To imitate these we must have a fluid circulating in tubes under pressure and separated by a porous membrane from a different fluid under less pressure. The interchange between these two fluids will be the sum of filtration and diffusion, a process which has been called transudation.

The amount of transudate formed experimentally under these conditions depends on three factors: (*a*) the difference between the hydrostatic pressure of the fluid inside and outside the membrane—*i.e.* the filtering force, (*b*) the permeability of the membrane, and (*c*) the difference between the osmotic pressure of the two fluids.

Theory of Ludwig.—In 1850 Ludwig published his celebrated mechanical view of lymph formation, into which only two factors entered, namely, filtration and diffusion.

According to this view lymph is essentially the fluid part of the blood filtered off by the capillary blood pressure through the capillary wall and altered by the membrane introducing a great resistance to the passage of proteids. In quantity it will vary directly as the variations in the capillary pressure. Its composition is the resultant of two variables: the composition of the blood,

which may be altered by food, excretion by the kidneys, &c.; and the composition of the tissue fluid, which is altered by metabolism in the tissues. In both the latter cases the changes taking place in the lymph are brought about by diffusion.

The experimental evidence brought forward by Ludwig in support of his theory was an attempt to show that there was a direct relationship between capillary blood pressure and lymph flow from a part. He and his pupils showed that lymph flow was increased by the following means: by obstructing the venous return, by overfilling the vascular system with either blood or normal salt solution. Vaso-dilatation brought about by section of vaso-constrictor nerves gave variable results, but active vaso-dilatation from stimulation of vaso-dilater nerves generally caused an increased lymph flow. Most subsequent observers have confirmed these results. But Lazarus-Barlow did not find that ligature of the femoral vein in a dog increased the lymph flow, although the pressure in the vein was from 50 to 75 mm. Hg for an hour. Although, in the extremities at any rate, no very striking parallelism between lymph flow and alterations of capillary blood pressure either from the arterial or venous side has been found to exist, there can be no doubt that variations in capillary blood pressure are associated with an interchange of fluid between the capillaries and tissue spaces. For, estimations of the corpuscular and hæmoglobin content of venous blood have shown that increased capillary blood pressure is associated with an increased concentration of the blood and a decrease of blood pressure with a decreased concentration.

With regard to diffusion between blood and lymph, it had been known long before Ludwig's theory was published that dyes, sugar, salts, &c., introduced into the blood or lymph soon appeared in the other.

Of these two factors, diffusion and filtration, no one, as we shall see, doubts that diffusion may play a not inconsiderable part in experimental and physiological lymph formation. But in regard to filtration both Heidenhain and Hamburger have denied that it is an important factor even in experimental lymph formation, and we must now consider their objections to Ludwig's view.

View of Heidenhain.—This view was published in 1891. Experimenting on the lymph flow from the thoracic duct in dogs, Heidenhain came to the conclusion that it was impossible to explain

his results by filtration and diffusion. He therefore assumed the intervention of the vital activity of cells, and put forward the view that lymph is secreted by the cells of the capillary wall. This secretory activity is aided by physical forces, but can and does override them. He further considered that he had shown that this secretion could be stimulated by chemical substances introduced into the blood, and suggested that in the body the products of cell katabolism had a similar lymphagogue action. He also suggested that the secretion might be under the direct control of the nervous system.

The experiments on which Heidenhain relied to show that blood pressure and lymph flow do not run parallel have been repeated by Starling, and, although the results have been confirmed, it has been shown that they are open to explanation on Ludwig's theory. Heidenhain's more important experiments were:—

(1) Ligature of the portal vein. This increased the lymph flow four to five times; the lymph contains less proteid per cent. than normal and is blood-stained. This result is obviously capable of explanation on the filtration theory, as due to an increased capillary pressure causing increased filtration and a decreased concentration of proteid in the filtrate.

(2) Obstruction of the thoracic aorta causes a great fall of blood pressure below the obstruction. The lymph flow is generally decreased, but may be unaltered or slightly increased, but in all cases the percentage of proteid is increased.

Starling pointed out that since Heidenhain considered change only in the arterial and not in the venous pressures, it was impossible for him to deduce the change in the capillary pressures. He showed that pressure in the portal vein falls greatly, but in the inferior vena cava is unaltered or slightly increased. Therefore the pressure in the intestinal capillaries is very far below normal, but in the liver capillaries is either unaltered or slightly increased. By ligaturing the hepatic lymphatics he stopped the flow along the thoracic duct absolutely, showing that in this experiment all the lymph was coming from the liver. By this and other experiments he demonstrated that the lymph in the thoracic duct is a mixture of two fluids, one with a high percentage of proteid coming from the liver and the other with a less percentage from the intestines.

(3) Obstruction of the inferior vena cava between the heart and hepatic veins causes a great fall of arterial pressure, the intestines becoming blanched. The lymph flow is increased ten to twenty times, and contains the same increased percentage of proteids as in the previous experiment.

Starling showed that the same fallacy as before underlay Heidenhain's interpretation of the result. The pressure in the aorta may fall to a third, but that in the portal vein and inferior vena cava is considerably increased. The capillary pressure in the intestines is probably decreased on the whole, but that in the liver must be increased three to four times. Ligature of the liver lymphatics stops all flow along the thoracic duct in this experiment.

(4) Injection of any of Heidenhain's second class of lymphagogues, which includes various crystalloids, such as sugar, urea, salts, &c. The injection of these in concentrated solution causes an enormously increased flow of less concentrated lymph and a more watery condition of the blood. The arterial pressure may be slightly increased, but it is not proportional to the lymph flow.

Heidenhain rejected a physical explanation of the action of these substances, in spite of the fact that the increase in lymph flow was known to be proportional to the osmotic pressure of the solution used. The physical explanation he himself proposed and rejected was that the injected crystalloid rapidly diffused into the tissue spaces and attracted fluid there from the tissue cells and fibres. His reason for rejecting it was his observation that directly after the injection was over the percentage of the lymphagogue began to fall in the blood and rise in the lymph, until the lymph contained a greater percentage than the blood. This condition of things he considered incompatible with diffusion, and only capable of explanation by the secretion of the crystalloid into the lymph by the capillary walls.

Leathes had shown that the injection of such solutions increased the volume of the circulating blood by attracting fluid into it—in other words, caused a condition of hydræmic plethora. Starling demonstrated the influence of this condition on capillary blood pressure by measuring the pressures in the aorta, the portal vein, and inferior vena cava simultaneously. He found that while the aortic pressure was but little increased, there was a marked

and short rise in the inferior vena cava and a marked and more prolonged rise in the portal vein. He considered the increased capillary pressure sufficient to explain the whole lymph flow as the result of increased filtration. He further showed that if the plethora be prevented by bleeding the animal previous to the injection, the presence of the crystalloid in the blood failed to increase the lymph f[illegible]. This last observation disposes of Heidenhain's view of the a[illegible]n of these bodies.

Starling's explanation of the lymph flow solely as the result of hydræmic plethora is also no longer tenable. Lazarus-Barlow confirmed Starling's results, but objected to his interpretation of them. He pointed out that the increased lymph flow lasted much longer than the rise in venous pressure. Asher has since shown that the increased lymph flow will continue long after the death of the animal, and must then be independent of capillary pressure.

Heidenhain's second point, that some time after the injection the percentage of the substance may be greater in the lymph than in the blood, has been confirmed by subsequent observers. But Cohnstein has pointed out that it is impossible to infer from this observation that the same difference exists on the two sides of a capillary wall. For, substances injected into the blood take some time to reach the thoracic duct, and will take different lengths of time from different areas. Therefore by examining the lymph in the thoracic duct it is impossible to say what percentage of the substance is present in tissue fluid outside a capillary. He therefore compared, not simultaneous specimens of blood and lymph, but the maximal percentage attained in the lymph, which he found was not higher than that in the blood. This result has been denied by Mendel, who found that the maximal percentage of NaI was greater in the lymph than in the blood, but not if the kidneys were ligatured.

(5) Injection of Heidenhain's first class of lymphagogues, which included such substances as extract of leeches, mussels, and crayfish, Witte's peptone, egg-albumin, &c. To this list has subsequently been added nuclein, various bacterial products and toxins, and extract of strawberries. Their injection causes an increased flow of more concentrated lymph. The arterial pressure is either lowered or unaltered. The blood and lymph lose their coagulability, and the blood becomes more concentrated from loss

of plasma. In proof of the lymphagogue action of these substances Heidenhain further pointed out that after prolonged obstruction of the aorta they cease to act as lymphagogues, owing, he considered, to the death of the capillary endothelium.

Starling showed by ligaturing the liver lymphatics that the increased lymph flow comes chiefly from the liver, which accounts for its higher concentration. He found the pressure in the inferior vena cava unaltered, but that in the portal vein increased owing to general vascular dilatation. He considered the rise in portal pressure insufficient to account for the lymph flow for two reasons: it would not very materially increase the pressure in the liver capillaries, and it was found to last only about half as long as the increased flow of lymph. He pointed out that all these bodies are highly poisonous, and therefore unlikely to stimulate a secretion, and explained their action by an alteration in the other factor concerned in filtration, namely, the permeability of the membrane. He considered that the power of these substances to increase the permeability of the capillaries of the liver, and to a less extent those of the intestines and skin as well, is analogous to the action of curare and scalding on the limb capillaries. He has explained that the reason why prolonged obstruction of the aorta destroys the action of these lymphagogues, is, not because a short obstruction does not lead to an increased permeability of the hepatic capillaries, but is due to the fact that prolonged obstruction so damages the liver endothelium that the blood flow through the liver is seriously interfered with.

Starling's interpretation of the action of these substances has been criticised by Asher on the grounds that obstruction of the aorta, while it prevents their action, can yet be shown to lead to greatly increased capillary permeability. He showed that an injection of normal salt solution, which alone produced no effect, after occlusion of the aorta caused transudation into the serous cavities. Asher, however, admits that peptone alters lymph flow by influencing the liver, and there is nothing in his observation bearing upon either the blood flow through the liver or the permeability of its capillaries.

A more serious criticism of Starling's explanation is that it is no explanation but simply a restatement of the result. For, except by the result, we have as yet no means of judging of the permeability of living membranes. And when, as here, more than

one factor concerned in lymph formation might be altered, it is impossible to say that a change in permeability is the explanation. Thus, Cohnstein accounted for the action of these substances by alterations not in the capillary wall but in the blood. Changes in coagulability and destruction of leucocytes are known to follow injection. And he showed that the blood serum of dogs after injection transuded more rapidly through dead membranes than normal serum.

View of Hamburger.—Independently of Heidenhain, Hamburger came to the same conclusion that lymph formation could not be explained as a physical process, and must therefore be a secretion by the cells of the capillary wall. This secretion was stirred up by substances formed during tissue activity and reaching the capillary wall by way either of the tissue fluid or blood stream.

He worked at the lymph flow along the main cervical trunk of the horse. He looked upon this lymph as arising solely from the muscles of the neck, and overlooked the fact, as Asher pointed out, that it is also derived from the salivary glands, thyroid, brain, &c. His more important experiments were the following:—

(1) Specimens of normal lymph and serum from the jugular vein taken simultaneously may show very different percentage compositions, and further, the osmotic pressure of the lymph may be higher than that of the serum.

Cohnstein's criticism of conclusions drawn from differences in the percentage composition of simultaneous specimens of lymph and serum have already been referred to. Leathes has confirmed Hamburger's observation that lymph from a lymphatic vessel constantly under all conditions has a higher osmotic pressure than serum. This is no objection to a physical theory of lymph formation, but, as Leathes and Starling have pointed out, only to be expected from the fact that products of cell katabolism must pass into the tissue fluid; a point which will be dealt with later.

(2) With the lymph from a horse at rest he compared that obtained when the horse ate, and when, with its neck at rest, it did work with the rest of its body. The "food" and "work" lymphs were increased in quantity and had the same composition. But the carotid pressure was different in the two experiments; it rose in the first and fell in the second. He concluded that as "work" lymph could not be caused by filtration, it must be due to a secretion stirred up by products of metabolism from the trunk

and extremities reaching the neck capillaries by way of the circulation. He further showed that the greatest lymph flow was produced when a horse ate during compression of the jugular veins, a result which he explained by the accumulation of metabolic products leading to increased secretion of lymph.

These experiments are obviously inconclusive and open to other interpretations. Observations on the carotid pressure give no information about capillary pressure; during exercise of the trunk and legs it is extremely unlikely that the neck was completely at rest, and no account was taken of the influence of increased respiratory movements causing increased lymph flow along the cervical trunk: compression of the jugular veins will increase the capillary pressure of the part.

Hamburger has, however, stated later that during exercise of the body with the neck at rest there is a fall in pressure not only in the carotid but also in the jugular, and yet the lymph flow is increased three to five times. It is impossible to explain this lymph flow by filtration: but until we know that in this experiment the muscles of the head and neck are not doing work, it is impossible to say that the lymph flow is not caused by increased tissue activity.

(3) Another important observation made by Hamburger against a pure filtration theory of lymph formation was the length of time a lymph flow could continue after death, a phenomenon which will be considered later and shown to be due probably to osmosis.

When we consider Heidenhain's and Hamburger's experiments as a whole, we may certainly conclude that they fail to bring forward any evidence which necessitates a belief in a secretory activity of the capillary wall. Their experiments are capable of a simpler physical explanation, which has been summed up by Starling as follows: "The formation of lymph and its composition, apart from the changes brought about by diffusion and osmosis between it and the tissues it bathes, depend entirely on two factors—(1) The permeability of the capillary wall; (2) The intracapillary blood pressure." But, whilst we may admit that the explanation of these and similar experiments is probably to be found in the alteration of mechanical factors, it does not follow that alterations in the same factors bring about the normal variations in lymph formation. For these experiments introduce changes so gross that they might well overwhelm and mask normal

vital action of the capillary wall and the tissue cells in controlling and regulating lymph formation.

Asher has given a wholly different explanation of these experiments. For he has attempted to prove the view that tissue activity is under all circumstances the sole cause of lymph flow, and that any effect, whether on blood pressure, diffusion, or osmosis, will affect lymph formation only indirectly, by altering tissue activity. In order to prove this view it is necessary to prove two things. Firstly, that increased lymph flow invariably accompanies increased tissue activity—this he has shown, as we have already seen; and secondly, that without increased cell activity there never is increased lymph flow, even experimentally. In showing this Asher has been much less successful, and especially in his attempt to explain Heidenhain's experiments in accordance with his view.

(1) Heidenhain's first class of lymphagogues, according to Asher, act by increasing the activity of the liver, just as do bile and hæmoglobin. He experimented with peptone, and found that it causes an increased flow of bile from a permanent biliary fistula. He has since stated that the result can be obtained but is less constant with a temporary fistula. Ellinger has, however, shown that peptone does not lead to an increased secretion of bile with either a temporary or permanent fistula, but only to an emptying of the gall bladder. For he found that peptone caused no increased flow of bile after ligature of the cystic duct. He found that leech extract had the same negative effect of bile secretion. Bainbridge, using a temporary fistula, was also unable to confirm Asher's result with peptone. He further pointed out that the lymph obtained after peptone is of higher concentration than that after injections of bile salts or hæmoglobin, and consequently that there is no reason for believing with Asher that they all act in the same way. Some recent work by Knsmine throws light on the mode of action of peptone, leech, and crayfish extracts. She found that their injection causes profound changes in the microscopical appearances of the liver cells, and therefore presumably in their metabolism. The histological appearances of the cells suggest in the main an acute degeneration. It hardly seems right in the face of this to account for the increased lymph flow by saying that these substances increase the activity of the liver cells, if by this is meant increase in physiological activity, such as we believe

is produced by an injection of bile or hæmoglobin. It seems probable that Heidenhain's first class of lymphagogues are liver poisons as opposed to cholagogues, and that they alter lymph production in at least two ways; by producing a pathological katabolism of the cell protoplasm whereby the osmotic pressure of the tissue fluids would be greatly increased; and by altering the capillary walls, and to a less extent the capillary blood pressure.

(2) Asher suggests that obstructions of the inferior vena cava increases lymph flow by increasing the activity of the liver, on the grounds that it causes the same change in the blood and lymph as does an injection of peptone. Since there is no reason for thinking that peptone acts in the way he suggests, there is equally no reason for thinking that obstruction of the inferior vena cava does. In fact, Asher on several occasions comes perilously near to arguing in a circle. He observes an increased lymph flow, and explains it by the very thing he has to show, namely, an increased tissue activity. It seems probable that obstruction of the inferior vena cava does alter the lymph flow by the same means as Heidenhain's first class of lymphagogues, but there is no reason for thinking that the means is increased physiological cell activity.

(3) With regard to the action of Heidenhain's second class of lymphagogues, Asher has tried to show two things—firstly, that it cannot be due to increased filtration, as Starling thought, and secondly, that it is probably due to increased tissue activity, aided perhaps by diffusion and osmosis.

Asher found, like Heidenhain, that intravenous injection of a concentrated sugar solution increased the flow along the thoracic duct, and, like Starling, that if plethora was prevented by previous bleeding, no increased flow took place. But he has also shown that if the injection is made a few minutes before the animal dies, the increased flow still continues for several hours, and may reach its maximum a quarter of an hour after death. It is clear that Starling's explanation by increased filtration due to increased capillary pressure does not suffice. Filtration may be a factor in the living animal, but there must be another as well to explain the *post-mortem* flow. Asher suggests that the older physical explanation of Heidenhain, namely, that the crystalloid rapidly diffuses out into the tissue space, and when there attracts water from the tissues, meets the case. He has tried to confirm this conclusion by the observation that when plethora is prevented by

venesection before the injection, the sugar concentration of the blood falls strikingly slowly—in other words, the diffusion of sugar into the tissue space is extremely slow. He does not attempt to explain this remarkable observation, which is wholly opposed to the results obtained by Leathes, who found that within a few minutes of injecting sugar its osmotic pressure in the blood and lymph was equal.

Asher makes the following points as showing that the injection of a strong sugar solution acts primarily by increasing tissue activity. Like Cohnheim and Lichtheim, who produced hydræmic plethora by injecting normal salt solution, Asher finds his injection also causes marked secretion of fluid from the mouth, nose, liver, kidneys, and into the alimentary canal; that these secretions, like the increased lymph flow, are absent if plethora is prevented by previous venesection; that the *post-mortem* lymph flow is accompanied by a parallel *post-mortem* salivary secretion, and like the latter must therefore be due primarily to increased tissue activity. He does not explain how sugar causes increased tissue activity, nor why this activity should be prevented by previous venesection, unless we are to ascribe it to the slow rate of diffusion of sugar into the tissue of spaces after venesection.

A review of the results of Asher's experiments shows that while he is certainly right in regarding tissue activity as a cause of increased lymph flow, it cannot be looked upon as the sole experimental means by which lymph formation can be increased. For the results of many of the experiments of his predecessors cannot be explained by alterations in physiological tissue activity. On the other hand, the experimental conditions under which most of these results were obtained, are purely artificial, and bear no obvious resemblance to conditions which exist in the normal body. We may therefore conclude in answer to the question, under what physiological conditions in the body is the formation of lymph altered, that only one such condition is known, namely, alterations in tissue metabolism.

In regard to the way in which tissue activity increases lymph flow, Asher points out that there are two possibilities. The first is that during tissue activity katabolic products are formed which, reaching the tissue fluid, must alter the osmotic pressure of lymph as compared with that of the blood. Starling had already pointed to the same fact in the following terms: "Since the final result of

metabolism in the animal body or in an animal cell is disintegration, a breaking down of large complex unstable molecules of high potential energy into a great number of small simple stable molecules of small potential energy, the total output of an animal cell must have a higher osmotic pressure than the total income, so that all the metabolic changes in the tissues would tend t increase the osmotic pressure of the lymph with which they are bathed." This increased osmotic pressure of the tissue fluid would lead to a flow of water from the blood into the tissue spaces, and so to an increased flow along the lymphatic vessels.

Asher, however, seems rather to favour the second possibility, namely, that lymph during tissue activity is formed by a process analogous to a secretion. That is to say, a gland cell when active turns out its specific secretion into its duct on one side and lymph into a tissue space on the other. His reason for adopting this view was his observations on the parallelism of the flow of lymph and saliv[illegible] *post-mortem*, already referred to. He concluded that both [illegible]na are due to the same forces, and that since the pour[illegible] of saliva is a secretion, so must also be the formation of l[illegible] This, however, does not necessarily follow; even supposing both phenomena are due to the same forces, it may equally well be that diffusion and osmosis cause the lymph flow and are also of great importance in this *post-mortem* gland secretion. A phenomena somewhat similar to this *post-mortem* salivary secretion has been observed by Mathews. If the blood supply is cut off for twenty-five minutes from the sub-maxillary gland and then readmitted, a marked vaso-dilatation takes place, and the gland secretes rapidly. He accounts for this by increased osmotic pressure within the cells.

Tissue activity may be held to increase lymph flow, in part at any rate, by raising the osmotic pressure of the tissue fluid; and this must be especially marked during proteid metabolism. For proteids have at most a minute osmotic pressure. And when the cell takes them up from tissue fluid it will not thereby materially decrease the osmotic pressure of that fluid, but by returning a number of smaller stable molecules it will greatly increase the osmotic pressure of tissue fluid and lymph. We should therefore expect to find that during tissue metabolism the osmotic pressure of the lymph would tend to keep above that of the blood. And this Leathes has found to be the case.

It is unnecessary to invoke secretion by the capillary wall to explain the phenomena, as Hamburger did.

During the death of a cell the disproportion between the osmotic pressures of its total income and output must be great. In this is probably to be found the explanation of the *post-mortem* flow observed by Hamburger. It will also take part in producing the *post-mortem* flow found to take place by Asher after the injection of sugar, and by Mendel and Hooker after injections of strawberry extracts or peptone.

We may therefore adopt the working hypothesis that tissue activity alters lymph flow by physical means, and we shall consider the process in detail when we discuss how the tissues are nourished.

Experiments dealing with Lymph Absorption

Absorption from the Connective Tissue Spaces.—There are many old and recent observations to show that dyes, salts, and other foreign substances in solution are rapidly taken up by blood-vessels, when introduced into tissues. This absorption by blood-vessels is due really to diffusion taking place between the extra-vascular fluid and the blood as long as any difference in their composition exists.

Heidenhain denied that it was possible for the blood-vessels to absorb normal extravascular fluid, because blood only differed from tissue fluid in containing more proteid, and therefore it was impossible for this fluid to return to the blood-vessels by any process of diffusion or absorption.

Starling was the first to offer conclusive proof that the blood-vessels can absorb an isotonic salt solution. He carried on an artificial circulation of a dog's own defibrinated blood separately through each hind leg, one of which was made artificially dropsical by the injection into it of a sodium chloride solution isotonic with the circulating fluid, and the other as a control. The serum was circulated from twelve to twenty-five times, and the percentage of oxy-hæmoglobin and total solids were estimated in it before the experiment and in the fluid which had circulated through each of the legs. He found that the serum which had circulated through the control leg had become slightly concentrated,

but that which had passed through the œdematous leg invariably became less concentrated, showing that fluid had been taken up.

Starling has also pointed out that the old observation that after venesection the blood becomes less concentrated, was to be explained in exactly the same way, by the taking up of isotonic fluid from the tissue spaces; a conclusion which is confirmed by Lazarus-Barlow's observation that after bleeding the sp. gr. of the tissues rises, and by Hamburger's observation that the Δ of the blood serum is unaltered.

Lazarus-Barlow's observations further show the part played by the tissue fluids in keeping the volume of the circulating blood normal. He found that if the hind legs of a dog were tightly bandaged from below upwards, the then overfilled vascular system got rid of its surplus fluid into the tissue spaces within an hour; for the sp. gr. of the tissues fell and that of the blood plasma rose. Conversely when the bandages are taken off the now underfilled vascular system takes up fluid from the tissue spaces, for the sp. gr. of the plasma falls and that of the tissues rises within an hour to normal.

Absorption from the serous cavities might differ materially from that taking place from the connective tissue spaces. For not only are there stomata opening direct into the lymphatics, but material would have to pass through the endothelium lining the cavity before it could reach the capillary.

Starling and Tubby showed that methylene-blue or indigo-carmine introduced into the serous cavities appeared within five minutes in the urine, and not in the thoracic duct for half-an-hour or more. This result was denied by Adler and Meltzer on mistaken grounds, and was confirmed by Mendel and by Starling himself. Absorption by blood-vessels was therefore proved.

The importance of experiments on absorption from serous cavities has not been in deciding whether blood-vessels absorb at all or can absorb an isotonic fluid, but in showing the interchanges between the blood and fluid which take place during absorption, and the relative importance of blood-vessels and lymphatics.

The researches of Leathes and Starling, Hamburger, and Roth and many others, have shown the nature of the interchanges between the blood and fluid injected into the pleural or peritoneal cavity. If the fluid is a hypotonic salt solution, the following

changes are observed in it: (*a*) The fluid rapidly decreases in quantity owing to water being taken up by the blood. This goes on until the osmotic pressure of the fluid left behind is raised to that of the blood. (*b*) At the same time the soluble constituents of the blood diffuse into the fluid, and the salt of the fluid into the blood, until there is equal concentration of all diffusible substances on the two sides of the capillary wall. If the salt solution introduced is hypertonic corresponding changes take place. If the fluid is isotonic, only the changes under (*b*) will take place; the fluid will remain throughout isotonic, but will be different in composition from that introduced.

Isotonic salt solutions are absorbed with rapidity from the serous cavities, but the conditions of the experiment render it impossible to decide finally whether they are absorbed only by blood-vessels or by both blood-vessels and lymphatics. Large quantities are absorbed without increasing the flow in the thoracic duct; but it is still possible, as Cohnstein maintained, that the fluid has been taken up by the subserous lymphatics and so has failed to reach the thoracic duct during the experiment. Starling showed that ligature of both thoracic ducts and the right innominate vein did not prevent absorption; but, on the other hand, that carmine injected with the solution could be traced to the lymphatic glands in the anterior mediastinum, showing that fluid had passed along lymphatics. It is probable, however, that in the absorption of salt solutions the blood-vessels play the more important part.

It has been shown that absorption can take place independently of the activity either of the endothelium lining the serous space or of that forming the capillary wall. Leathes and Starling found that scalding or killing the endothelium with a poisonous solution of sodium fluoride did not affect absorption. Hamburger showed that absorption took place from the abdomen of an animal twenty-two hours after death. Although absorption can take place independently of the activity of living cells, the explanation of how it takes place at all is much less clear.

Mechanism of the Absorption of Isotonic Salt Solutions.—Starling has pointed out that only two physical processes seem available for explaining absorption—backward filtration or osmosis with diffusion.

Backward Filtration.—The pressure under which tissue fluid normally exists has been estimated by Landerer at from $\frac{1}{2}$ to $\frac{3}{4}$

of the capillary blood pressure. Direct measurements gave 40 mm. H_2O for the liver and 140 mm. H_2O for the skin. If the capillary pressure fell considerably below that of the tissue spaces, backward filtration might theoretically take place for a time. If, however, this relative increase of the extravascular above the intracapillary pressure were communicated to the outside of the veins, they would be strangled, and the circulation through the part cease. Starling has pointed out that this result might not ensue, if the vessels were bound to the surrounding tissues by radiating fibres whose pull would tend to keep the vessels patent. He found that the injection of normal salt solution under high pressure into the subcutaneous tissue of a dog's leg greatly decreased the venous outflow. The same was true of the submaxillary gland and tongue. In these regions, at any rate, it would appear to be impossible for backward filtration to take place.

Osmosis with Diffusion.—Starling has shown that blood serum as against a non-proteid salt solution, otherwise isotonic with it, possesses an osmotic pressure of about 30 mm. Hg when separated by gelatine between two layers of peritoneal membrane. He supposes that the capillary wall, like such a dead membrane, is more or less impermeable to proteids, that there is in consequence a less concentration of proteid outside than inside the capillary, and that the osmotic pressure of proteids, although trifling when compared with that of crystalloids, can attract water from the tissue spaces into the circulation.

The absorption of an isotonic salt solution from the subcutaneous tissue or serous cavity would take place according to this view in the following way. Water would be attracted into the capillaries by the proteids, and as this would raise the concentration of the diffusible substances in the solution above that in the blood, they would diffuse into the blood until the fluid was again isotonic. Then osmosis would again come into play, and alternate with diffusion until the whole fluid had been absorbed. It has been shown by experiment that, when an isotonic solution containing more proteid than the plasma is introduced into the peritoneal cavity, an isotonic fluid passes from the blood into the cavity until the concentration of proteid in the cavity has been reduced to that of the plasma. According to Starling, therefore, while "capillary blood pressure determines

transudation, the osmotic pressure of the proteids of the serum determines absorption."

Lazarus-Barlow explains absorption by the same alternations of osmosis and diffusion, but considers that osmosis has a different origin. According to him, the fluid which contains a greater concentration of proteid will always in the end increase in quantity at the expense of a fluid which contains a less concentration, because of the different degrees to which the membrane is clogged on its two sides by proteid. The concentration of diffusible substances is in consequence slightly greater on the side with more proteid, and osmosis is started.

Starling has applied the same factors—the osmotic pressure of proteids and the relative impermeability of the capillary wall—to the explanation of the accurate regulation of the volume of the circulating blood. He points out that, granted his premises, there will always exist a relation between the capillary pressure and the osmotic attraction of the blood proteids for extravascular fluid. This relation must be one of balance, unless one force is stronger than the other, when either transudation or absorption will take place, as the case may be. If by any means the capillary pressure is increased, more fluid containing a less concentration of proteid will be transuded. This will go on until the increased filtering force is balanced again by an increased absorbing force, represented by the now greater difference in the proteid concentration on the two sides of the capillary wall. And when a fresh point of balance has thus been reached, the tissue spaces will be filled with a larger quantity of fluid derived from the blood. With a fall in capillary pressure exactly the opposite will happen. The decreased filtering force will become balanced by a diminution in the absorbing force. This will take place by isotonic fluid being absorbed into the capillaries, thus raising the proteid concentration in the extravascular fluid and reducing the difference in proteid concentration on the two sides of the capillary wall. When the decreased absorbing force again balances the decreased capillary pressure, the tissue spaces will contain less fluid than before and the blood more.

Starling has observed that lymph in the lymphatics from different parts of the body contains very different percentages of proteid. Lymph from a limb has 2 to 3 per cent., that from the intestines 4 to 6 per cent., and that from the liver 6 to 8

per cent. He associates these differences with different permeabilities of the capillary walls for proteid. Granted that this is so, certain corollaries follow. (1) The more impermeable the capillary the greater will the blood pressure have to be in order to drive proteid through. Correspondingly we find that the capillary pressure in a limb is about 20 mm. Hg, in the liver about 5 mm. Hg, and in the intestines something intermediate between the two. But it is equally true that, if the permeability of the capillary walls were the same in all three areas, the difference in capillary pressure would explain the different concentrations of colloid in the filtrate according to the laws of filtration. (2) The more impermeable the capillary wall the more will a rise in capillary pressure increase the force tending to absorption by decreasing the concentrations of colloid in the filtrate. In this way Starling explains the apparent fact that in a limb there may be no flow along the lymphatics although transudation is presumably taking place. On the other hand, in the areas drained by the thoracic duct there is a constant flow of lymph, which is rapidly accelerated by any increase in transudation. In the same way he would explain the different effects on lymph flow in a limb produced by ligature of the veins, and vaso-dilatation of the arteries. In the former there is increased transudation and decreased absorption, but in the latter increased transudation and a nearly corresponding increase in absorption.

These views of Starling on the part played by the osmotic pressure of proteids in determining absorption have been criticised by Moore and Parker. The premises necessary for his view are —a relative impermeability of the capillary to proteid, and consequent differences in concentration of proteid on its two sides. They point out that the former has never been shown by direct experiment, and that the latter is incapable of proof; for we cannot obtain tissue fluid, but only its overflow into the lymphatics after the tissues have removed some proteid from it. They would explain the different percentage of proteid in lymph as compared with blood, and in lymph from different parts of the body, by the varying amounts of proteid abstracted from the tissue fluid by the tissues. From this it would follow that anything which increased the rate of flow of tissue fluid through the tissues must also increase the percentage of proteid which the lymph contains. This is often the case, but by no means always

so, as the effect of injections of concentrated solutions of crystalloids and solutions of bile salts or hæmoglobin show.

They further point out that the absence of lymph flow from a resting limb and its presence in an active one can be explained, without invoking the impermeability of capillaries to proteid, by admitting exactly the opposite, namely, that the wall is readily permeable to proteids. For in the resting limb proteid, like O_2 and other substances in solution, would be conveyed to the tissue cells by diffusion without any accompanying transference of the solvent. But in the active limb, when this mode of providing nutriment would not suffice, there would be added pressure filtration, carrying proteid and the other dissolved substances along with the solvent and at the same rate. But this fails to explain why liver lymph is so wholly different in its proteid content from that obtained from a limb even during its greatest activity; especially when we consider how much lower the capillary pressure in the liver probably is. Further, it offers no obvious explanation of the action of curare, which reduces capillary pressure and yet raises the proteid content of the lymph from a limb up to that from the liver, a rise which no activity of the limb can bring about.

They attempt to show that even granting Starling's premises, the osmotic pressure of proteids could not effect absorption. They point out the obvious fact that the total osmotic pressure of plasma against an otherwise isotonic salt solution is not available for absorption, but has to be reduced because of the presence of proteid in tissue fluid, and because the capillary wall is partially permeable to proteid. It is impossible to estimate the magnitude of this correction; but they estimate that a force of only 6 mm. Hg is available for absorption. It is clear that absorption could only take place when the force tending to cause it is greater than the force tending to produce filtration, *i.e.* when the available osmotic pressure of the plasma proteid is greater, not than the capillary blood pressure as they state, but than the difference between the hydrostatic pressures of the intravascular and extravascular fluids, which is a very different thing. Considering our complete ignorance of the actual numerical values of the three factors concerned, it is impossible to say more than that Starling's view is not known to be impossible.

Waymouth Reid has brought forward evidence to show that

the actual proteids in serum do not exert an osmotic pressure, but he admits that serum contains something to which gelatine and parchment are impermeable and gives a readable osmotic pressure. His observations, therefore, do not render Starling's view untenable.

The Absorption of Serum.—Starling carried on an artificial circulation of defibrinated blood through a dog's leg, made œdematous with the same serum, but was unable to demonstrate any absorption by blood-vessels. But serum is absorbed from serous cavities, although it takes place more slowly than in the case of isotonic salt solutions. If this absorption of proteids took place by blood-vessels it could only be accounted for either by invoking a secretory activity by the endothelium or by backward filtration, a phenomenon which has not as yet been demonstrated, although Hamburger, Adler, and Meltzer found that moderate pressure does increase the rate at which fluid disappears from the abdominal cavity. Differences in osmotic pressure could not carry proteid through the impermeable capillary wall. Absorption of serum by blood-vessels might be possible to a small extent, according to Starling, by tissue cell eating up the proteid and a corresponding absorption of isotonic fluid taking place. But practically, on a mechanical view of absorption, it is necessary to believe that serum is carried away by the lymphatics. Attempts have been made to show that this is not the case. Orlow found that ligature of the thoracic duct did not prevent absorption from the abdominal cavity, and Hamburger obtained the same result after ligaturing the left innominate vein. These experiments are inconclusive, because the right thoracic duct was unligatured, and the fluid might have passed into the subserous lymphatics, a point which has already been dealt with in discussing the absorption of salt solutions.

Physical Factors available for the Production and Absorption of Tissue Fluid.—We have seen that the following factors have been considered to be available for explaining the experimental results dealing with lymph formation and absorption.

1. The intracapillary blood pressure—P.

2. The pressure of the tissue fluid—p.

$P - p$ constitutes the filtering force, $p - P$ a possible force of backward filtration.[1]

[1] See Editor's note, p. 618.

3. The permeability of the capillary wall.
4. The chemical composition of the blood plasma—O. P.
5. The chemical composition of the tissue fluid—O. L.

The differences in composition between the blood and tissue fluid are responsible for diffusion and osmosis taking place between them. O. P − O. L constitutes a possible absorbing force, which, however, could only act when it is greater than $P - p$.

It is necessary to consider how far we are able to estimate in the body these various factors.

Capillary blood pressure cannot be directly measured. We do not know, for instance, how much P is increased by hydræmic plethora, nor how much it is reduced by a hæmorrhage. We can alter it at will, and infer the magnitude of the alteration by simultaneously measuring the pressures in the corresponding artery and vein.

The pressure of tissue fluid cannot be accurately estimated. We know that it is usually less than the capillary pressure, and that it is derived partly from the capillary pressure and partly from the elasticity of the tissues. Whether it remains constant during alterations of capillary pressure and whether it can be greater than the capillary pressure are unknown.

The permeability of the capillary wall is a factor which we have no means of estimating. The nearest approach for experimental purposes to such a living membrane are various dead animal or vegetable membranes. But how far the unaltering properties of a dead membrane are like those of a capillary wall, and which membrane is least unlike it, are unknown. The permeability of different experimental membranes varies enormously. For instance, Moore and Parker found that fresh peritoneal membrane is permeable to proteids, peritoneum soaked in gelatine is not, vegetable parchment is permeable neither to proteids nor to soaps. Nor do we know how far the experimental means used for altering the permeability of the capillary wall have really brought about their result in that way. All that we actually know is that there is the result, that more lymph of a higher concentration has been formed. It is likely that the capillary wall is [illegible] impermeable to proteids, that the permeability varies in different parts of the body, and even in the same area, as the result of the action of metabolic products upon it. But the exact [illegible]tes are also possible.

The composition of tissue fluid is unknown. Since it is impossible to say at what point of time any specimen of lymph was drained off from the tissue spaces, we cannot compare the osmotic pressures of blood and tissue fluid more than approximately.

That owing the state of our knowledge about these physical factors, there is obviously room for great difficulty in interpreting the results of experiments in which more than one of these factors has probably been at work. We can, however, say that experiments have shown that the importance of osmosis and diffusion is relatively greater, and that of filtration relatively less, than was thought to be the case.

It is impossible at present to prove or deny a mechanical formation and absorption of lymph. As a working hypothesis the mechanical view is the better. It deals with processes which we understand in some measure, and, even if they are ultimately proved to be insufficient, there can be little doubt that they do play a large part in the history of lymph. To explain lymph formation and absorption as a secretion is at present no explanation at all, since it is to invoke a force or process of which we cannot yet form any conception. It implies, further, that we already know the exact conditions of the various physical factors in the body, and that experiments exist which conclusively demonstrate their insufficiency, neither of which is true.

We may briefly recapitulate the conclusions at which we have arrived regarding the formation of lymph in the normal body.

1. The formation of tissue fluid is determined by the metabolism of the tissue cells. The only exception to this is the fact that in order to keep the volume of the circulating blood constant fluid can be taken up from or given out to the tissue spaces.

2. Tissue fluid is not secreted by tissue cells, but is poured out from the capillaries in obedience to osmosis, diffusion, and filtration, which in turn are controlled and determined by the degree of activity of the tissues.

3. Tissue fluid is absorbed into the capillaries by a combination of osmosis and diffusion, but it is uncertain how these forces are brought into play.

4. The cells of the capillary wall do not secrete tissue fluid. There is no evidence that they play any but a passive part in the

formation and absorption of tissue fluid. Being living cells, they possess a wholly unknown permeability, which might be constant or variable.

The Relation of Lymph to the Nutrition of the Tissues

Interchange of material between tissue fluid and blood would be meaningless except as a means of making good an interchange which was taking or had taken place between the cell and the fluid which bathes it. The only exception to this would appear to be the case in which interchange took place, in order to keep the volume and composition of the blood uniform.

Living cells appear to determine themselves the rate at which they take up nourishment; it would therefore seem likely that they initiate the passage of nutriment from the blood to the tissue fluid, just as much as from the tissue fluid to themselves. And, if this passage is brought about by diffusion, osmosis, and filtration, some change in the cell would have to start these processes at work.

Internal respiration is considered to take place by diffusion. Oxygen passes from a point of high pressure in the blood to a point of low pressure in the cell across the tissue fluid, and at a rate determined by the difference in pressure between these two points. And since the oxygen pressure in arterial blood is approximately constant, the rate of diffusion is actually determined by the consumption of oxygen by the cell. CO_2 passes in an opposite direction, according to its rate of manufacture by the tissue. In this process no alteration in the volume of the tissue fluid is necessary. In an exactly analogous way it is possible for all diffusible substances to pass to and from the cell and blood according as the cell needs or manufactures them, and without altering the quantity of tissue fluid. We know that in the case of the limbs such an interchange does go on without any overflow from the tissue spaces into the lymphatics.

We have seen that the osmotic pressure of the total output of a cell must be higher than that of its total income, and especially during the breaking down of proteid; water will, there-

fore, be attracted by osmosis towards the cell. Presumably katabolic products will be at greater concentration within the cell than in the tissue fluid, and in the tissue fluid than in the blood. Consequently water would be attracted into the cell at a rate determined by the magnitude of the osmotic pressure maintained within the cell by its own metabolism, and from the blood into the tissue fluid by the rate at which the metabolic products pass from the cell into the tissue fluid. It is not, therefore, difficult to imagine a possible way in which the cell might regulate the passage of water and all diffusible substances from the blood.

Before we can hope to understand how this regulation is actually carried out we must have information on two other factors, namely, the exact chemical changes that go on in different cells, and the permeability of cells in both directions for various substances. It is possible that it is variations in the metabolism and permeability of cells, more than differences in the permeability of the capillary wall, which determine the variations in the composition and flow of lymph found in different areas, and that the objections which appear to exist to the view of Moore and Parker, that proteids are diffusible in the body, do not hold good.

If blood proteids are non-diffusible, they can only pass through the capillary wall by the force of filtration. By analogy it would seem likely that cells would have the power of regulating this passage from the blood, and if so, they must be able to alter the main factor in filtration, the capillary blood pressure. It is usual for arterial dilatation to accompany tissue activity, and it is probable that arterial vaso-dilatation cannot cause increased tissue activity, therefore tissue activity must cause the vaso-dilatation, or both must be due to a common cause. The latter appears to be the case in the salivary glands; but in muscle and other important tissues no such nervous arrangement is known. In the case of muscle it has been suggested that products of its own metabolism other than acid or CO_2 may cause the vaso-dilatation, and more recently Bayliss and Starling have found evidence that the products of tissue metabolism exert a specific local vaso-dilator action.

This filtration of proteids entails the passage of large quan-

tities of the solvent into the tissue spaces at the same time. According to Starling's view much of this in a limb would be absorbed by the capillaries, and all the more if the tissues actively used up proteid and so increased the effective osmotic difference between the blood and lymph. We should, therefore, expect that vaso-dilatation accompanying tissue activity in a limb would cause a smaller flow of lymph than vaso-dilatation alone ; which would be the exact opposite of the experimental result. It would seem likely that during tissue activity it is the raised osmotic pressure of the tissue fluid which retards reabsorption by the capillaries, and so leads to an overflow along the lymphatics.

The importance of vaso-dilatation to tissue activity is not confined to providing a means by which more proteid can be passed to the tissues. Just as the increased velocity of blood flow aids internal respiration by keeping the pressure of O_2 as high and that of CO_2 as low as possible in the blood. so it will also help the passage of all diffusible foods and metabolic products to and from the cell.

Note.—The Editor would put forward for the reader's consideration a view which he holds, viz. that such thing as a filtration pressure is impossible in the body. In the case of a limb enclosed in the skin, or kidney enclosed in its capsule, the whole semi-fluid mass must be at capillary pressure, just as much as the brain and cerebro-spinal fluid are at capillary pressure—this he has determined experimentally. A filtration pressure can only exist, in the Editor's opinion, when the body is opened at any point. The heart opens into the cœlomic cavity of a simple tubular animal and circulates the cœlomic fluid by its systole and diastole. The whole animal is at the same mean fluid pressure. In the higher animals capillaries take the place of the cœlomic cavity, but the physical conditions are unchanged. The whole of the body fluids, unless influenced by gravity, or the localised compressive action of muscles, or the secretory activity of the cells, are at the same pressure, viz. that of the capillaries. Any organ, such as the kidney, or liver, compressed by muscular action is at the same pressure throughout.

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CHAPTER XVIII

THE MECHANISM OF ABSORPTION FROM THE SMALL INTESTINE

Water and material in solution pass from the lumen of the small intestine through its epithelial lining into the subepithelial lymphatics and blood capillaries. In this, as in every other instance of a similar phenomenon in the body, two opposing explanations of the means by which it is brought about have been put forward. On the one hand, there are those who attempt to explain the result by diffusion, osmosis, filtration and other physical processes, and who look upon the epithelium as an inert membrane with a permeability which is peculiar to itself, and which is constant so long as the physical conditions of the cells remain unaltered. On the other hand, there are those who consider that while the epithelium makes use of these physical processes, it can always control them, and may bring about results in direct defiance of physical laws. The point to be discussed is which of these two views is the nearer to the truth in the case of the small intestine.

In considering experiments on intestinal absorption it is necessary to bear in mind continually certain obvious facts. Normally the fluid of the two sides of the gut epithelium is of very different composition. We have therefore to try and estimate how far the end result of an experiment is due to this difference in the fluid on the two sides of the membrane and how far it may be due to a specific action of the membrane itself. Again, it is necessary to remember that the epithelium lining the intestines, no matter whether it acts as a physical membrane or not, is composed after all of living cells, and although they must be adapted to having on their gut side solutions of very various composition, there must be limits within which alone the epithelium can function in a strictly normal way. When, therefore, we introduce into the gut a

wholly abnormal fluid, such as 0·9 per cent. sodium chloride solution, we may ignore perhaps the effect of the cells upon the salt, but we cannot ignore the possible effect of the salt on the cells. It is certainly a euphemism to call such a solution normal saline; it may be isotonic with the blood, but we know that sodium chloride is by no means an indifferent salt to tissues. And in regard to the effect of ions upon the functional activities of cells, we know that different ions have different effects upon a given cell, and also that the same ion may affect tissues in different ways. In fact, no mixture of salt solutions can be made normal for all tissues. In researches dealing with intestinal absorption, as with many other physiological subjects, the experimental conditions are often so abnormal that, even if some mechanism used in response is clearly brought to light, it cannot be safely assumed that the same mechanism is in constant use in the normal body. No part of the body can have had a more varied education than the alimentary canal, but it is clear that the only strictly physiological experiment on absorption would be one studying the absorption of the normal products of digestion in concentrations which do occur in the alimentary canal. But here we are met by the serious difficulty that between the gut lumen and the subepithelial capillary or lymphatic, the final products of digestion undergo change by ferments, such as lipase, maltose invertin, and erepsin. The ferments very likely act both within and without the very cell whose properties in absorption we wish to investigate. These considerations make the use of solutions of soap, maltose, or peptone objectionable. Dextrose has suggested itself, and being a non-conductor it undergoes no ionisation; but it is not by any means certain that dextrose is a normal constituent in the alimentary canal, at any rate in large quantity. These ferments, when compared with living cells, are extremely resistant to the action of chemical poisons, &c., and it may be stated safely that, long before the ferments were destroyed, the living cells would be killed. In the matter of what concentration can be used with safety we are profoundly in the dark. For, we have no means of knowing at what point the intestinal epithelium ceases to function normally. Experiment has gradually evolved a criterion by which we can say with certainty that the cells are no longer normal; but

whether the cell may be considered normal until that point is reached is unknown.

In considering intestinal absorption, therefore, it is obviously necessary to remember the elementary facts connected with the osmotic laws and their general relation to bioplasm; and a short account of them will help materially the discussion of the experiments to be considered.

Osmosis.—By osmotic pressure is meant the pressure which a diffusible substance in solution exerts on a semi-permeable membrane, *i.e.* one which is permeable to the solvent, but absolutely impermeable to the substance in solution. One physical method of measuring osmotic pressure is to separate in a copper ferrocyanide membrane osmometer a solution from its solvent, and to measure the pressure exerted directly in mm. Hg. This method, however, gives only approximate values, for, the membrane is not strictly impermeable to most substances, although nearly so to cane-sugar. The law of Avogadro-van 't Hoff states that at the same osmotic pressure and the same temperature equal volumes of all dilute solutions contain the same number of molecules; that is to say, at the same temperature equimolecular solutions of different substances in the same solvent must be isotonic with each other. This law opens up another and more accurate physical method of measuring osmotic pressure, namely, by the determination of the depression of the freezing-point. For, the freezing-point of a solution is lower than that of the pure solvent, and the depression of the freezing-point, Δ, is found to be proportional to the concentration of the dissolved substance. It follows, therefore, that if a gram molecule of any substance is dissolved in 100 grm. of the same solvent it ought to have the same Δ. And this is found to be the case. When the solvent is water $\Delta = -18{\cdot}6^{\circ}$ C. Solutions of various substances dissolved in water having the same Δ will consequently have the same osmotic pressure; and this osmotic pressure will correspond to the pressure which the solution would exert on a true semi-permeable membrane. But it was soon found that while equimolecular solutions of some substances, such as sugars, urea, &c., gave their theoretical Δ, this was not so in the case of solutions of the strong inorganic acids, bases, and salts, all of which gave larger actual values than they theoretically should. Thus, a

solution of NaCl, 0·682 grm. in 100 grm. water, was found to give an actual Δ which was 1·95 times greater than its theoretical value. In other words, the actual Δ corresponded to a solution having 1·95 times more molecules in solution than were apparently there. Arrhenius accounted for this discrepancy by believing that these substances underwent electrolytic dissociation or ionisation, and that the ions conducted themselves as independent molecules in the solution. If, then, equimolecular solutions of two substances be made, one of which undergoes ionisation and the other not, the former will contain a larger number of molecules in a given volume, and will therefore have an osmotic pressure greater by the degree of ionisation which it has undergone. By determining the Δ of a solution we ascertain the number of inactive molecules plus the number of active molecules or ions, if any, present in a given volume of the solvent. The dissociative power of solvents, *i.e.* their ability to split up a substance into its ions, is very different. Hence the osmotic pressure of equimolecular solutions of the same substance in various solvents may be very different. Water has the greatest dissociative power of any solvent.

The question arises, how is it known when a substance in solution has undergone ionisation and the degree to which it has taken place? Now, it is the possession of free ions which makes a solution an electric conductor or electrolyte. Ions are the electrically charged particles which together constitute a molecule of an electrolyte, and under the influence of an electric current the anions migrate towards and discharge at the anode, and the kations at the kathode. It must be understood that free ions are actually present in the aqueous solution of an electrolyte because of the dissociative power of the solvent, and that they are not first produced by the action of the electric current. Ions move freely in all directions through a solution until under the influence of an electric current they are driven in two definite directions. Solutions of various substances, therefore, may be divided into conductors and non-conductors, according as the substance undergoes ionisation or not. Thus, 100 per cent. sulphuric acid is a non-conductor, but if water be added it undergoes ionisation and conducts. Up to a certain limit the greater the dilution the greater the ionisation. Since the possession of ions makes a

solution an electrolyte, the degree of ionisation of a given solution can be measured by observing its electrical conductivity, *i.e.* the resistance offered by the solution to the passage of an electric current.

Diffusion.—When the concentration of a dissolved substance is not the same at two points in a solution, the difference of concentration, *i.e.* of osmotic pressure, between the two points moves the substance from the point of higher to the point of lower osmotic pressure by the process of diffusion, and at a velocity proportional to the difference of pressure. Graham divided substances into crystalloids, which diffuse rapidly, and into colloids, which diffuse extremely slowly. A little consideration shows that differences in diffusibility must be a property of molecular weight. For, according to Avogadro-van 't Hoff's law, 1 grm. mol. of a substance dissolved in water and made up to a volume of 22·4 litres exerts at 0° C. an osmotic pressure of 760 mm. Hg; and therefore it follows that the greater the molecular weight the less is the osmotic pressure exerted by each molecule. Now, colloids are substances with very high molecular weight, and therefore they depress the freezing-point of a solution but little, and have a correspondingly low osmotic pressure and diffusion velocity. They are non-conductors. While it is roughly true that crystalloids diffuse through colloidal membranes, such as animal or gelatin membranes, &c., and that colloidal solutions do not, yet there can be no very sharp line between the two; for the diffusion of many crystalloids is hindered by colloidal membranes, and there is evidence that colloids can diffuse into colloids. In the body simple diffusion between two solutions without an intervening membrane is rare. A membrane would complicate diffusion only if it were not equally permeable to all diffusible substances in solution. In the body this complication is added, and it is clear that diffusion must be greatly influenced by the particular permeability of the membrane present.

When we turn to the investigation of the osmotic properties of such physiological fluids as blood, urine, &c., we see that the physical methods yield information which we could not otherwise obtain. Chemical analysis alone cannot yield definite information about the osmotic properties of such solutions; for, their osmotic pressure depends upon the number

of molecules and ions in the solution, and this cannot be determined by chemical analysis. Further, organic substances, such as proteids, present in physiological fluids yield on incineration organic acids, &c., capable of exerting a considerable osmotic pressure, which would have been impossible in the original solution. If, therefore, we wish to know the osmotic pressure of one of these fluids we must determine its Δ. From what has been said it would appear that we could then determine the concentration of the electrolytes present by estimating the electrical conductivity of the fluids. This is true for such a fluid as urine, but in the case of blood, lymph, and other fluids containing large quantities of proteid it would not hold good. For it has been found that the presence of colloids, whilst not altering the diffusion velocity of an electrolyte, does diminish its electrical conductivity. In order to carry out a complete osmotic analysis of serum, &c., it is necessary to employ complicated indirect methods which cannot be discussed here.

When the osmotic pressure of a fluid has been determined, we know only the pressure which the solution would exert on a true semipermeable membrane; but this tells us nothing about how much of this pressure would be exerted on a cell wall, unless we know the permeability of tissue cells for the various substances in solution. Of the actual permeability of tissue cells for various substances we know very little. De Vries estimated the osmotic pressures of various solutions by immersing in them tangential sections of Tradescantia leaves. When the solution had an osmotic pressure just higher than the contents of the cell, the protoplasm was observed to undergo plasmolysis and shrink up. There is no doubt that this vegetable cell wall is a more or less semipermeable membrane for some sugars and salts. In the case of animal cells we know little of the permeability of any, excepting red blood-corpuscles. Hamburger estimated osmotic pressures by adding red blood-corpuscles to various strengths of different salt solutions. If the solution is hypotonic to the corpuscles, they swell up, and their hæmoglobin is liberated. If two strengths of a solution are found, one of which just does and the other does not produce hæmolysis, then the mean strength would be isotonic with the animal's blood plasma. Another method of determining the same thing is to measure directly the volume of red corpuscles in the

capillary tube of a haemocrit; any solution in which the corpuscles do not alter in volume will be isotonic with the plasma. Although these physiological methods, as a way of estimating osmotic pressures, are open to many limitations and fallacies, they do show that the red corpuscle behaves to some substances in solution as a partial semipermeable membrane. But the actual permeability of red corpuscles can be estimated in the following ways. Hedin's method consists in dissolving equal quantities of the same substance in whole blood and in plasma; the corpuscles are centrifugalised away in the one case, and the Δ of both lots of plasma is observed. If the Δ of the centrifugalised plasma is less than that of the other plasma, then some of the dissolved substance has been taken up by the corpuscles. Oker-Blom's method depends upon the fact that electrolytes contained in red blood-corpuscles or taken up by them from the plasma do not influence the electrical conductivity of blood. If a conducting substance is dissolved in blood and the observed electrical conductivity of the solution compared with what it ought to be if none of the substance has passed from the plasma into the corpuscles, it can be ascertained whether or not the corpuscles are permeable to this substance. By these means it has been shown that red corpuscles are practically impermeable to NaCl, KCl, $NaNO_3$, KNO_3, KBr, K_2SO_4 and a few ammonium salts, but are readily permeable to NH_4Cl, NH_4Br, NH_4NO_3 and most other ammonium salts. Of the permeability of other cells we know practically nothing, but it is clear that in any question dealing with filtration, diffusion, or osmosis, through living membranes, the permeability of those cells is of fundamental importance.

When we consider the ways in which various solutions could produce an effect upon living cells, two obvious possibilities at once present themselves: (1) that they act in virtue of their osmotic pressure, and (2) by ionic action. That osmotic pressure does affect the properties of living cells is best shown in the case of non-electrolytes; for, by using them we exclude the possibility of ionic action. Mathews has shown that non-electrolytes in isotonic solution will not stimulate a sciatic nerve, Loeb and others have shown the same thing for muscle. On the other hand, any non-conductor in solution having an osmotic pressure of about 14 atmospheres will stimulate a nerve. The same

is true of similar solutions of electrolytes, no matter what property a more dilute solution has. The way in which these hypertonic solutions cause stimulation is uncertain, but it seems likely that they act largely by the extraction of water setting up a definite change in the colloids of the cell.

When we turn to solutions of electrolytes containing ions, we see that they might act on living cells, apart from osmotic pressure, in two ways either chemically or electrically. The subject is still highly theoretical, and nothing more than a very brief outline can be attempted. The subject has been investigated by Hardy, Loeb, and many other observers, and recently by Mathews, Garrey, and Benedict for nerve, skeletal muscle, and cardiac muscle respectively; and in their papers the necessary references to previous workers can be found. Electrolytes, like non-electrolytes, can stimulate nerve by an osmotic abstraction of water. Mathews found that nearly all electrolytes in solutions having an osmotic pressure of about 14 atmospheres will produce stimulation. But electrolytes, unlike non-conductors, may also produce stimulation as isotonic solutions, therefore it is clear that they have some further power of stimulation, which might be either chemical or electrical; and we have to discuss which it is. Now, since nearly all sodium salts stimulate nerve the chemical composition of the anions in this instance must be unimportant; and the stimulating power of these salts might be looked upon as a peculiarity of the cation Na. But we find that many salts of ammonium, potassium, barium, lithium, and rubidium share their power with sodium salts: and we are obliged to conclude that this power does not appear to be a peculiarity of the chemical composition of the cation any more than of the anion. Solutions of KOH or NaCl produce the same stimulatory effect on nerve, and yet the only thing they have in common is nothing chemical, but the point that both are electrolytes. Both solutions contain positive and negative particles, and since the chemical composition of these ions appears to be a matter of indifference, we can conclude only that their properties depend upon their electrical condition; in other words, we come to the conclusion that chemical stimulation is essentially electrical stimulation. Now, if this is so, we should expect that the anion of NaCl would be the stimulating agent; for, having a negative charge it would correspond to the cathode or negative

electrode of a constant current, and we should expect for the same reason the cation Na to have a depressing action owing to its positive charge. The truth of this supposition is well shown by the different action of acids and alkalies upon nerve; hydr[illegible] stimulate and acids depress it. The action of a hydrate [illegible] to the predominance of negatively charged OH ions which [illegible] contains, and the action of an acid is similarly due to a preponderance of positively charged H ions. Mathews, in fact, has reached the generalisation that all anions have a stimulating action, and all cations a depressing action, not only upon nerve but upon bioplasm in general. The available evidence on the whole strongly supports Mathews's view.

A salt consists of both an anion and cation. From what has been said it would follow that the factor which determines whether a salt as a whole stimulates or depresses, must be the relative efficiency of its anion and cation; if the anion predominates, as in the hydrates, the salt will stimulate, and if the cation predominates the salt will depress.

It would seem to follow also that there must be a connection between the valency of an ion and its physiological action; for, the greater the valency the greater is the number of electrical charges which the ion possesses. And on the whole this is found to be the case; the depressant action of a salt increases with an increase in the number of charges on its cation, and the stimulating power increases with the valency of the anion. But if the valency of the ions were the real and sole explanation of the physiological action of a salt, we should at once be landed in a difficulty. For salts with ions of the same valency should stimulate or depress equally; but this is not so. For instance, NaCl as a whole stimulates nerve, *i.e.* the Cl overbalances the action of the Na, but AgCl depresses nerve, *i.e.* the Ag overbalances the Cl. We have therefore to explain why the charge on Ag appears to be so much more efficient than that on Na. Mathews has pointed out that the efficiency of an ion depends upon the potential of its charge, its ionic potential, that is, upon the tendency of the ion to give up its charge and to change its electrical state; or, to put the point in another way, those elements which are the most stable and hold their charges most firmly, will have the least physiological action. Now the greater the affinity of an element for its ionic charge, the greater will be

the voltage required to separate its charge from it. This voltage when measured will also give the solution tension of the element, that is, the tendency of the element to go into solution and acquire a charge. Solution tension will therefore be the reciprocal of ionic potential. In other words, the greater the solution tension of an ion, the less will be the ionic potential; the electrical stability of the ion will be greater, and the physiological action less. Now Ag has a much lower solution tension than Na, therefore it possesses a greater ionic potential and a more powerful depressant action. In this way Mathews explains the fact that AgCl depresses nerve while NaCl stimulates it. Similarly for any other salt we may [illegible] the general proposition that the more stable the anion [illegible] is the stimulant action of the salt, and the greater the electrical stability of the cation the less is the depressant power of the salt. Mathews has shown that the physiological action of an ion is modified also by its velocity, weight and volume in such a way that the faster it moves the more powerful it is, and the heavier it is the less is its power. But these points cannot be discussed here. Mathews has summed up this part of the subject as follows: Ions are minute, freely movable electrodes, of different voltages. The physiological action of any ion depends upon (1) its concentration, (2) the sign of its electrical charge, and (3) its electrical stability or ionic potential. The physiological action of an ion is therefore dependent on electrical state and stability, and is independent of chemical composition except in so far as this may influence the velocity and weight of the ion. The physiological action of a salt depends upon the sum of the physiological actions of its component ions.

Thus far we have seen that chemical stimulation is electrical and independent of the chemical composition of the ions which cause it. But this does not exclude the possibility that ions might have a chemical as well as an electrical action. And that they had, was thought to be shown most clearly by a consideration of the toxic and antitoxic actions of salts. Ringer made the original discovery that the action of K salts on the heart could be antagonised by Ca salts. His observations have been extended by Loeb, MacCallum and others, for other salts and for other tissues and cells. Further, it has been shown that salts may antagonise the action of drugs other than salts. For instance, the

whole group of drugs which act somewhat like barium chloride, *e.g.* digitalis suprarenal, &c., can be antagonised largely by Ca salts. The same is true of the purgative cascara and of some bacterial hæmolysins. Such observations suggest the possibility that the antagonistic action of ions might be of a chemical nature. And this was supposed to be the case until Loeb made the important suggestion that it was the valency of the cation which determined antitoxic action. Loeb started at least two views to account for his own observations on antitoxic action, and left the subject in a condition of great uncertainty, which still exists more or less in spite of the recent work of Mathews and Lillie. It is possible to give here only the general conclusions at which Mathews has arrived. According to his view the physiological action or toxicity of a salt is due to the sum of the solution tension and not to the valency of its ions, that is to say, toxicity is a property of both ions. Similarly, he believes that the antitoxic power of a salt is a property of both its ions, and is not confined to an antagonism between cations of different valency, as Loeb thought. It is as yet uncertain what determines the antitoxic power of a salt; for it is not in all cases known to be related to the sum of the solution tensions of the ions.

When we try and get an insight into the way in which salts exert their toxic and antitoxic powers on bioplasm, we find that our knowledge is extremely meagre. The experimental evidence is confined largely to observations on the ova and ciliated larvæ of marine animals. The observations show that the toxic action of a salt may be associated with the following gross changes in bioplasm. (*a*) Plasmolysis. Many salts, such as NaCl, KCl and LiCl, are toxic to ova when the osmotic pressure of their solutions is greater than that of sea-water. The egg membrane is relatively impermeable to those salts, and if the osmotic pressure outside is sufficiently great, the cell undergoes plasmolysis. A calcium salt is able to neutralise this action completely by altering apparently the permeability of the membrane to the toxic salt; in this way the effective osmotic pressure of the toxic solution is reduced and plasmolysis prevented. (*b*) Coagulation. Other salts, such as those of Mn, Co, Ni, are toxic in concentrations far lower than would be necessary to produce plasmolysis of the cell. The salts produce a slow coagulation of the cell contents owing to the gradual passage of the salt through the egg membrane. The

antitoxic action of a calcium salt appears to be due to its power of reducing the permeability of the membrane to the toxic salt. How these changes in permeability are brought about we do not know, any more than we know whether or not permeability is a chemical or physical phenomenon. (*c*) Liquefaction of the cilia of larvæ is observed to take place in some solutions and to be prevented by the presence of other salts. Besides these gross toxic changes, however, it is likely that ions can influence bioplasm in many other ways, such as alterations in the state of colloidal aggregation, alterations of surface tension, &c., and perhaps chemically; but these are largely theoretical and cannot be discussed.

Thus far we have dealt entirely with the action of ions on living matter. But it must not be thought that their field of action is confined to bioplasm, and that because ionic action may be demonstrated to have taken place, it is any proof that the material, on which it is acting, is living. Hardy and others have demonstrated the power of solutions of electrolytes to produce coagulation of colloidal solutions It is a matter of secondary importance whether the coagulative power of ions is related to their valency as opposed to their ionic potential. The results suggest that the power of salts to alter the permeability of cells and the physiological activity of bioplasm may be connected in part with a power to alter the physical, perhaps electrical, condition of the protoplasmic colloids. Cole and others have investigated the action of ions upon the activity of unorganised ferments. Cole came to the general conclusion that the activity of a ferment is stimulated by anions and depressed by cations, the effect of the ions being determined by their activising power. His results have been confirmed and extended by McGuigan, who concluded that the inhibitory power of any salt is inversely proportional to the sum of the solution tensions of its ions; a result which is identical with that arrived at by Mathews for the action of ions on nerve. Neilson and Brown have shown that the catalytic decomposition of hydrogen peroxide by platinum black is stimulated by anions and inhibited by cations. This action of ions upon unorganised ferments must be of great importance in relation to experiments on absorption. For, during absorption, the products of digestion undergo change by ferments; and further, it is now thought that many of what used

to be considered vital properties of bioplasm, such as synthesis, oxidation, and reduction, are due to the presence of unorganised intracellular ferments.

We thus arrive at the general conclusion that a solution of an electrolyte may alter the properties of cells, (*a*) by osmosis, (*b*) by ionic action, which is certainly electrical and may be also chemical; whereby the solution alters the permeability, the state of colloidal aggregation, and the other uncertain physical and chemical conditions, &c., upon which the normal physiological activity of bioplasm depends; (*c*) by alterations in the activities of unorganised ferments.

When discussing such a subject as whether absorption is due to physical forces or to the vital activity of cells, it is impossible to ignore the questions, what do we understand by vital action? and at what point do we consider it necessary to say that a phenomenon is due to it? Vital activity cannot be held to mean any and all the properties exhibited by living cells, for the same cells when dead may still exhibit some of these properties, which must then be held to have a physical or chemical basis. We shall consider as vital such properties of a cell as are changed in kind and not merely in degree when the same cell is dead. This definition leaves open the question how far vital activity, as defined, may still have a physical and chemical basis. It is simple to interpret a comparison between the properties of the same cell when living and when dead; but the case is altogether different when we allow solutions of electrolytes and poisons to act upon living cells. Our ignorance of the nature of the changes produced in bioplasm by ions and poisons renders it impossible to interpret the results in terms of vital activity. And to a less extent the same must be true of any other solution which is foreign to the cells in question.

We must now turn to the more important experiments which deal with absorption by the small intestine.

Hamburger's Experiments.—The older observers had suggested that filtration, diffusion, osmosis, and imbibition might explain intestinal absorption. But Hamburger is the chief modern observer who has attempted to explain by physical processes all the phenomena of intestinal absorption. He has suggested that physical factors other than those enumerated above may play a part, namely, the osmotic pressure of proteids, aspiration

produced by the blood flow in the capillaries, and the pumping action of the villi, which is supposed to be like that of both a suction and a force pump. It is necessary to say a word about the relative importance of these various factors according to Hamburger's view, and to mention the experimental evidence on which the view as a whole is based.

All observers admit that diffusion and osmosis may play a part in absorption, but they differ in the importance which they ascribe to them. Even on Hamburger's view diffusion and osmosis, like imbibition, play a relatively secondary rôle. Two varieties of imbibition are recognised, molecular and capillary. By molecular imbibition is meant the taking up of fluid by homogeneous non-porous masses, such as gelatine and agar plates, and by capillary imbibition the taking up of fluid in the pre-formed spaces or pores of a material. The tissues of the body have the power of taking up much more fluid than they normally possess, and Hamburger assumes that this may take place by imbibition. He supposes that during absorption solutions are taken up into the epithelium by molecular imbibition and are then passed by capillary imbibition through the connective tissue spaces of the subepithelial tissue, where they are taken up by the capillary wall again by molecular imbibition and finally passed by capillary imbibition into the interior of the capillary. The fluid reaching the blood-stream is constantly carried away, and this makes a continuous absorption by imbibition possible. There is some evidence to show that imbibition may be a selective process and that gelatine and pieces of dried tissue are by no means indifferent to the solutions in which they are soaked. It has been shown, for instance, that they will take up much more of a solution of NaCl than of a solution of Na_2SO_4. Sulphates are known to be but slowly absorbed from the alimentary canal, and it has been suggested that the different rates at which salts are absorbed may be due in part to the varying degree to which they can be imbibed. It has been found that dead tissues may show considerable imbibition, but how far living tissues show the same phenomenon it is impossible to say.

It is impossible to take very seriously the aspiration produced by the blood-stream as a force which is to be capable of absorbing fluid from the gut; for the suction produced by a fluid of the specific gravity of blood travelling at the rate of about

1 mm. per sec. must indeed be small. Hamburger apparently looks upon it as a force which determines, not so much the absorption of fluid from the lumen of the gut, as the passage of fluid from the connective space into the blood capillary rather than into the lymphatic; for the rate of flow in the blood capillary is probably far greater than in the lymphatic.

Filtration is, according to Hamburger's view, the most important force in absorption. Lenbuscher had previously maintained that a slight increase of intra-intestinal pressure favoured absorption, and he explained the result not by filtration, but by an unfolding of the mucous membrane increasing the absorbing surface. Hamburger eliminated this factor by enclosing a coil of intestine in a rigid tube which was so arranged that the coil, whilst filling the tube, retained its normal blood supply. The coil was filled with ·9 per cent. NaCl solution and connected with a reservoir of the same fluid. The maximal intra-intestinal pressure used corresponded to about 10 mm. Hg. and he found that up to that point the rate of absorption varied directly with the pressure. In other experiments the intra-intestinal pressure was raised by blowing air into a closed abdominal cavity: the maximal pressure used was about 8 mm. Hg. and the result on absorption was the same as before. In yet another series of experiments he investigated the effect on absorption of reducing the intra-intestinal pressure to zero. For this purpose he sucked the gas out of a coil, and, in order to prevent the coil from collapsing, he introduced into it a cage made of aluminium wire. He found that no absorption of ·9 per cent. NaCl solution took place when the pressure was zero or negative, but that if the intra-intestinal pressure were raised to as little as ·04 mm. Hg. absorption began and increased with the pressure up to 17 mm. Hg., the maximal pressure used. If these experiments were true and contained no fallacy, they would militate greatly against Hamburger's own view. For, when the pressure was zero and no absorption took place, imbibition and aspiration by the capillary blood flow could still occur; and yet not a drop of fluid was absorbed. Further, osmosis and diffusion could still take place, for it is well known that if a ·9 per cent. NaCl solution is separated from serum by a membrane permeable to NaCl and water, such as he supposes the gut to be, absorption readily takes place. The fallacy of the experiments with reduced pressure would appear

to be that, when the pressure was zero or negative, the mucous membrane would be pressed against the cage and absorption stopped by interference with the circulation, and that when the mucous membrane was lifted off the cage by the slightest positive pressure, absorption began again. The intestines during life undoubtedly contain a positive pressure, but for filtration to be possible, the intra-intestinal pressure would have to be greater than that in the venous end of a capillary. It is, therefore, necessary to inquire how great these two pressures are. Hamburger considered that intra-intestinal pressure was due to three causes—respiratory movements, peristalsis, and gravity. During inspiration the diaphragm is thrust down into the abdomen and will exert pressure upon the abdominal viscera. He measured the respiratory rise in intra-intestinal pressure, and the highest value obtained was about 8 mm. Hg. During the peristalsis of one coil pressure will be exerted upon neighbouring coils, and gravity could affect intra-intestinal pressure by the weight of coils pressing on each other and by acting also upon the contents of the individual coils. In order to eliminate these three forces, Hamburger introduced an aluminium cage into one coil, and found that the absorption from it was still considerable, although less than from a neighbouring coil. This experiment would appear to show that intra-intestinal pressure is not really of great importance to absorption, and the result obtained is in marked contrast to those of Hamburger's previous experiments, in which the importance of filtration was insisted on. Hamburger never measured simultaneously the fluid pressure in the gut and the blood pressure in the mesenteric vein, and has consequently brought forward no real evidence that filtration could ever take place. Reid, however, has determined these pressures simultaneously, and found that during absorption the pressure in the mesenteric vein may be four times greater than the fluid pressure in the gut. We may therefore conclude that absorption may take place when filtration is impossible, and that no one has yet brought forward evidence to show that the intra-intestinal pressure is ever sufficiently great to make filtration possible. But we cannot conclude that the intra-intestinal pressure has no influence on the rate of absorption. Both Leubuscher and Edkins have found, like Hamburger, that a pressure of about 8 mm. Hg is the optimum; above that, absorption falls off and the outflow from

the mesenteric vein decreases, which suggests that a reduction of circulation is the cause of the decreased rate of absorption; also below 8 mm. Hg absorption decreases, possibly because the gut is less unfolded and the absorbing surface diminished.

In order to prove that absorption was a purely physical process, Hamburger carried out experiments on animals which had been dead for varying periods up to twenty-five hours. The rate of absorption could not be expected in the absence of a circulation to be as great as during life, and if the difference were in degree only, such experiments might be held to show that physical processes play a correspondingly important part. If, however, the absorption after death were found to differ in kind also, then the experiments would prove nothing, but might be held to indicate that vital activity of cells was essential to normal absorption. Hamburger obtained results which appeared to show that *post-mortem* absorption differed only in degree from absorption during life. He found that 1·5 per cent. NaCl solution was absorbed at a rate of about ·15 cc. per cm. of gut per hour, which is less than a tenth of the rate of absorption during life; further, whilst in the living animal the osmotic pressure of the solution in the gut becomes nearly isotonic with the blood serum in twenty-five minutes, after death the osmotic pressure of the solution had fallen only to that of a 1·3 per cent. NaCl solution in two hours. With blood serum in the gut the actual rate of absorption was even less, and corresponded to about a fifth of the absorption in a living animal. Hamburger concluded that absorption in a dead animal differed in degree only from that in a living animal, and took place presumably by imbibition and filtration in the case of serum, and by diffusion and osmosis as well in the case of anisotonic salt solutions. Cohnheim, from his experiments, came to a very different conclusion, and believes that there is a real difference in kind between absorption by living and by dead or damaged epithelium. He studied the absorption of dextrose solutions, and at the end of the experiment estimated the dextrose and NaCl in the unabsorbed fluid. He found that with a normal intestine not more than about ·2 per cent. NaCl was to be found in this fluid at the end of an experiment lasting two hours. He considered that this NaCl was due, not to diffusion from the blood, but to a secretion of succus entericus having taken place, and as Na_2CO_3

appeared in the fluid at the same time, this is probably true. On the other hand, when the epithelium was damaged by using strong dextrose solutions (15 per cent.), or solutions heated to 90° C., or by additions of NaF or liquor arsenicalis, this power of the epithelium to keep back the blood NaCl was lost, and the fluid in the gut might contain more than ·6 per cent. NaCl within twenty-five minutes. From this he concluded that the normal living epithelium possesses an almost completely one-sided permeability for NaCl of such a nature that the epithelium is readily permeable from the gut towards the blood, but impermeable in the opposite direction. He repeated Hamburger's experiments on dead animals, using dextrose solutions, and found the same slight absorption that Hamburger did; he also found that the NaCl in the solution at the end of the experiment was under ·2 per cent., in fact was not above that found in experiments with living gut. He explained this result as being due, not to the epithelium retaining after death its one-sided permeability, but to there being no circulation, and consequently no renewal of NaCl to diffuse into the gut. In order to prove this point he repeated the experiments with an artificial circulation of ·9 per cent. NaCl carried on through the mesenteric vessels. He then obtained a very different result; the percentage of NaCl in the fluid remaining in the gut was about ·7 per cent., no absorption took place, and the osmotic pressure of the solution introduced into the gut always rose, and did not tend to approximate to that of the serum, as Hamburger found. Cohnheim, in response to Höber's criticisms, has confessed that these experiments on dead animals with an artificial circulation are unsatisfactory, because the circulation was carried on too long and at too high a pressure; consequently the epithelium may have been much altered, water-logged, and unable to absorb. Höber has repeated Hamburger's experiments on dead animals, using dextrose solutions. He was able to verify Hamburger's results, but not those of Cohnheim, and he came to the conclusion that absorption in a dead intestine differs from that in a living one quantitatively but not qualitatively. Nevertheless, Cohnheim's experiments prove that the normal epithelium possesses a one-sided permeability, and in this he is confirmed by Reid. We may therefore say that when the epithelium loses this property in the course of an experiment, it is no longer in

a normal condition. But Cohnheim's experiments do not prove, and we do not know, that this property is peculiar to the living epithelium, and that poisonous solutions would not produce just the same loss of it in a dead epithelium.

It is necessary to discuss briefly the explanation and significance of this remarkable one-sided permeability to NaCl. Hamburger and Hober consider that it has a physical basis, and Cohnheim that it depends upon a vital activity of the cells. Considering our complete ignorance of what the permeability of any membrane to a solution means, it is obvious that it is no more an explanation to say that it is due to a vital activity of the cell than it would be to explain it as a physical peculiarity of the cell. With regard to the significance of the phenomenon, it is unfortunate that the evidence should be conflicting as to whether it is confined to the living or is equally shared by dead epithelium; for, if it were confined to the living epithelium, we should ascribe it to vital activity. But it must be remembered that even if it is confined to the living epithelium, it is not a final proof that it is due to an active thrusting back of NaCl in one direction. Nor does the fact that various ions and drugs can annul the property prove that they do so by inhibiting a vital activity and not by causing a physical alteration in the cell. To say, as Cohnheim did, that only living organised material can be poisoned, is in the present state of our knowledge untrue; for we have seen that the properties of dead materials can be altered by chemical agents. The fact that the decomposition of hydrogen peroxide by platinum black, and the activity of digestive ferments can be inhibited and stimulated by various ions, would, according to Cohnheim's line of argument, indicate that platinum black and these ferments must possess vital activity. It is true that we know of no physical membrane with such a one-sided permeability, but that does not prove that a cell could not have such a physical constitution as to allow of such a property being exhibited. Instances of other tissues with similar one-sided permeabilities are known. Reid has shown that the permeability to fluid of the living frog's skin is greater from without inwards than in the reverse direction; and that when the skin is dead the permeability does not become equal in the two directions, but is now greater from within outwards. Hill and Ridewood have

shown that the lung of a frog is much more permeable to CO_2 in the direction from pleura to bronchus, and that when the lung is killed an excessive permeability still exists in the same direction. These experiments, if true, certainly suggest that a one-sided permeability in animal tissues may have a physical or chemical basis.

Höber's Experiments.—Hamburger's work is not the sole evidence that physical processes may play an important part in intestinal absorption. Hober compared the rates of absorption of a large variety of salts by Heidenhain's method. For this purpose a known quantity of the solution is placed into an intestinal loop of known length and ligatured at its two ends, the loop is put back into the abdomen for a given time, and at the end of the experiment the quantity of fluid in the gut is measured. The Δ of the serum before the experiment, of the original solution and of the fluid in the gut at the end of the experiment is taken. He found, like previous observers, that isotonic as well as anisotonic solutions were absorbed, and that anisotonic solutions during absorption became more or less isotonic with the animal's blood serum. In this way he investigated the absorption of many cations and of but few anions. Wallace and Cushny observed by similar methods the absorption of a great variety of anions and of but few cations. Their results were identical with those of Hober when they overlapped, so that the results of both series of experiments may be given together. They show that both cations and anions differ enormously in their rates of absorption. The various anions can be divided into four groups with decreasing rates of absorption: (1) chloride, bromide, and iodide; (2) nitrate, salicylate, &c.; (3) sulphate, phosphate, tartrate, citrate, &c.; and (4) fluoride and oxalate. The members of this last group injure the gut very materially. The cations may be similarly divided into four groups: (1) NH_4, together with urea; (2) K, Na, Li; (3) Ca, and (4) Mg.

The cause of these great differences in absorption rate might be vital or physical. Heidenhain had suggested that the different rates of absorption of isotonic NaCl and $MgSO_4$ solutions was due to the latter depressing the vital activity of the intestinal epithelium by which the cells transported solutions from the gut towards the blood-vessels. Such a vital explanation, however, can be arrived at only by excluding every possible physical one.

Of the factors involved in Hamburger's scheme of absorption, the only ones which could be influenced by change in the solution to be absorbed would be either molecular imbibition or diffusion depending on differences in diffusion velocity or in the permeability of the cells to the substance in solution. Molecular imbibition is influenced by salts; for Hofmeister found that gelatine plates absorbed less water when soaked in a sulphate, tartrate, or citrate than in a chloride, bromide, or nitrate. But considering the uncertainty how far living tissues show molecular imbibition at all, much stress cannot be laid on Hofmeister's observation. He also showed that the same division of anions indicated the relative power of different salts to precipitate egg albumin or gelatine; and in this way there might exist some general relation between the power of salts to precipitate colloids and to pass into living cells. But this is not the case. For Leathes and Starling found that the pleural endothelium absorb solutions of $MgSO_4$ or Na_2SO_4 just as rapidly as those of NaCl; and we have already seen that the relative permeability of red blood-corpuscles to salts is not the same as the relative rates at which the same ions are absorbed from the intestine.

Hober has put forward an explanation founded on the observation that with few exceptions a parallelism exists between the diffusion velocity of a crystalloid and its rate of absorption from the gut. From this it would follow that the epithelium acted like an indifferent membrane; but the exceptions to the rule are sufficient to show that this cannot be the case. The more important exceptions are, in the first place, dextrose, cane-sugar, galactose, and urea, which are absorbed more rapidly than corresponds to their diffusion velocities. In the case of the sugar, the certainty that ferment action takes place during absorption introduces an unknown factor which may or may not explain the discrepancy. Höber gave an explanation for urea which will be referred to later. The second important group is formed by chlorides, bromides, and iodides, which have practically the same diffusion velocities, and yet show decreasing rates of absorption in the order named. Finally, there came the fluorides and oxalates, and salts of arsenic and quinine, all of which damage the epithelium, and are absorbed more slowly than would be expected from their diffusion velocities.

Wallace and Cushny made a different suggestion, namely,

that the permeability of the intestinal epithelium to various anions was related to the solubility of their calcium salts. They believed that the precipitation of an insoluble calcium salt in the epithelium would retard absorption. The main evidence against the view is the fact pointed out by them, that the relation between solubility of the calcium salt of an anion and its absorption rate is not absolute. The same view has been put forward to account for the power of many salts with a polyvalent anion, like citrate and oxalate to stimulate nerve and muscle. It is supposed that these salts act, not by means of the many negative changes which they possess, but by precipitating the calcium in the tissues, and so neutralising the depressing action of the calcium salts. Although there exists a general parallelism between the stimulating power of anions and the insolubility of their calcium compounds, there are too many exceptions to make it likely that the one property is the cause of the other. We have seen that the stimulating power of an anion is determined by its solution tension, and it is probable that the same factor helps to determine its power of precipitating calcium. These considerations point to the idea that the rate of absorption of different anions might be related to their ionic potential. Our knowledge of the solution tension of anions is very imperfect; but to some extent their rate of absorption is related to their solution tension in such a way that anions with the lowest solution tensions, which are therefore the most physiologically active or toxic, are the least rapidly absorbed. In the case of cations the relation between rate of absorption and solution tension is much closer. The relation is such that cations with the highest solution tension, which are consequently least depressant, are the most rapidly absorbed. Such facts suggest that the factor which determines the rate at which a salt is absorbed, is the sum of the physiological actions of its ions. Until we understand more of ionic action upon bioplasm generally it is idle to speculate upon the nature of the action of ions upon the properties of the intestinal epithelium. And we are not in a position at the present moment to say whether the rate of absorption of an electrolyte is determined by vital action or not.

From what has been said it is clear that the slow absorption of a salt might be due to its anion alone, *e.g.* sodium sulphate,

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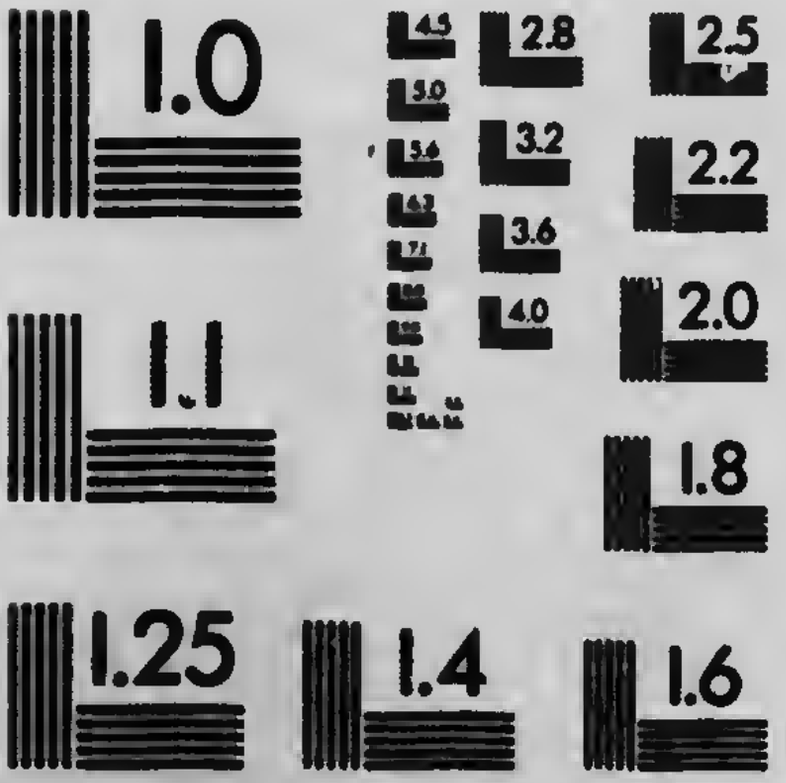

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phosphate, or citrate; or to its cation alone, *e.g.* magnesium oxide; or to both its anion and cation, *e.g.* magnesium sulphate. Such slowly absorbed salts constitute the saline purgatives which are of importance in medicine and deserve consideration here.

The Action of Saline Purgatives.—We have seen that solutions of some salts appear to be absorbed more slowly than others, and that this conclusion is based chiefly on the fact that at the end of the experiment more of a fluid isotonic with the serum is found in the gut in some cases than in others. Höber, Wallace, and Cushny have satisfied themselves by analysis that in cases of slow absorption less of the salt has actually been absorbed, and they have assumed that all the fluid remaining in the gut is a solution of the corresponding unabsorbed salt. The question whether this is true or not is a point of some importance; for the slowly absorbed salts constitute the great class of saline purgatives, and the answer to the question involves the explanation of their mode of action. Wallace and Cushny believe that these salts produce a watery condition of the fæces because they are absorbed with difficulty, and therefore keep back with them in the gut enough water to make their solution isotonic with the serum. There is no doubt that their purgative action can be altogether independent of osmosis; for they still act as purgatives when placed in the gut as solutions isotonic with the serum. In order to prove this Wallace and Cushny introduced into the stomach isotonic solutions of various salines, and measured the fluid obtained within an hour from an opening into the cæcum. When 100 c.c. of a NaCl solution was introduced into the stomach no fluid was obtained from the fistula, but with the same quantity of a solution of sodium sulphate or citrate in the stomach 75 c.c. were obtained from the fistula. From this they concluded that whilst all the NaCl was absorbed, 75 per cent. of the purgative solutions was not. This conclusion is not now justified in the light of MacCallum's experiments. He found that much more of the saline purgative had to be administered, and that it took longer to act when given by the mouth than when given subcutaneously, and that their intravenous injection required a still smaller dose and acted much more rapidly. These results suggested that the salts can act after absorption; and he proved that this was so by showing that these solutions set up local

peristalsis of the gut within a minute of being painted on its peritoneal surface. He found that Ca salts applied locally would prevent the peristalsis, just as Loeb had found that Ca salts can stop contact irritability of muscle and the hyper-sensitiveness of the nervous system induced by various salts. MacCallum showed further that besides inducing peristalsis these purgatives may increase the normal secretion of the intestinal glands ten-fold, and that this effect as well as the normal secretion may be inhibited by Ca salts. We may therefore conclude that solutions of the saline purgatives are slowly absorbed, but that the portion which is absorbed rapidly leads to an increased secretion of fluid from the intestinal glands, and that consequently the quantity of fluid obtained from the gut at the end of an experiment is not an accurate measure of how much of the original solution remains unabsorbed. MacCallum has also shown that when large quantities of NaCl solution isotonic with the plasma are injected intravenously, there is a rapid secretion into the intestine of fluid containing ·25 per cent. dextrose. When we recollect Colenheim's experiments on the absorption of dextrose with an artificial circulation of NaCl solution, MacCallum's results emphasise the impression that in the present state of our knowledge solutions of electrolytes are unsuitable for investigating the mechanism of intestinal absorption.

The Path of Absorption.—It has frequently been assumed that all material is absorbed through the epithelial cells. But the epithelial layer consists of cement substance as well as cells; it is therefore possible that material might pass through the cement substance by a physical process, and that such material as passed through the cells might be transported by their vital activity. Our knowledge of the actual path taken by different substances is extremely meagre. We know that the products of fat digestion pass into the epithelial cells, but we know nothing of the path taken by the products of proteid and carbohydrate digestion. Heidenhain placed solutions of methylene-blue in the intestine, and found two hours later the dye both within and between the cells. From this he concluded that water can be absorbed by both routes. MacCallum has definitely shown that iron salts can pass into the epithelial cells. He does not discuss the possibility of their passing between the cells as well; but, from the fact that he sometimes found none in

the cells and large quantities in the subepithelial layer, it seems possible that such may be the case. Höber extended MacCallum's research and failed to demonstrate the salts of the other heavy metals, Hg, Pb, Cu, &c., within the cells by micro-chemical tests, although they are known to be absorbed. He treated sections of epithelium which had absorbed NaCl or $MgSO_4$ with alcoholic $AgNO_3$ and $BaCl_2$ solutions respectively; but the results were inconclusive as to whether or not any of the salts had passed into the cells. He demonstrated within the cells certain basic dyes, such as methylene-blue, which are known to produce intravital staining of cells, but others which have not this property were found only intercellularly. In order to explain the path taken by different substances Höber adopted Overton's theory of the chemical permeability of the superficial layer of cell protoplasm. According to this theory only such substances can pass through this layer as are soluble in mixtures of cholesterin, lecithin, cerebrin, protagon, &c.—Overton's lipoids. Such substances are water, alcohol, some basic dyes, urea, ammonium salts, and the products of fat digestion. On the other hand, most salts and carbohydrates are insoluble in lipoids. Höber therefore explained that urea was absorbed at a rate greater than corresponded to its diffusion velocity, because, unlike most salts, it could be absorbed by the cells as well as by the cement substance. The difficulties of this view are obvious. For instance, inorganic iron salts are not soluble in lipoids and yet are absorbed intracellularly. But the greatest difficulty of all is the one-sided permeability of the intestine to NaCl. If this salt passes only by an intercellular path, then we have to believe that the apparently structureless and dead cement substance is of such a nature that it allows normally the passage of NaCl in only one direction. Such a mechanism may of course exist, but we have no knowledge of a physical membrane with such a one-sided permeability. Although some substances may be absorbed through the intercellular cement, Höber's view cannot be held to explain satisfactorily the different absorption rates of various substances. And it is better in the present state of our knowledge and for the sake of simplicity to consider that the main path of absorption of all substances is through the cells.

We must now deal with the experiments which have been considered to be inexplicable by physical processes, and which

therefore show that the vital activity of the epithelial cells controls absorption.

Heidenhain's Experiments are of importance in the history of intestinal absorption, because they were the first experiments on the subject performed after a knowledge of osmotic phenomena had begun to be applied to physiology. His method of experimenting has already been described. His discovery that water was absorbed more slowly from a $MgSO_4$ solution than from a solution of NaCl of equal concentration, and his explanation of the fact, have been already mentioned. He knew by analyses that the $MgSO_4$ was less rapidly absorbed; therefore it would be expected on physical grounds that less water would be absorbed from the $MgSO_4$ solution. In most of his experiments he used solutions of NaCl, and the two other results which he considered were inexplicable by osmosis and diffusion were the following—(1) When a hypertonic NaCl solution (1·5 per cent.) was placed in the gut, no preliminary attraction of water into the intestine took place, but both water and salt began to be absorbed at once. He considered that the salt absorption was due to diffusion owing to the partial pressure of NaCl being greater in the solution than in the blood (·65 per cent.), but that the simultaneous absorption of water could not be due to osmosis. (2) When a hypotonic solution (·3 to ·5 per cent.), having a less partial pressure of NaCl than the blood, was introduced, not only water but salt also was absorbed. He accounted for the water absorption by osmosis, but he considered that the absorption of salt must be physiological; and in order to demonstrate this physiological factor he added NaF to his solutions and found that absorption was then interfered with. Heidenhain, however, in this explanation overlooked two important facts. In the first place, if blood serum is separated from a solution of NaCl isotonic with it by a dead animal membrane, which is permeable to NaCl in both directions, the water of the solution does pass into the serum and the solution is absorbed. Starling has demonstrated beyond doubt that the living blood capillaries can absorb an isotonic salt solution, and a physical explanation of the fact has been given, namely, that serum contains substances possessing an osmotic pressure to which the membrane is impermeable. In the second place, Heidenhain was unaware of the fact subsequently discovered by Cohnheim, that the normal gut possesses a one-sided

permeability to NaCl, and would therefore be in a peculiarly good position to absorb solutions of this substance in any concentration by diffusion and osmosis. And the action of NaF in showing absorption could be readily explained by its destroying this one-sided permeability.

Cohnheim's Experiments.—Besides the experiments already referred to, Cohnheim made a considerable study of the effect of various poisons upon the absorption of dextrose solutions. He found that anisotonic solutions became, during absorption in a normal gut, isotonic solutions of dextrose, either by the absorption of sugar or water, as the case might be; and he considered that this conversion of an anisotonic into an isotonic dextrose solution was due to diffusion and osmosis. When, however, the epithelium is severely poisoned by NaF or As, it loses its special peculiarities, and becomes more like an ordinary dead permeable membrane. Consequently, NaCl diffuses from the blood into the gut, and the intestine, instead of containing an isotonic solution of dextrose, is filled with an isotonic fluid containing a lower concentration of dextrose and a much higher concentration of NaCl. The rate of absorption of this abnormal fluid is very slow. This might be explained on the grounds that normally it is the partial pressure of NaCl in the blood which determines the absorption of water from a dextrose solution. But Cohnheim has brought forward evidence that this physical explanation may not be the whole truth; for he has shown that the power of taking up water from a dextrose solution may be interfered with without at the same time destroying the one-sided permeability of the epithelium to NaCl. He found that at a certain stage of poisoning with NaF, quinine, or potassium acetate, the percentage of NaCl in the gut might be scarcely raised at all, and yet the absorption of water was greatly interfered with. He looks upon this result as strong evidence that fluids are transported by the vital activity of the epithelium. Hamburger has pointed out an obvious objection to all these experiments with poisons. It is not justifiable to assume tacitly that the action of a poison is confined to the intestinal epithelium and does not affect also the capillary walls and the circulation through the part. Further, we are not in a position to say that the epithelial cells are still normal because the one-sided permeability to NaCl is still retained; and that any observed alteration

in absorption is due to a change in the vital activity and not in a physical condition of the cells.

We have still to answer the question whether the uptake of water is, or is not, conditioned solely by the difference of osmotic pressure maintained on the two sides of the intestinal epithelium. Leubuscher answered this in the negative, because he found that a ·25 per cent. or a ·5 per cent. solution of NaCl was absorbed more rapidly than distilled water. But Reid has shown that distilled water damages the epithelium and destroys its one-sided permeability, which amply accounts for Leubuscher's result. Reid, also, believes that when the gut is absorbing a solution, which does the epithelium no damage, the uptake of water is independent of the initial and final concentrations of the solution. He quotes the observation that water is absorbed at the same rate from 2 per cent. solutions of dextrose and maltose, although both substances are absorbed at the same rate, and the osmotic pressure of the dextrose solution on an impermeable membrane is about double that of the maltose solution. On the other hand, there are results among Reid's experiments which point just as much to the opposite conclusion. For instance, he found that, after a loop had been washed out with a ·9 per cent. NaCl solution, distilled water was absorbed more rapidly than a ·9 per cent. NaCl solution. Again, he found that water was absorbed more rapidly from a 2 per cent. solution of dextrose with a final $\Delta = ·440$ than from a solution of the same percentage of dextrose in a ·6 per cent. NaCl solution with a $\Delta = ·550$.

After a brief consideration of the available evidence we are bound to believe that osmosis does play a considerable part in the absorption of water. And although it is impossible to look upon the intestinal epithelium as a simple osmotic membrane, it is equally impossible to say how far these cells have the power of sucking up fluid and passing it over to the blood in defiance of osmosis.

Waymouth Reid's Experiment.—Like Heidenhain and Cohnheim, Reid has come to the conclusion that absorption is not a purely physical process, but depends upon a physiological factor as well. His experiments are of very unequal values, and we must consider how far they necessarily bear the interpretation he has put upon them. He has compared the diffusion velocities of peptone and dextrose through parchment into serum with the rates at

which these substances are absorbed from the intestine. He found that the diffusibilities of dextrose and peptone through parchment were in the proportion of 2 to 1, but that in the intestine peptone was slightly the more rapidly absorbed. In the same way he has shown that the relative diffusibilities of dextrose and maltose through parchment into serum are as 1·8 to 1, and in the intestine as 1·05 to 1. From these results he concludes that diffusion is not a prepotent factor in absorption. But these experiments are open to two objections. In the first place, the use of peptone and maltose is objectionable, because they certainly undergo fermentative change during absorption, and their observed absorption might depend just as well on diffusion and [illegible] ferment action as upon any cell activity. And in the second place, the permeability of two physical membranes for a given substance need not be the same, any more than a given membrane need be equally permeable to all substances. Reid's experiments prove that absorption in the gut is not the same thing as diffusion through the parchment, but they do not prove that the absorption rates of different substances does not accurately represent their diffusion rates through the intestinal epithelium. He also performed experiments to show the effect on the absorption of dextrose, maltose, and peptone solutions of the removal of the epithelium which is caused by clamping the mesenteric arteries for half-an-hour. He found that the rates of absorption of these substances then more nearly corresponded to their diffusion rates through parchment into serum. But, again, all that this necessarily proves is that the intestinal epithelium and parchment are two very different membranes, and that when the epithelium is removed, the rest of the intestinal mucous membrane has properties something like parchment.

Three series of Reid's experiments are of great importance.

(1) *Absorption of a Dog's own Serum.*—The one really important set of experiments performed by Heidenhain were those in which he observed the absorption of horse's or another dog's serum from the small intestin The chief objections which can be raised against his conclusion that absorption in this case could not be a physical process, are that he took no account of the possibility of filtration and imbibition, and that the serum was that of another animal. Reid repeated Heidenhain's experiments, and at the same time appeared to exclude most of the known

physical processes which could be held responsible for the results. By using the dog's own blood serum, he considered that he had excluded osmosis and diffusion; by simultaneously measuring the pressures in the mesenteric vein and the gut he was able to exclude filtration, for he found that the pressure in the vein was about four times greater than that in the gut, and the pressure in the mesenteric capillaries must have been still greater. He also tied the mesenteric lymphatics in order to prevent the pumping action of the villi; whether this would bring about the desired result is unknown, but the procedure had no effect upon the absorption. It is impossible to exclude imbibition experimentally; Reid attempted to do so by washing the loops with serum before the experiment began, and found that it did not influence the result; but it is clear that such a procedure could not prevent a continuous process such as imbibition is supposed to be by Hamburger. The results obtained by Reid may be gathered from a typical experiment. 50 c.c. of the dog's own serum were placed in a loop of ileum 80 cms. long; at the end of an hour the pressure in the mesenteric vein was 13·5 mm. Hg, and that in the gut 4·0 mm. Hg; there had been absorbed 56 per cent. of the water, 58·5 per cent. of the salts, and 30 per cent. of the organic solids. At the end of the experiment the alkalinity and the partial pressure of the NaCl in the dog's plasma and in the serum in the gut were the same; this excludes the possibility that the one-sided permeability of the gut to NaCl could be made responsible for the absorption. In the absorption of the serum work had been done, and, according to Reid, with no apparent force to do it excepting the physiological activity of the living intestinal epithelium. But it seems that Reid has really proved too much, for it would follow from these results that in the absence of this cell activity no absorption could take place. And this he found to be the case in some experiments in which the epithelium was poisoned with NaF. But in an exactly similar experiment, in which the dog was in unusually good condition, the NaF reduced absorption scarcely at all. Again, Hamburger had shown that serum was absorbed from the intestine of a dog which had been dead four hours, and at a rate which corresponded to about a third of that observed by Reid in normal animals with an intact circulation. Reid himself obtained much the same result when the epithelium had been destroyed by

anæmia, or poisoned with osmic acid, &c. It seems clear, therefore, that in the absence of a normal living epithelium, considerable absorption of serum can still take place, possibly by imbibition, but at any rate by some physical process.

Further, it does not seem by any means certain that osmosis and diffusion are really excluded in these experiments. Reid seems to assume that the serum proteids are taken up by the epithelial cells without digestion. The possibility of such an occurrence has long been maintained, but recent work has thrown very grave doubts upon it. For instance, Mendel and Rockwood compared the absorption of casein and caseose from the small intestine. The loops of gut were washed out very thoroughly beforehand with salt solution. At the end of five hours they recovered 91 per cent. of the casein unabsorbed and only 3 per cent. of the caseose. The slight absorption of casein may well have been due to digestion, for casein can be split up by erepsin as well as by trypsin. Reid in his experiments only washed out the loops of gut with salt solution sufficiently to remove débris of food and worms, and does not mention that he continued the washings in order to remove digestive ferments. Unless it is certain that in these experiments the proteid was not absorbed by digestion, the absorption of the salts and . . ter could be accounted for by osmosis and diffusion. And there is strong presumptive evidence that the proteid must have been absorbed by digestion.

At first sight, however, there appears to be a difference in kind as well as in degree between the absorption of serum from a damaged and a normal loop. Reid found that the anæmia produced by excitation of the mesenteric nerves reduces the rate of serum absorption to half the normal, but does not otherwise alter the relative proportion in which the water, salts, and organic solids are taken up. On the other hand, when the epithelium is further damaged by clamping the mesenteric arteries or by poisons, not only is the rate of absorption further reduced but the quality is altered; for the organic solids are taken up relatively to the salts and water more freely than normal. Nevertheless he looks upon this result as physical and directly connected with the removal of the epithelium. We can conclude only that however likely it may be *a priori* that the living epithelium does actually transport material, these experiments bring forward no evidence in favour of such a process.

(2) *Absorption of Defibrinated Blood by the Surviving Intestine.* — The object of these experiments is the same as the last with the added possibility of eliminating imbibition. The method consists in decapitating a cat, taking out a length of small intestine as rapidly as possible, and filling it with a known quantity of the animal's own defibrinated blood; the gut is placed in a beaker containing the same fluid through which oxygen is bubbled, and the whole is placed in a thermostat kept at the body temperature. Cohnheim had performed a large number of similar experiments, using sometimes the same salt solution inside and outside the gut, and sometimes different fluids. His results showed that in an hour as much as two-thirds of the fluid in the intestine might disappear. Osmosis and diffusion are excluded when the same fluid is on both sides of the intestine. Filtration was not excluded in many of the experiments, because in them the gut showed lively peristalsis, but in other cases peristalsis was absent and yet the fluid disappeared. Cohnheim was never able to demonstrate directly where the fluid disappeared to, but he excluded disappearance through solution by weighing the gut before and after the experiment and finding that its increased weight would not account for all the fluid which had disappeared. He concluded, therefore, that the fluid passed through the wall of the gut from the epithelium to the serous surface and so into the surrounding solution, and that this transference was due to the vital activity of the cells. This was further proved by the observation that the whole process was stopped by NaF. Hamburger repeated one of Cohnheim's experiments, using a ·9 per cent. NaCl solution inside and outside the gut. He found that at the end of $1\frac{3}{4}$ hours 50 per cent. of fluid had disappeared, but he also found that the weight of the gut had increased by 6·2 grm., and concluded that this had been absorbed by imbibition. Reid has confirmed Cohnheim's observations; he used the animal's own defibrinated blood diluted with ·6 per cent. NaCl solution on both sides of the intestine; but he did not weigh the gut before and after the experiments, and there is therefore no certainty about the destination of the absorbed fluid. On turning the gut inside out he was unable to show that fluid passed now in the reverse direction. Reid, however, has attempted to demonstrate by a special apparatus the actual transference of fluid across surviving gut in the direction

of normal absorption. His original experiments were performed long before Cohnheim's, and he has since amplified them. The apparatus consists essentially of two horizontal glass tubes containing ·9 per cent. NaCl solution; these when clamped together are separated only by a disc of intestinal wall. The amount of fluid on the two sides of the membrane could be accurately measured. With absolutely fresh intestine he found that for a little more than ten minutes fluid passed from one side of the membrane to the other in the direction of normal absorption, but that after that interval fluid passed from both sides into the membrane. In this way he claims to have demonstrated a vital transference of normal salt solution to normal salt solution across surviving rabbit's gut at an equality of hydrostatic pressure on the two sides of the membrane. The actual quantities of fluid passing across the membrane were necessarily minute, but the work done could have been performed only by living cells, if the physical condition on the two sides of the membrane were really identical. There is, however, a fact in Reid's experiments which raises a doubt on this point. He could obtain a positive result only when the membrane was taken from an animal in full digestive activity. Gut taken from a fasting animal did not show the phenomenon at all, but from the very beginning of the experiment fluid passed from both sides into the membrane. Considering the rapidity with which salt solution is absorbed by a normal fasting intestine, this total difference between the gut when digesting and fasting is suggestive that there may be some factor other than cell activity at work. The whole phenomenon might equally well be due to osmosis. In the fasting gut as the cells died the osmotic pressure in it would rise and fluid be attracted into it from both sides. But the intestinal wall of an animal in full digestion would contain the products of digestion, and, if these diffused out into the salt solution more readily on the serous than the epithelial side, a transference of fluid in the direction of normal absorption would be observed until the dying condition of the gut was able to determine a passage of fluid from both sides into the membrane. And that something wholly abnormal is taking place in these experiments is shown by the consideration that the fluid absorbed by a normal intestinal epithelium does not pass right through the wall of the gut and does not appear on its serous surface.

(3) Reid's third series of experiments was devised with the object of testing how far diffusion and osmosis play a part in absorption, under conditions removed as little as possible from the normal. The practical difficulty is how to devise experiments which shall give definite results and not at the same time injure the epithelium. As has been pointed out, the only criterion of epithelial injury at present available is the retention by the epithelium of its one-sided permeability to NaCl. But this at best is a very rough guide, for Cohnheim showed that it was possible to poison the cells up to a certain point without depriving them of this power.

The first experiments dealt with the absorption of water, and were based on the following considerations. If water is taken up from a solution in the gut by osmosis, then the amount absorbed must bear relation to the sustained excess of osmotic pressure on the blood side of the membrane, due to substances to which the membrane is impermeable existing in solution in the blood. If, then, the osmotic pressure of the blood were raised by an addition of NaCl, which is a normal constituent of the blood and cannot diffuse into the gut, water should be absorbed from a solution in the intestine more rapidly after than before the addition of NaCl to the blood. Reid injected slowly into the circulation enough of a 5 per cent. solution of NaCl to raise the percentage of NaCl in the blood by about 0·1 per cent. He injected actually 0·3 grm. NaCl per kilo of body weight, which was about 100 c.c. of the solution. He placed in the intestine a 4 per cent. solution of dextrose. Comparing the absorption before and after the injection of the NaCl, he found after the injection that the absorption of water was unaltered. The absorption of dextrose was generally decreased, but this point is only incidental to the other. As the percentage of NaCl in the intestinal contents did not rise after the injection, Reid considered that the epithelium was uninjured, and concluded from his results that the uptake of water is not a simple function of the osmotic pressure of substances to which the normal epithelium is impermeable from the blood to the gut side. In these experiments it is clear that the epithelium had escaped gross injury, but it is not so obvious that it would be uninfluenced by the NaCl injection. It is only necessary to remember MacCallum's experiments to see that the epithelium is just as susceptible from the blood as from the gut side; and it

is possibly more so. MacCallum injected 1000 c.c. of a ·9 per cent. NaCl solution into a rabbit and found a large secretion of fluid containing ·25 per cent. dextrose in the gut. Reid injected about 100 c.c. of a 5 per cent. solution of NaCl into a dog and found that the absorption of water was not increased and the absorption of dextrose was diminished. It would be difficult to say that these two results have no connection with each other, and that the explanation of the failure of an increased concentration of NaCl in the blood to increase correspondingly the absorption of water is not to be found in MacCallum's experiments.

In the other experiments of this series Reid dealt with the absorption of substances in solution, and sought an answer to the question whether the rate at which a substance in solution is absorbed is determined by the relative partial pressures of the substance on the two sides of the intestinal epithelium. Heidenhain showed that when either a 1 per cent. or a ·3 per cent. solution of NaCl is being absorbed, the concentration of NaCl in the solution becomes rapidly altered until it is equal to the concentration of NaCl in the blood, and that when that point of equilibrium has been reached, water and salt are absorbed at the same rate. Hamburger and Höber consider that the solution during concentration becomes isotonic with the blood before the substance and solvent are absorbed at the same rate. The difference of opinion between these observers is immaterial in this connection. It is clear that the whole phenomenon can be explained by diffusion and osmosis; the alteration of the concentration of the solution up to the point of equilibrium could be accounted for by the rapid diffusion into the blood of either solvent or substance in solution; and the final absorption of the solution as a whole could be accounted for by the osmotic pressure of substances in the blood to which the intestinal epithelium is impermeable. Reid found, like Cohnheim, that the point of equilibrium for a solution of dextrose in water was a concentration of about 4 per cent., and that if a stronger or weaker solution was used, its concentration alters until this point of equilibrium was reached. Thus, if a 2 per cent. solution of dextrose in water is placed in the intestine, it becomes a 4 per cent. solution within half-an-hour. He further found that if 2 per cent. of dextrose was dissolved in a ·6 per cent. NaCl solution, the concentration of dextrose remained unaltered and

did not rise during absorption. This result could be explained as due to the water absorption being so delayed by the osmotic pressure of the NaCl that the uptake of dextrose could still keep pace with it. Now, from these data it would be expected that if the absorptions of 2 per cent. solutions of dextrose in water and in ·6 per cent. NaCl solution were compared, a greater quantity of dextrose would be absorbed from the former solution than from the latter; because the concentration of dextrose in the former solution will rise during absorption up to 4 per cent., but will remain unaltered in the other. This expectation, however, was not fulfilled. Reid found in most of his experiments that just as much dextrose was absorbed from the salted as from the unsalted solution, *i.e.* from the low as from the high concentration of dextrose. And in two animals which were in full digestion with white lacteals he found that much more dextrose was absorbed from the salted solution. He associated this unexpected result with the presence of NaCl in one of the solutions, and in support of this idea he found that more dextrose was absorbed from a solution salted with KCl than from one salted with NaCl. He sees in these results a clear indication of ionic action; and every one will agree with him. But when he goes on to explain the ionic action as due to its producing a stimulation of a specific cell activity, he may be right, but he is going far beyond what is justified by our knowledge of ionic action in general. In fact, these experiments are but another instance of the impossibility of interpreting at present the results of experiments on intestinal absorption where solutions of electrolytes are allowed to influence the absorbing cell membrane.

Conclusion.—We have reviewed the more important experiments dealing with the mechanism of absorption from the small intestine with the object of finding out how much is left unexplained by known physical processes, and therefore how much must be attributed to vital action. We have seen that, whilst there is no one experimental result which can be pointed to as indicating with absolute certainty vital action, there are many which cannot be satisfactorily explained by our present knowledge of physical processes in relation to living cells. Nevertheless we have seen that physical processes can explain much; and in forming a working hypothesis on intestinal absorption we are driven at present to leave vital activity largely out of account

and to explain as much as we can by the ordinary physical forces. And of these osmosis and diffusion appear to be the most important. But before we can apply these physical forces to absorption we must know the physical conditions which exist in the blood and lymph, into which material is to be absorbed, as compared with those in the gut. That is to say, we must have at least roughly the following data—the exact products which have to be absorbed, and the concentrations in which they exist in the gut; the form in which they reach the blood and lymph, and the concentration in which they already exist in those fluids; the nature and site of the changes which they undergo between the gut and the blood or lymph. It is almost unnecessary to point out how meagre is our information on these points. Water and salts alone of our food-stuffs reach the blood unaltered, and their absorption need not be further discussed. The others undergo at least a double change. One of these changes certainly takes place in the gut; the chemical nature of the final products is not beyond dispute, physically they are probably more or less diffusible; but their concentration in the gut is unknown. The second change takes place during absorption, but its site is uncertain; it must be somewhere between the gut lumen and the subepithelial capillaries, and we may place it provisionally in the epithelial cells. The nature of these chemical changes is not known for certain; and with the exception of fat the same is true of the form in which the absorbed substances reach the circulation. But it is clear that we have to look upon the absorption of fats, carbohydrates, and proteids as generally taking place in two stages; in the first the digested material has to pass into the epithelial cell and undergo change, and in the second this new product has to leave the cell and pass into the circulation.

Before considering the absorption of the food-stuffs separately, one feature common to all may be referred to. There is no evidence in the case of any one of them that absorption is in any way regulated by the needs of the body; whatever quantity can be digested will also be absorbed, no matter whether the body wants it or not. And the same is true of the absorption of water; but in the case of salts there is evidence that the needs of the body may play a part in absorption. This behaviour of the intestine is in strong contrast to that of the kidney, the main excretory organ of the body.

Absorption of Fat.—It would be simple to suppose that fat was absorbed in some such way as the following. Fat is first digested into soap or a solution of fatty acids. This solution diffuses into the epithelial cells, and is there converted by lipase into neutral fat. This conversion would keep down the concentration of soap, &c., in the cell, and the absorption would continue because of the difference of concentration within the cell and in the gut. Lipase having a reversible action, converts the fat in the cell into a soluble form, and this diffuses out of the cell because of the difference in concentration of this soap within the cell and in the subepithelial spaces. Here the soap is again converted by lipase into fat, which passes into the lacteals. It would be necessary to imagine that the epithelial cell has a one-sided permeability for soap, otherwise it would begin to diffuse back into the gut as well as into the subepithelial spaces. Even such a rough working hypothesis as this necessitates several considerable assumptions. It supposes that fat is digested into a soluble substance and absorbed in solution; this on the whole is probable. It also assumes that this substance in solution will diffuse into the epithelial cells. We have no direct evidence on this point. The cells might be permeable to soaps, &c., in a chemical way in accordance with Overton's views, or in a mechanical way. As against this latter idea we have the observations of Moore and Parker. They found that solutions of soaps in water did not diffuse through parchment paper; that is, they did not behave like crystalloids, because presumably they existed as solution aggregates formed by the aggregation of many molecules. Provided, however, that soap or some such substance existing in a more or less mono-molecular form could diffuse into the cell, it might well undergo aggregation within the cell, and in this way keep up absorption; for the absorbed substance would no longer exist as such in the cell. This change of the absorbed substance from a diffusible to a non-diffusible form might also determine an osmotic absorption of water; for Moore and Parker have shown that soap solutions can exert an osmotic pressure upon a parchment membrane to which they are impermeable. The reversible action of lipase might be to produce first an aggregation of molecules which proceeded until the dissolved solution aggregates passed into the granular form, and then a change in the reverse direction, the granules passing into colloidal

solution and the less aggregation proceeding until the molecules could diffuse out of the cell again.

Absorption of Proteid.—We may form a working hypothesis of this process which would begin as follows. Proteids must be digested before absorption, that is, they must be dissolved and undergo hydrolytic cleavage, at least as far as the albumose stage. Albumoses are diffusible through parchment paper, and we may believe that they might diffuse into the epithelial cells, undergo change there, and so keep up absorption. But when we have got as far as this we are faced by the difficulty that we have no certain knowledge of what change proteids undergo in the epithelial cells. If the view of Folin is right, that the major part of the absolved proteid is immediately broken down further, we can still proceed. For we may believe that albumose is further hydrolysed by erepsin and split into amido-acids, that the NH_2 groups of these acids are split off, and that the ammonia and non-nitrogenous moieties pass into the portal blood by diffusion. There can be little doubt that Folin's view explains the known facts, but it is doubtful where these changes actually take place, whether in the lumen of the gut or in its mucous membrane. If, on the other hand, we believe that any of the absorbed albumose undergoes aggregation within the cell and is there built up into a complex proteid, then the passage of such a substance into the blood becomes difficult to explain by a physical process, unless we believe that the cell on its blood side and the capillary wall are permeable to proteids as complex as the blood proteids.

Absorption of Cabohydrates.—The chief end product of starch digestive appears to be maltose. We may imagine that this diffuses into the epithelial cell and becomes converted by maltose invertin into dextrose, which in turn passes by diffusion into the blood. As in the case of fat and proteid absorption, it is necessary to believe that the new product formed in the cell cannot diffuse back into the gut. Lactose and cane-sugar similarly are changed by ferments during absorption. We have seen that a solution of dextrose is absorbed as rapidly as one of maltose, and yet dextrose undergoes no change by ferments during absorption. We have not the data which enable us to discuss how far the absorption of dextrose could be a physical process. We are uncertain how far dextrose in the portal blood is in a free con-

dition uncombined with colloid; we are faced with the possibility that dextrose in the epithelial cell is not free, but is possibly linked on to proteid, and that this loose combination can again be split in two, the dextrose passing into the blood and the proteid molecule remaining intact and capable of combining with fresh dextrose.

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CHAPTER XIX

THE FORMATION OF UREA

Urea or carbamide, $CO.(NH_2)_2$, is in mammals the form in which the major part of the nitrogen absorbed from the alimentary canal leaves the body in the urine. The proportion of the total urinary nitrogen excreted as urea and other substances is by no means constant even in health. The following table shows the effect produced upon the distribution of the urinary nitrogen in a healthy man by altering the total nitrogen of the food.

Quantity of urine	1170 c.c.	385 c.c.
Total nitrogen	16·8 grm.	3·6 grm.
Urea-nitrogen	14·7 grm. = 87·5%	2·2 grm. = 61·7%
Ammonia-nitrogen	0·49 grm. = 3·0%	0·42 grm. = 11·3%
Uric-acid nitrogen	0·18 grm = 1·1%	0·09 grm. = 2·5%
Kreatinin-nitrogen	0·58 grm. = 3·6%	0·60 grm. = 17·2%
Undetermined nitrogen	0·85 grm. = 4·9%	0·27 grm. = 7·3%
Total SO_3	3·64 grm.	0·76 grm.
Inorganic SO_3	3·27 grm. = 90·0%	0·46 grm. = 60·5%
Ethereal SO_3	0·19 grm. = 5·2%	0·10 grm. = 13·2%
Neutral SO_3	0·18 grm. = 4·8%	0·20 grm. = 26·3%

(Folin.)

It will be seen that when the total urinary nitrogen has been greatly reduced the nitrogen excreted as urea falls to about 60 per cent. of the whole, and that there is a corresponding rise in the nitrogen excreted as ammonia, kreatinin, and undetermined bodies and also in the exeretion of neutral sulphur. On a liberal nitrogenous diet the urea-nitrogen forms at least 85 per cent. of the whole.

The problem to be discussed is, what tissues produce urea and out of what do they form it.

According to the theories both of Voit and of Pflüger the bulk of the nitrogen of the food after absorption is built up either into proteid or into bioplasm, and this is broken down in

the tissues into simpler compounds, which are ultimately converted into and leave the body chiefly as urea. At the same time it has been supposed that an unknown proportion of the total urea-nitrogen may have had a different history, and that it is derived from the breaking down of proteids not in the tissue but in the alimentary canal. Folin has recently put forward a wholly different view of proteid metabolism, and divides the nitrogenous katabolism into endogenous and exogenous. The endogenous or tissue metabolism is represented in the urine by kreatinin, uric acid, neutral sulphur, and possibly a very small proportion of the total urea. The bulk of the urea and the inorganic sulphate represent the exogenous katabolism, that is, the proteid which is broken down during digestion and absorption and never built up again into proteid or bioplasm. According to this view urea is not the chief end product of nitrogenous tissue metabolism, as in views of Voit and Pflüger, but is the form in which the nitrogen of the food which is in excess of the tissue needs is passed out of the body after having been broken down in the alimentary canal. According to all of the three views urea possibly has a double origin, the tissues, and the alimentary canal apart from the tissues.

It is impossible to follow in detail the breaking down of proteid in the alimentary canal of a living animal, and our knowledge is derived chiefly from the action outside the body of digestive ferments and other hydrolytic agents. It is even more difficult to follow in the living animal the steps in the disintegration of tissue proteid which might lead to the formation of urea. The views which have been held as to what substances are the immediate precursors of urea, and by what process urea is formed from them, have been founded both on chemical and experimental evidence. Proteids have been oxidised by various agents and hydrolysed by digestive ferments, acids, or alkalies; many of the simpler nitrogenous products so obtained have been chemically converted into urea; the same products have been placed in the alimentary canal, or injected into animals, or circulated through their organs, and the formation of urea investigated. The subject has been rendered more difficult partly because of our ignorance of the actual constitution and mode of cleavage of the complex proteid molecule, and partly because both the end and intermediate products formed by the action of

various oxidising and hydrolysing agents have been so numerous and diverse. Recently some light has been thrown on this subject from two directions. On the one hand, our knowledge of the constitution of the proteid molecule has received considerable additions, partly from the work of Fischer and others who have synthesised proteid-like bodies, the polypeptides, and partly from the work of Kossel and many others who have studied in detail the hydrolytic disintegrations of proteids. On the other hand, it has been discovered that tissues contain proteolytic and other ferments, which presumably exert their action during the normal metabolism of the tissues. The autolytic action of these ferments outside the body on their corresponding tissues is being studied by numerous observers, including Leathes, Hedin, Dakin, Levenne, and others in England and America. As far as the results at present show, the products of autolysis do not differ greatly from those produced by the digestive ferments and other hydrolytic agents.

The various products into which proteid may be broken up either by oxidation or by hydrolysis with acids, alkalies, or ferments may be roughly classified for our present purpose as follows:—(1) The base, ammonia; (2) the mono-amino acids, *e.g.* glycin, alanin, leucin, aspartic acid and glutamic acid; (2*a*) the aromatic mono-amino acids, *e.g.* tyrosin and phenyl-alanin; (2*b*) the mono-amino acids, containing sulphur, cystein, and cystin; (3) the diamino-acids, *e.g.* arginin, lysin, ornithin, and histidin; (4) the chromogenic group, indol and pyrol derivatives; (5) the purin and pyrimidin bases; and (6) the carbohydrate group.

The N of most proteids is found on complete artificial hydrolysis to be distributed over the first three groups and in the following proportions. By hydrolysis of various proteids with acid it has been found that ammonia N forms 8 to 13 per cent., diamino-acid N 20 to 30 per cent., and the mono-amino acid N 55 to 75 per cent. of the whole. Cohnheim found that when erepsin acted upon peptone, made from syntonin by pepsin, the distribution of N was much the same; the ammonia N formed 7 per cent., the diamino-acid N 30 per cent., of which a third was present as arginin, and the mono-amino acid N 63 per cent., of the whole. Members of other groups besides those of the first three can be made chemically to yield urea, which is then ob-

tained indirectly from proteid. But urea has been obtained directly from proteid in two ways, either by oxidation in the presence of ammonia or by prolonged heating with baryta.

We shall first consider the various views which have been held as to the nature of the immediate precursors of urea in the body, and the process by which their conversion into urea is carried out, and then deal with the seat of urea formation. Unfortunately much of the work on this subject is now known to be unreliable because of the methods used in the detection and estimation of urea. The methods of Bunsen and Schöndorff were extensively used; they are now only of historical interest, and need not be further discussed than to say that there is no guarantee that the substance being estimated is wholly urea. Salkowski's modification of Bunsen's method is more satisfactory. For in it the ammonia as well as the CO_2 split off from the separated substance are estimated, and if these two bear a certain proportion to each other, the substance from which they are derived is presumably urea. Even the more modern method of Mörner and Sjöquist is not free from serious fallacy. The principle of the method is that a mixture of alcohol and ether after the addition of a solution of barium chloride and barium hydrate precipitates all nitrogenous constituents except urea and ammonia. The urea-nitrogen is determined in the concentrated filtrate, after driving off the ammonia, by Kjeldahl's method. It is now known that hippuric acid, kreatinine and traces of allantoin are estimated with the urea, and there is the further possibility that the nitrogen of other less well-known substances may be simultaneously estimated. A more reliable method is that of Folin. This is based on the fact that when urine is heated with hydrochloric acid and crystalline magnesium chloride the nitrogen of urea, but not of other substances, is decomposed into ammonia. The ammonia produced is distilled off and determined by titration. The preformed ammonia in the urine is separately estimated and the urea-nitrogen calculated by difference. Even more trustworthy is a combination of the methods of Mörner and Folin. The preliminary precipitation is carried out by Mörner and Sjöquist's method, and the concentrated filtrate so obtained is then treated by Folin's method. The most certain method of detecting urea is to separate crystals of it, or of one of its compounds, and determine their melting-points, or

subject them to elementary analysis. As a method of estimation, the preparation of urea crystals could yield only minimal figures.

The Precursors of Urea

(1) *Hofmeister's view*: the formation of urea by an oxidation synthesis.—He showed that urea could be obtained directly from egg-albumin or gelatine when they were oxidised with potassium permanganate in the presence of ammonia. He further showed that urea could be obtained by the same method from certain products of proteid hydrolysis, the mono-amino acids glycin, leucin and aspartic acid. It has not yet been shown that diamino acids can by the same means be converted into urea, although it would seem probable that they could. He experimented in the same way with a large number of substances, and found that only certain arrangements of the C atom would yield urea when oxidised in the presence of ammonia. He found that the acid radicle $-CHOH.COOH$, or the amino-acid radicle $-CH_2.NH_2.COOH$, readily yielded urea, and that in certain other groups the same was true of the simpler compounds and not of the more complex members of the same series. Thus with the $-CO.NH_2$ group, formamide ($H.CO.NH_2$) yielded urea but not acetamide; with the $-CN$ group, formo-nitrite ($H.CN$) but not aceto-nitrite; and with the $-CH_2.OH$ group, methyl alcohol but not ethyl alcohol. From these various radicles he supposed that a radicle $-CO.NH_2$ was produced either as oxamic acid ($CO.NH_2.COOH$) or as formamide ($H.CO.NH_2$), and that this radicle combined with NH_2, derived from the oxidation of ammonia, to produce urea.

$$H.CO.NH_2 + NH_3 + O = NH_2.CO.NH_2 + H_2O.$$

This theory supposes that in the body proteids are broken down into mono-amino acids and ammonia, which together yield urea by undergoing further oxidation and a final synthesis of their nitrogenous products. All the conditions necessary for this view are present in the body. It is obvious that urea, which contains two NH_2 groups, could not arise direct from a mono-amino acid except by some process of condensation of two molecules into one, and it has been found that in the absence of ammonia oxidation of proteid with potassium permanganate does not yield

urea. There is nothing inherently improbable in such a [illegible] taking place as a final step in proteid katabolism. Simi[illegible] syntheses are well known in the production of hippuric [illegible] ethereal sulphates, and the conjugated compounds of gl[illegible]ome acid.

(2) *Drechsel's view*; the origin of urea from arg[illegible] H[illegible] showed that urea could be directly produced from pr[illegible]ds [illegible] heating them for a long time with baryta. He found [illegible]hat t[illegible] intermediate step in this direct conversion was formed [illegible] body, lysatin, which he produced by hydrolysing prote[illegible] wi[illegible] acid, and which yielded urea when heated for half [illegible]our[illegible] baryta. Hedin showed that lysatin was a mixture [illegible] the hexone bases, lysin and arginin, and that the urea was de[illegible] only from the arginin, which splits on hydrolysis into urea ornithin.

$$\begin{array}{l} \qquad\qquad\quad NH_2 \\ NH:C \\ \qquad\qquad\quad NH-C_3H_6.CH.NH_2.COOH + H_2O \\ \qquad\qquad\quad (Arginin) \\ = CO(NH_2)_2 + NH_2.C_3H_6.CH.NH_2.COOH. \\ \quad (Urea) \qquad\qquad\qquad\quad (Ornithin) \end{array}$$

This reaction is of considerable theoretical importance.

It has been shown that the combinations of C within the proteid molecule occurs probably in two types. The first is seen in hippuric acid and the synthetic polypeptides of Fischer. In this type a single NH group unites two C chains together and has a CO group next to it (1). The second type consists of a guanidin group $-NH.C.NH-$ which either joins as before two

$$\ddot{N}H$$

C chains together (2), or is at one end of a carbon chain (2′).

$$\begin{array}{ll} (1) & -CO.NH.C- \\ (2) & -CO.NH.C.NH.C- \\ & \qquad\quad \ddot{N}H \\ (2') & NH_2.C.NH.C- \\ & \qquad\quad \ddot{N}H \end{array}$$

Hydrolysis of (1) or (2) by acids splits off only the CO group,

and the rest of the combination holds together, producing (1*a*) and (2*a*).

$$(1a)\quad -COOH + NH_2.C-$$

$$(2a)\quad -COOH + NH.\underset{\ddot{N}H}{C}.NH.C-$$

$$(2'b)\quad NH_2.CO + NH_2.\underset{\dot{N}H_2}{C}-$$

As examples of (1*a*) we may take the hydrolysis of hippuric acid and of glycyl glycin by an acid. Hippuric acid is hydrolysed into benzoic acid and glycin.

$$C_6H_5.CO.NH.CH_2.COOH + H_2O$$
$$= C_6H_5.COOH + NH_2.CH_2.COOH.$$

In the same way glycyl glycin, the simplest polypeptide, splits up into glycin.

$$NH_2.CH_2.CO.NH.CH_2.COOH + H_2O$$
$$= NH_2.CH_2.COOH + NH_2.CH_2.COOH.$$

In the case of (2*a*) it is clear that hydrolysis by acid will have produced a free amino-acid and some combination with the guanidin group. If, however, the substance containing the guanidin group is heated with baryta, a more profound change is produced. The guanidin group itself is broken up and urea formed, (2′) becoming (2′*b*). For example, guanidin acetic acid treated with an acid yields guanidin and oxalic acid.

$$NH_2.\underset{\ddot{N}H}{C}.NH.CH_2.COOH + H_2O = NH_2.\underset{\ddot{N}H}{C}.NH_2 + COOH.COOH.$$

But when the same substance is heated with baryta, it splits up into urea and glycin.

$$NH_2.\underset{NH}{C}.NH.CH_2.COOH + H_2O = NH_2.\underset{\dot{N}H_2}{CO} + NH_2.CH_2.COOH.$$

Corresponding to this difference in the hydrolytic action of acids and baryta, the imidolytic ferments of the body may be divided into two groups. (1) Those which act like acids, namely, trypsin, erepsin, and some of the proteolytic and other autolytic tissue ferments. (2) Those which can split up the

guanidin radicle and p[illegible]urea. Such a ferment has been described by Kossel and [illegible]kin and called arginase.

Arginase in a few hours splits arginin, a compound of a guanidin radicle with α. amino-valeriamic acid, into urea and ornithin (diamino-valerianic acid). This ferment has been found in greatest quantity in the liver, in less quantity in the kidneys, thymus, lymphatic gland, and mucous membrane of the alimentary canal, and possibly in the blood and muscles. It appears to be absent from the spleen, suprarenals and pancreatic juice. Arginase is not capable of attacking such simple proteids as the protamins, and is presumably therefore incapable of splitting urea from the more complex proteids. This ferment can explain only the second step in Drechsel's view, the production in the tissues of urea from arginin. But the proteolytic tissue ferments have been found to produce arginin during the autolytic decomposition of tissue proteids. It is possible, therefore, to imagine the production of urea from proteid taking place in the tissues in two stages as the result of the action of two tissue ferments.

The possibility exists, however, that the tissues may contain a ferment capable of splitting off urea or some such body direct from proteid. For Kossel and Dakin have described a ferment in the wall of the small intestine which can attack the arginin groups contained in a protamin in such a way that the ornithin portion of the arginin remains attached to the rest of the proteid molecule. It is not yet known in what form the guanidin radicle of the arginin is split off, whether as urea, guanidin, or some similar body, nor have they yet shown whether this ferment can attack in the same way proteids more complex than protamins. Kutscher and Otori have shown that guanidin is produced during the autolysis of the pancreas, presumably by the action of a ferment. They have found that it is also produced when gelatine, arginin, or guanin, derived from nucleo-proteid, are oxidised with a permanganate. They suggest that it is a product in normal tissue metabolism and might have three possible fates in the body. It may pass out unchanged in the urine and up to now have escaped detection; it may be converted into urea, possibly by arginase; or in the muscles it may be synthesised into kreatin, for in them arginase is either not present at all, or only in very small quantity.

It has been tacitly assumed that autolysis represents a normal tissue metabolism, and that, if a substance is formed during autolysis, we may believe that it occurs also during nitrogenous katabolism in the living body. This idea, although not universally accepted, is in the present state of our knowledge justifiable. Thus arginin, like the amino acids, is formed during autolysis, and is to be found in the urine during phosphorus poisoning. From this we may infer that arginin is an intermediate product of normal metabolism which under these circumstances has failed to be converted into its usual end product, urea.

The possibility and likelihood of urea being formed in the tissues in accordance with Drechsel's view has been amply demonstrated. We have to consider now what proportion of the total urea has this origin. The available data are as follows. We have already considered the distribution of arginase in the tissues. Arginin has been demonstrated among the products of the autolysis of almost every tissue It has been missed in the case of the kidney, thymus, and wall of the alimentary canal, but as these are now known to contain arginase, the absence of arginin may be only apparent. Kossel has shown that the pure proteids of beef, milk, or bread when hydrolysed by acids yield about 5 per cent. by weight of arginin, and would therefore yield less than 2 per cent. by weight of urea from arginin. Levenne has found that liver hydrolysed by acid yields about 0·5 per cent. by weight of arginin, and that pancreas and spleen yield about half that amount. From such data it is impossible to answer the above question; we do not know how much urea is formed in the body in this way, but it does not seem likely that there is enough arginin in the body to yield a very high proportion of the total urea. It has been found that during complete starvation, when the body proteids are being used up, the urea-nitrogen may form only 1·5 per cent. of the total nitrogen in the urine. It appears, therefore, that the endogenous formation of urea cannot be a very important one. Drechsel calculated that less than 4 out of the 34 grm. of urea theoretically obtainable from 100 grm. of proteid could be formed from arginin, and he stated a second view to account for the formation of the balance.

(3) *Drechsel's view;* urea from ammonium carbamate.—He pointed out that when free ammonia (NH_3) unites with CO

ammonium carbamate is produced. It has been found, however, that when solutions of ammonia (NH_4OH) are mixed with soluble carbonates, little or no carbamate but carbonate is produced, unless the ammonia solution be in great excess. Ammonium carbamate is also produced when proteid, mono-amino acids, tartaric or oxalic acid, are oxidised according to the method of Hofmeister with a permanganate in the presence of ammonia solution. Ammonium carbamate can be converted into urea by heating it to 135° C. Drechsel carried out the conversion at a low temperature by passing an alternating electric current through its solution; this produces an alternating oxidation and reduction according to the following formulæ—

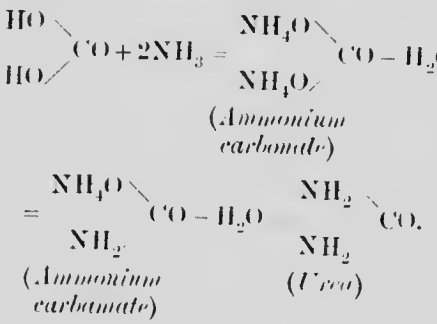
$$NH_4O.CO.NH_2 + O = NH_2O.CO.NH_2 + H_2O.$$
$$NH_2O.CO.NH_2 + H_2 = NH_2.CO.NH_2 + H_2O.$$

There is no necessary antagonism between this view of the origin of urea and that of Hofmeister. Ammonium carbamate might arise by a process of oxidative synthesis, and this be subsequently dehydrated into urea.

There can be little doubt that carbamates are a constituent of the normal body; but all the quantitative estimations made are now known to be unreliable because Drechsel's method of estimation is open to the objection that carbamate may be formed during the manipulations. Macleod and Haskins have lately published what promises to be an accurate method of estimation, but it has not yet been applied to the elucidation of this subject. Carbamates have been found in traces in the blood serum and urine of normal dogs, and in considerable quantities in the alkaline urine of the horse. Large quantities have been found in the urine of dogs which had been fed on lime until their urine was alkaline to litmus. Apart from the theoretical possibility that ammonium carbamate could be a precursor of urea, the only experimental evidence which supports it is that afforded by Pawlow, Massen, Hahn, and Nencki. They showed that, after an Eck's fistula, an anastomosis between the portal vein and inferior vena cava, had been established in dogs, and especially if the hepatic artery was tied as well, large quantities of carbamate were present in the urine and the urea was reduced.

(4) *Schmiedeberg's view; Origin of Urea from Ammonium Carbonate.*—It has been shown that ammonium carbonate can be

produced artificially from proteids, and that when heated to 140° C. it loses two molecules of water and is converted into urea.



According to this view the N in such a group as CH_2-NH_2 in the amino-acid is converted into ammonia, combines with carbonate acid to form ammonium carbonate, and is dehydrated into urea. A similar terminal synthesis with loss of water is known to take place in the production of hippuric acid from benzoic acid and glycin. There is no necessary antagonism between the views of Schmiedeberg and Drechsel, for ammonium carbamate could be looked upon as an intermediate product in the dehydration of ammonium carbonate to urea.

We have abundant evidence that ammonium salts are constantly formed in the body, and, being highly poisonous, are converted into a relatively innocuous body, urea. Ammonia is formed during the autolysis of tissues, arterial blood contains 0·4 mgr. per cent., the urine contains only small quantities, and not more than about 5–10 per cent. of its total nitrogen is present as ammonia. When the liver is put out of circulation or extensively destroyed, the ammonia content of the blood and urine is increased and the urea decreased. Schröder has proved that ammonium salts circulated through the surviving liver of a dog are converted into urea. He found that not only the carbonate but any ammonium salt whose acid radicle could be oxidised in the body, such as the formate, lactate, citrate, and acetate, was converted into urea. We have, therefore, conclusive evidence that ammonia is one of the immediate precursors of urea, but we have no evidence which shows how this conversion is carried out, and whether it takes place by an oxidative synthesis or by a process of dehydration.

(5) *Origin of Urea from Ammonium Cyanate.*—This salt has been produced artificially from proteid, and Wöhler made the

classical discovery that it became converted into urea gradually, at ordinary temperatures, and at once at 100° C. Dry ammonia and cyanic acid (CN.OH) together produce ammonium cyanate, and the view supposes that during proteid katabolism in the body these two substances are formed. Cyanates are produced by the oxidation of cyanides, and several observers have obtained prussic acid during the oxidation of proteid. Plimmer has recently shown that many proteids when oxidised with equal parts of strong nitric and sulphuric acids yield constantly more than 0·5 per cent. by weight of prussic acid. He further showed that the products of hydrolysis of the proteid gave the same yield. He found that the prussic acid came neither from the mono-amino acids nor the bulk of it from the hexone bases, and he was unable to determine its origin further than by showing that tyrosin yielded appreciable quantities. Oxidation of the same proteids with chromic acid gave constantly a greater yield than oxidation with the nitric acid mixture, and in this case he was able to show that none of the prussic acid arose from tyrosin but chiefly from the amino-acids, glycin and aspartic acid.

Although this view is chemically possible, it lacks the necessary physiological evidence that cyanates do occur in the body.

Besides arginin and the various ammonium compounds already considered, there are other intermediate products of nitrogenous metabolism which can be converted into urea by chemical means, and which are either possible sources of urea in the body or are already known to be converted into urea or some similar substance.

(*a*) *The Mono-amino Acids.*—There are two places in the body where mono-amino acids might be formed from proteid, the alimentary canal and the tissues. In the alimentary canal we know that these bodies are produced, and we have already seen that erepsin can outside the body convert more than 60 per cent. of the nitrogen of peptone into the form of mono-amino acid; but we have no information to what extent this change goes on during normal digestion. Since normal urine contains at most traces of these bodies, it seems likely that any amino acid produced in the alimentary canal must be either synthesised during absorption and made use of by the body or else excreted as some other substance. There are experiments to show that glycin, leucin, or aspartic acid given to an animal by the mouth

are excreted in the urine as urea when estimated by Bunsen's method. Salkowski, using his own modification of Bunsen's method, has recently shown that aspartic acid introduced into the stomach of a rabbit is excreted chiefly as urea and not as the corresponding amide, asparagin. But some of the aspartic acid absorbed was excreted not as urea but probably unaltered. Although we can be certain that mono-amino acid absorbed from the alimentary canal is excreted mainly as urea, we are completely in the dark as to how much of the urea has this origin.

We know but little about the production of mono-amino acids in the tissues. They occur abundantly during the autolysis of organs, and their formation has been ascribed to the action of proteolytic tissue ferments, resembling trypsin. Vernon has lately described tissue ferments which resemble erepsin in their action—*i.e.* they are incapable of attacking most native proteids, but are able to hydrolyse albumoses or peptones into mono-amino acids, ammonia and diamino-acids. He found them most plentiful in the kidney, intestinal mucous membrane, pancreas, spleen, and liver; but they were present in the heart, muscle, brain, and all other tissues investigated. We are bound, therefore, to believe that the formation of mono-amino acids represents an important step in the disintegration of tissue proteid, and consequently in the ultimate production of urea from proteid.

We have some evidence how a mono-amino acid is further dealt with in the body in the process of its conversion into urea. We have already dealt with the chemical possibilities exemplified in the view of Hofmeister, Schmiedeberg, and Drechsel. Jacoby found that a water extract of fresh liver tissue mixed with a mono-amino acid converted it into ammonia. Lang greatly extended these observations. He pounded up various organs with salt solution, toluol, and the substance to be investigated. He showed that besides the liver many other organs, such as the kidneys, pancreas, wall of the alimentary canal, &c., were capable of converting the NH_2 group of glycin into ammonia. He further showed that the same was true for many other amido substances, such as tyrosin, asparagin, glutamin, &c. These experiments suggest that the power of tissues to convert the NH_2 group of a mono-amino acid into ammonia is

probably due to a tissue ferment. Salaskin and Kowalesky have shown that when glycin is injected into a systemic vein of a dog, only a small part appears unaltered in the urine. The rest soon disappears from the blood and is not to be found in the tissues. And as the ammonia content of the blood is increased, they suggest that the tissues have probably converted the glycin into some ammonium compound. There are several pathological conditions of the body in which mono-amino acids are excreted in the urine. The best known of these is severe destruction of the liver, such as occurs in the acute yellow atrophy of that organ. But there are other more general conditions known in which the same thing occurs, such as gout, phosphorus-poisoning, pneumonia, and leucocythaemia. This suggests, like the experiments of Lang, that the conversion of this amido-N into ammonia is a property, not of a single tissue, the liver, but of the tissues in general.

(*b*) *The Diamino-Acids.*—We know that they are formed from proteid both by the digestive ferments and during the autolysis of organs. We have but little information as to their fate in the body, with the exception of arginin. More attention has been paid to the possibility that their non-nitrogenous part may be synthesised into a carbohydrate than to the fate of their nitrogen. Mayer has experimented with diamino-propionic acid. He injected it subcutaneously into a rabbit, and was able to demonstrate small quantities of glyceric acid in the urine.

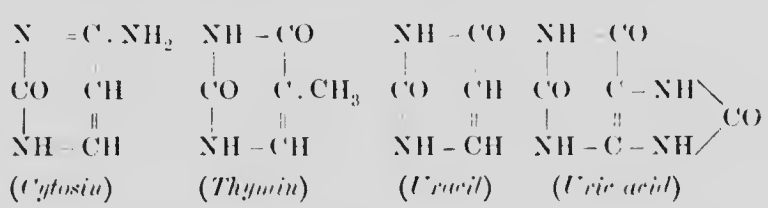
$$\begin{array}{l} CH_2 . NH_2 \\ | \\ CH . NH_2 \\ | \\ COOH \end{array} + 2H_2O = \begin{array}{l} CH_2 . OH \\ | \\ CH . OH \\ | \\ COOH \end{array} + 2NH_3$$

This reaction entails the splitting off of amido-nitrogen as ammonia, and this presumably would be converted into urea. The reaction also illustrates the power the tissues have of splitting off the amido-nitrogen from comparatively complex bodies without necessarily at the same time breaking up their non-nitrogenous portions. Thompson has recently brought forward proof that a diamino-acid is converted into urea in the body. He fed dogs with arginin, and also injected it subcutaneously, and

found that from 70–95 per cent. of its nitrogen was excreted in the urine as urea. Since, as we have seen, arginin is split into urea and ornithin, and each of these contains half of the arginin-nitrogen, it is clear that a large part of the ornithin-nitrogen must be excreted as urea. Thompson has since shown that ornithin injected intravenously reappears in the urine in part at any rate as urea.

(*c*) *The Purin and Pyrimidin Bases.*—These bodies are produced during the autolysis of glandular organs; they are also obtained by the hydrolysis with acids of nucleo-proteids, and are known to be derived from the nucleic-acid portion of those proteids.

The pyrimidin bases, cytosin, thymin, and uracil, as their formulæ show, have an obvious relation to the purin bodies. For purin can be synthesised from pyrimidin, consequently the pyrimidin ring is contained within the purin ring.



We know nothing about their production in the body, but it is possible that they represent a stage in the disintegration or synthesis of the purin bases. Thymin and uracil, as their formulæ suggest, have been synthesised from urea, and might possibly yield urea in the body, but as yet we know nothing of the fate of their nitrogen.

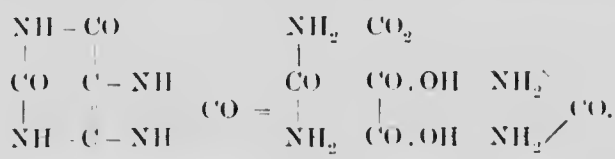
The purin bases, adenin, guanin, hypoxanthin, and xanthin, although they occur plentifully during the autolysis of tissue nucleo-proteids, are largely replaced in the urine by another purin base, uric acid. The total purin N in the urine may not amount to more than 1 to 3 per cent. of the whole urinary N. It has two origins, the purins of the food and the disintegration of nucleo-proteid in the body. The possibility of uric acid also having a synthetic origin in mammals as in birds need not be considered here. By placing a man on a purin-free diet it is found that the endogenous purin N in the urine rapidly becomes

constant in quantity and forms about a half to two-thirds of the total urinary purins excreted by the same man on a mixed diet. But the amount of exogenous purin found in the urine is much less than that in the food. Burian and Schur showed that, when a known quantity of a purin was given by the mouth, a man excreted 50 per cent. of it, a rabbit 16 per cent., and a dog only 5 per cent. of the amount introduced. They further found that, with the exception of the methyl derivatives of purin, whichever purin body was given it was excreted in the urine as uric acid. From this they concluded that the uric acid formed from the exogenous purins of the food is largely destroyed in the body. Probably the same is true of the endogenous purins also, but it is much more difficult to demonstrate experimentally. Perhaps the most convincing evidence for this supposition is the fact that many tissues possess ferments which can convert the various purins into uric acid and further break it up. Schittenhelm found that watery extracts of the liver, spleen, lungs, and muscle possessed ferments which converted adenin into hypoxanthin and guanin into xanthin, and then these into uric acid. He found, like previous observers, that the liver, kidney, and muscle possess a ferment or ferments which can break up uric acid.

It seems pretty clear that the purin N in the urine does not represent the total purin N metabolised in the body, and there can be no doubt that the bulk of the rest of the metabolised purin is excreted as urea in mammals. The older experiments are not conclusive because of the methods of urea estimation used. Salkowski has given uric acid by the mouth to a rabbit and found that the greater part of the absorbed uric acid was excreted as actual urea, a small portion unchanged and another small portion possibly as allantoin. It is not known what proportion of the whole urea is thus produced from uric acid, but it is not likely to be large. For, the purin content of food is comparatively small, and the total quantity of nucleo-proteid metabolised in the tissues is likely to be small when compared with that of other proteids.

It is necessary to consider the chemical changes involved in the breaking up of uric acid. The structure of uric acid suggests that urea might be obtained from it direct, just as from the pyrimidin bases. This can be readily brought about by its

oxidation and hydrolysis, when urea, oxalic acid and CO_2 are finally produced.



A different decomposition of uric acid is brought about by heating it with strong hydrochloric acid in sealed tubes at 170° C., when it is split into glycin, ammonia, and CO_2. It seems likely that the splitting of uric acid by the tissue ferment resembles this last mode of decomposition, but the information on the subject is as yet meagre. It has been shown that ammonia is produced when uric acid is decomposed with extracts of liver, kidney, spleen, and muscle. It has also been found that when uric acid is digested with kidney a formation of glycin takes place. We may conclude provisionally that, when uric acid is converted into urea in the body, the immediate precursor of the urea is ammonia.

(*d*) *Kreatin and Kreatinin.*—They deserve a word in this connection because they bear such an obvious chemical relation to urea and are formed abundantly in the body. Kreatin (methyl-guanidin acetic acid) is split by boiling with alkalies into urea and sarcosin. Muscles contain about 0·4 per cent. of kreatin and kreatinin together, and after severe muscular exercise or prolonged starvation this figure may be more than doubled. It is easy to calculate that the muscles of a man may contain a good deal more than 50 grm. of these substances. The quantity of these substances in the urine is about 2 grm. a day, and it seems likely that, as in the case of uric acid, the urinary kreatinin has two distinct origins, an exogenous and an endogenous. Kreatin given by the mouth to man is excreted as kreatinin in the urine, and it is certain that in meat-eaters some of the urinary kreatinin is derived from the meat of the food. But, in rabbits recent experiments have failed to show that more than a minute portion of the kreatin administered was excreted as kreatinin, and further the balance could not be found in the urine either as ammonia, urea, or uric acid.

Folin has recently investigated the excretion of kreatinin in the

urine in man on a meat-free diet. He found, like Macleod, that the excretion was still considerable, about 1·5 grm. a day, and that this endogenous kreatinin was constant for the individual and quite independent of the total nitrogen of the food. In different individuals the total amount of endogenous kreatinin excreted appears to be determined by the body weight and by the fatness or thinness of the person, the excretion per kilo of body weight being less in fat than thin persons. It seems probable that hard muscular exercise which increases the kreatin content of muscle also increases the excretion of kreatinin in the urine. From these observations it seems clear that some of the kreatin or kreatinin of muscles leaves them unchanged and is excreted as kreatinin. But considering the quantity present in the muscles as a whole, it would seem *a priori* likely that, as in the case of uric acid, the excretion of kreatinin does not represent the total formation, and that kreatin leaves the muscles also in some other form. It does not seem likely that kreatin leaves the muscles partly as urea, for, quite apart from the question whether the muscles can form urea at all, they contain but little, in fact less than the blood. From analogy we should imagine that muscles would contain a ferment capable of splitting off the amido-nitrogen of these substances as ammonia; but there is no evidence in favour of such an idea except the fact that muscles appear to produce ammonia in large quantities, as is shown by the estimation of the ammonia content of blood going to and from masses of muscles and of the muscles themselves.

On the other hand kreatin, as its formula shows,

$$\begin{array}{c} NH_2 \quad CH_3 \\ | \quad\quad | \\ NH=C-N-CH_2.COOH \end{array}$$

although chemically not unlike arginin, is peculiar in that it contains the group $\left(\begin{array}{c} CH_3 \\ | \\ -N- \end{array}\right)$, which the body apparently cannot easily deal with. Thus glycin is converted in the body into urea, but its methyl derivative, sarcosin, is excreted unchanged. Ordinary proteids do not yield kreatin on autolysis, possibly because they do not possess the necessary $\left(\begin{array}{c} CH_3 \\ | \\ -N- \end{array}\right)$ group, but myosin does yield

kreatin, presumably because it contains the necessary methylated N. It would be possible, therefore, to look upon kreatin as a normal metabolite of muscle proteid which resisted further change in the body excepting dehydration; and if it could be shown that kreatin possesses a physiological action, it might explain why such comparatively small quantities of kreatin should leave the body. Again, there is the further possibility that kreatin is a substance developed *de novo*, like adrenalin, in muscle by synthesis of guanidin in order that it may exert a physiological action. It has been shown that it increases the irritability of the motor nerve endings, and guanidin that of the muscle fibres. It seems possible that when the nerve impulse reaches a muscle it may set up chemical changes there with the production of substances which increase the excitability of nerve and muscle. Such considerations raise a doubt whether it is necessary to believe that endogenous kreatin does leave muscle as something other than kreatin and is a precursor of urea.

The Seat of Formation of Urea

That portion of urea which is produced direct from arginin must have its seat of formation limited to the distribution of arginase. This ferment has been found in the liver, kidneys, thymus, lymphatic glands, and mucous membrane of the alimentary canal; it is absent from the spleen and suprarenals, and, most significantly of all, the muscles.

We have now to deal with the seat of production of urea from ammonium compounds. It might have been expected that organs which are the seat of urea formation would contain on analysis more urea than those in which no such formation was taking place. Many of the older analyses have shown that the liver contained more urea than other organs. Schöndorff was unable to confirm this. In the case of a dog fed on meat, he found that the blood and all the other organs, including the liver, contained the same percentage of urea, namely, 0·12 per cent. There were only two marked exceptions; the muscles with 0·08 per cent., which was less than the blood, and the kidneys with 0·67 per cent., which was much higher, and probably to be accounted for their being the seat of excretion. Schroder was the first observer who made a systematic experimental investigation of the

possible seats of urea formation, using methods of estimation which were conclusive as to the presence of urea. He obtained a positive result with one organ only, the liver.

The Formation of Urea in the Liver.—Schröder circulated through the surviving liver of a dog blood to which ammonium carbonate or formate had been added. The urea in the blood was estimated after the blood had been circulated once through the liver, in order to wash out any urea present, and again after circulating for four hours. In most of his experiments he estimated the urea by Bunsen's method, but in one experiment he separated it as crystals of urea nitrate, and demonstrated finally that the liver can form urea from ammonium salts. In this experiment he found that the blood had gained 1 grm. of urea, representing an increase of 220 per cent. Schröder's experiment has been extended to the herbivora, and found to give the same result in the sheep.

We have already seen that some of the intermediate products of proteid katabolism are converted into ammonia, not only in the liver but also in other tissues. The question arises, does much N reach the liver in the form of ammonia, and, if so, from what tissues does it come? Analyses of the ammonia content of the blood from different parts of the body by Horodynski, Salaskin, and Zaleski have thrown light on the answer to this question. According to their analyses, arterial blood contains about 0·4 mgr. per cent. of ammonia. The blood from a peripheral vein contains about twice as much as the corresponding artery, and muscles contain even more than the venous blood. From this it seems clear that muscles manufacture ammonia and give it off freely to the blood. They also found that the portal blood of a starving animal contained three times as much ammonia as the blood in the hepatic vein or an artery. When the animal was fed on meat the ammonia content of the portal blood was four to five times greater than that of the blood in the hepatic vein. These experiments show that the liver abstracts ammonia from the blood, and that, besides the general tissues, the portal area is an important source of ammonia which might have two origins, the tissue of the alimentary canal and proteid food. We know that the digestive ferments trypsin and erepsin can outside the body form ammonia from proteid, but we do not know to what extent they do so in the body. The rise

in the ammonia of the portal blood from 1·3 mgr. per cent. during starvation to 1·8 during the digestion of proteids might be accounted for in this way; but it is not the only possibility. Horodynski, Salaski, and Zaleski found that the pancreas and mucous membrane of the stomach and intestine contained a higher percentage of ammonia, even during starvation, than any other structure in the body. This percentage was greatly increased during digestion and even during Pawlow's "sham-feeding" experiments. This would seem to show that the tissue metabolism of these digestive glands is marked by a considerable production of ammonia. And it is striking that Vernon should have found tissue erepsins more plentifully in the intestinal mucous membrane, the pancreas and spleen, than in any other tissue except the kidney.

The importance of digestion in the alimentary canal as a source of the precursors of urea is shown in some of Schröder's experiments. He found that when the blood of a fasting animal was circulated through its own liver, the urea content of the blood was diminished by 5 per cent. If an ammonium salt was then added to the blood and circulated through the liver for the same length of time the urea content was increased by 153 per cent. When he circulated the blood of a well-fed dog in digestion through its own liver he found that the urea content of the blood was increased by 27 per cent. in the same time.

The relative importance of the alimentary canal and the other tissues of the body as sources of ammonia is shown by the experiments of Hahn, Massen, Neneki, and Pawlow, and also by those of Nencki, Pawlow, and Zaleski. They were performed on dogs with a simple Eck's fistula. In this case the liver still remains supplied by the hepatic artery, but the portal blood passes direct into the inferior vena cava, and so into the general circulation. Many of the dogs recovered completely from the operation and lived for months without showing any symptoms of ill-health. It was found, however, that when these dogs were fed on meat or received glycin, salts of ammonium or carbamic acid by the mouth, they rapidly became convulsed; but that normal dogs under the same circumstances showed no abnormal symptoms. When the dogs were in convulsions, an examination of the blood and urine showed that the ammonia content of the arterial blood was raised very nearly to that of the portal vein, and that

in the urine the percentage of the total nitrogen excreted as urea was decreased, whilst the percentage present as ammonium salts was increased sufficiently to render the urine alkaline. Among other ammonium salts present was the carbamate. From these results it was concluded that the convulsions were due to poisoning by salts of ammonium or carbamic acid. On the other hand these observers found that, if dogs with an Eck's fistula were fed on a mixed diet containing but little proteid, the ammonia content of the blood and urine might be normal and no convulsions ensue. The explanation of the different result in these two cases appears to be that the liver, when supplied only by the hepatic artery, is capable of converting into urea the ammonia slowly produced by the tissues generally, but that when larger quantities of ammonia enter the portal vein rapidly during digestion, and especially the digestion of proteids, the liver does not receive this increased supply of ammonia sufficiently quickly to prevent poisoning. From these experiments we may draw the following conclusions: that the alimentary canal is an important source of ammonia; that the digestion of proteids increases the ammonia content of the portal vein more than the digestion of other food-stuffs; and that the liver is the chief, if not the only organ, which can convert ammonium salts into urea.

The overwhelming importance of the liver in this conversion is shown by other experiments. The analyses of Horodynski, Salaskin, and Zaleski show that the liver is the only organ which abstracts ammonium salts from the blood. Some experiments by Schröder suggest that the liver alone is capable of carrying out this conversion. He found that extirpation of the kidneys in dogs leads to a slow accumulation of urea in the body, and that it took twenty-seven hours for the urea content of the blood to be increased by four and a half times. If after the nephrectomy ammonium salts or a carbamate were injected into the animal, the urea content of the blood might be increased 100 per cent. in an hour. When, however, the liver was put out of circulation in addition to the removal of the kidneys, the injection of ammonium salts led to no increase of urea in the blood within the period of the experiment, one and a half hours.

We have already seen that there is conclusive evidence that mono-amino acids given by the mouth are excreted chiefly as

urea. We have also seen that there is some evidence that the liver and other tissues can convert the N of these acids into ammonia, and presumably by the action of a ferment. But conclusive experimental evidence that the liver is the organ which converts the mono-amino acids into urea is still wanting. Löwi showed that glycin digested with a liver extract is converted into an amide, but he did not determine its nature. Salaskin carried out experiments in which he circulated through a surviving dog's liver blood to which glycin, leucin, or aspartic acid had been added. In each case he found increased urea values in the blood. Unfortunately his estimations were done by Schöndorff's method, and, however likely, it is not absolutely certain that urea and not some other amide was actually produced.

The same is true in the case of uric acid. We have seen that it is partly destroyed and largely converted into urea in the body. We have seen that the liver and other organs possess a ferment which can break up uric acid, but we have no conclusive evidence that the liver actually does manufacture urea out of uric acid. Hahn, Massen, Nencki, and Pawlow observed in dogs, with ligature of the hepatic artery in addition to an Eck's fistula, the usual changes in the urinary ammonia and urea, but in addition an increased excretion of uric acid both actual and relative to the urea. This increase they ascribed to a greater destruction of nucleo-proteid in the tissues. But it might equally well be due to the failure of the liver to convert uric acid into urea. Ascoli carried out a series of experiments in which he circulated through a dog's liver blood containing known quantities of uric acid. He found that within an hour a loss of uric acid took place which was in excess of the loss due to the action of the blood on the uric acid. He further showed by Schöndorff's method increased urea values in the perfused blood.

Is Urea formed in Tissues other than the Liver?—The fact that many organs yield arginin on hydrolysis and also possess arginase suggests that the answer to the question must be in the affirmative, so far as this origin of urea is concerned. But, as has been pointed out already, we have no knowledge of the relative magnitudes of this and of other methods of urea production, and we have still to discuss the question whether or not tissues other than the liver can form urea from ammonia or some other substance.

The question of the possible origin of urea from ammonium salts could be investigated in two ways, either by perfusing blood containing ammonium salts through various surviving organs, or by eliminating the liver in an otherwise intact animal. Both methods have been used. Schröder perfused blood containing ammonium salts through the surviving kidneys of dogs with negative results. He performed similar experiments, using the lower half of a dog's body, also with negative results. With regard to the other method of experiment, it is manifestly impossible to prove a negative, that no other organ than the liver can produce urea from ammonium salts. But the experiments already referred to of Hahn, Massen, Nencki, and Pawlow on dogs with an Eck's fistula, the analyses of Horodynski, Salaskin, and Zaleski, and Schröder's experiments on nephrectomised dogs, make it extremely unlikely that ammonium salts can be converted into urea elsewhere than in the liver.

There still remains the possibility that the other tissues might be able to produce urea from some substance other than ammonium salts or arginin. We have seen that there is evidence that some of the intermediate products of the metabolism of tissue proteids are converted into ammonia previous to their conversion into urea; but in some cases the evidence of this is slight, and in other cases we have no evidence at all. Further, our knowledge of what are the actual intermediate products of nitrogenous metabolism in the body is meagre, and there is no *a priori* improbability of there being substances, other than those already considered, which are converted into urea in the tissues. No systematic series of experiments have been carried out in which blood containing mono-amino acid or uric acid, &c., has been circulated through various organs or muscles. We have, therefore, no direct evidence whether tissues other than the liver can or cannot produce urea from anything else than ammonium salts or arginin. Such evidence as we have on this point has been obtained in the following indirect way. If the liver is the sole seat of urea formation it would follow that putting the liver out of the circulation, or removing it, ought theoretically to make the urea completely disappear from the urine. Hahn, Massen, Nencki, and Pawlow carried out experiments to test this point. Their method was to make an Eck's fistula in a dog, and subsequently either to tie the

hepatic artery or to remove the liver. They found that removal of the liver caused the death of the animal within a very few hours. After ligature of the hepatic artery most of the dogs died within fifteen hours, and it is possible that these lived so long only because some collateral circulation had been set up in the interval between the two operations. Owing to the unsatisfactory results of their first series of experiments, Nencki and Pawlow carried out a further series, but with no better result to the duration of life. One dog after ligature of the hepatic artery in addition to an Eck's fistula lived ten hours. After the operation, the blood contained the same percentage of urea and ammonia as before it; the urine still contained 4·1 per cent. of urea, and the urea formed the same percentage of the total urinary nitrogen as before the operation. They estimated the urea by Schöndorff's method. From these results they concluded that the liver cannot be the only tissue which produces urea. These experiments cannot be considered conclusive. For ligature of the hepatic artery in addition to an Eck's fistula does not necessarily put the liver wholly out of the circulation; a certain amount of collateral circulation may still be possible through the liver, and this has been found to vary in degree in individual dogs. The only conclusive method could be the more serious operation of complete removal of the liver. The most recent and successful attempts to carry out this operation have been made by Salaskin and Zaleski. Their method was to make an Eck's fistula, ligature the portal vein between the fistula and the liver, and then ligature off the liver bit by bit. Their most successful result was in a dog which lived 13 hours after the operation. Previously the dog had been starved for 10 days. For $8\frac{1}{2}$ hours after the operation the dog remained in fairly good condition and had a blood pressure of 100 mm. Hg. At $10\frac{1}{2}$ hours tetaniform convulsions started and lasted until death. The total urine passed subsequent to the operation was 118·5 c.c., of which nearly the whole was passed within the first 8 hours. It contained lactic acid and 4 grm. of urea in all. The urea-N fell from 89 per cent. to 70 per cent. of the total N, and the ammonia-N rose from 3·5 per cent. to 15·5 per cent. of the total N. In this experiment the urea was estimated by Schöndorff's method, but in others it was estimated by the Mörner-

Sjöquist method, and similar results obtained. The urine remained strongly acid to the end, although a very large dose of alkali, 10 grm. of sodium carbonate, had been introduced into the stomach before the operation. At the *post-mortem* examination it was found that 28 grm. of liver, or 5·1 per cent. of the intact organ, had not been included in the ligatures, and was still in connection with the circulation. The peritoneal cavity contained 500 c.c. of blood. The ammonia content of the blood, brain, and muscles was estimated and found to be within the normal, but in some other experiments it was found to be moderately increased. In none of the experiments was the removal of the liver complete, and never less than about 5 per cent. of the organ was unligatured.

These experiments show that for a few hours after the removal of the bulk of the liver the urine still contains large quantities of urea; that the percentage of N excreted as urea is decreased, and that the percentage excreted as ammonia is increased, but not quite correspondingly to the decrease as urea. These facts are clearly open to more than one interpretation. They might be explained by saying that after the removal of the liver urea is still produced by some organ other than the liver from some substance other than ammonia. But these experiments cannot be held to prove it. For, not only was an appreciable amount of liver still connected with the circulation, but there is nothing to show that urea was produced after the operation, and that the urea excreted was not already preformed in the body. Even if urea had been formed after the operation it might still have arisen from arginin. Again, the experiments might be considered to show that ammonium salts are the chief immediate precursors of urea, and, as we have seen, are converted into urea solely in the liver; in fact, the results might appear to be very similar to those obtained when a dog with an Eck's fistula receives a meal of proteids. For in both cases the dogs became convulsed, and the urine is found to contain more ammonia relatively to the urea. But, as Salaskin and Zaleski pointed out, the resemblance is merely superficial and the two conditions are fundamentally different. In these experiments the convulsions could not be due to ammonia poisoning, for in some of them the percentage of ammonia in the blood and brain remained

normal, and yet convulsions occurred just the same. Other points of contrast between the two sets of experiments are as follows: in their experiments practically no bile could have been produced, the urine was not alkaline but extremely acid and contained lactic acid. There can be little doubt that they were right in ascribing the symptoms in their dogs to an acid intoxication by intermediate metabolic products.

The numerous observations on men the subjects of liver destruction have equally failed to bring forward any conclusive evidence that the liver is not the only organ which can form urea. The changes in the urine observed in cases of advanced destruction of the liver by acute yellow atrophy or cirrhosis are similar to those in Salaskin and Nencki's dogs. But the actual degree of liver destruction is in those cases even more uncertain, and a condition of acid intoxication almost certainly exists in them. We are bound to conclude that there is at present no proof that tissues other than the liver can manufacture urea from anything but arginin. There is another difficulty in the interpretation of observations on destruction of the liver which is important. The various steps in the breaking down of tissue proteid, which we now ascribe to the activity of numerous tissue ferments, cannot be looked upon as a number of isolated phenomena which can take place in the body quite independently of each other. They are to be looked upon rather as a series of events in a continuous cycle of changes, and, if one is prevented, the whole cycle may be interfered with. We know that in the liver many of the terminal changes in proteid katabolism take place. When that organ is largely destroyed we cannot assume either that any alterations in metabolism which ensue represent nothing but the series of changes which would be normally carried out in the liver, or that proteid katabolism goes on in all other tissues in an undisturbed and normal manner. This assumption becomes all the more impossible when we know that many of the intermediate products of proteid metabolism are poisonous to the tissues.

Acid intoxication or acidosis, one of the auto-intoxications by intermediate metabolic products, may be briefly considered here, because it throws a side-light on the relation of ammonia to urea in the body. The acids of which we know most in this

connection are sarcolactic acid and β-oxy-butyric acid with its derivative aceto-acetic acid. It is probable, however, that oxalic acid and a large number of less well-known acids are also produced during metabolism. The history of sarcolactic acid in the body is only imperfectly understood. In health it is produced in muscles and other tissues, and is found in the blood but not in the urine. It must therefore be oxidised in the body. But it is found in the urine in a large number of conditions of disturbed metabolism; the only one which interests us in this connection is destruction of the liver. It is found in the urine of men suffering from acute yellow atrophy of the liver, and we have already seen that Salaskin and Zaleski found it in the urine of their dogs. Minkowski demonstrated its presence in the urine of geese after the liver had been either excluded from the circulation or excised. He showed that geese might live for twelve or more hours after the operation, and might pass as much as 3·5 grm. of sarcolactic acid. At the same time the uric acid was almost completely replaced by ammonia. β-oxy-butyric acid is not known for certain to be a normal metabolic product, but it may be found in the blood and urine in severe cases of human diabetes and in pancreatic and phloridzin diabetes. In man the amount passed in a day may be as much as 100 grm. Acidosis has also been produced experimentally by placing mineral or organic acids in the stomach of an animal. In all the experimental and pathological acid intoxications it is found that the excretion of ammonia is increased both actually and relatively to the total N, and that this increase of ammonia is in mammals at the expense of the urea. In order to account for this the view has been put forward that ammonia is produced in metabolism with the special object of neutralising acids simultaneously formed, and of so saving for the body the important bases Na, Ca, K, &c., which would otherwise have to be used. The view is applied not merely to abnormal but also to normal metabolism, and the ammonia in healthy urine is looked upon as base which was required to neutralise acid and could not be allowed to be converted into a neutral body like urea. That ammonia can spare the bases of the body, and that this defensive mechanism against acidosis is more highly developed in man and carnivora than in the herbivora, has been shown

by experiment. That ammonia does act as a base in an acid intoxication seems to be shown by the fact that its excretion can be very greatly reduced by putting into the animal's body a sufficient quantity of some other base. But that ammonia is specially produced either in health or disease with the object of neutralising acid is open to question. For the view implies that the quantity of ammonia produced is determined by the amount of acids formed and the quantity of other available base which can be spared by the body. The evidence on this point is not sufficient to enable us to form a decided opinion, but so far as it goes it is against this view. If we take the percentage of ammonia in the blood as the only rough guide we have to the amount produced, then it is not found that the quantity of ammonia formed bears any necessary relation to the degree of acid intoxication. Salaskin and Zaleski found in their experiments that a dog might be in convulsions and passing lactic acid in its urine, and yet the percentage of ammonia in the blood and tissues be normal. The same thing has been noticed in cases of severe diabetes. The abnormality in an acidosis appears to be, not in the quantity of ammonia formed, but in the degree to which the ammonia is converted into urea. When in addition to an acidosis there is destruction of the liver, it is obvious that the conversion of ammonia into urea may almost cease. The relation of the liver to acidosis may, therefore, be twofold: on the one hand, destruction or gross impairment of the liver interferes with the oxidation in the liver of lactic and possibly other acids, and an acid intoxication results. On the other hand, when an acid intoxication has arisen from some cause not primarily connected with the liver, the conversion of ammonia into urea by the liver is suspended in a degree corresponding to the acidosis, necessary base is thereby obtained, and a corresponding proportion of the total N in the urine appears as ammonia instead of urea. Even in health something less than about 5 per cent. of the total N is excreted as ammonia. But the whole of this cannot be looked upon as being present only because it is required as a base. For, if a healthy man is given sufficient sodium bicarbonate by the mouth to make his urine for days strongly alkaline, the ammonia in it does not disappear, it is only greatly reduced. The ammonia now present cannot be looked upon

as necessary base, and may possibly represent ammonia from blood which has passed through the kidney before going to the liver. It would be interesting to know whether the effect of alkalies on the urinary ammonia is due to decreased formation of ammonia or to its more complete conversion into urea; there are, however, no estimations of the ammonia contents of the blood to show.

Means by which the Liver converts Ammonia into Urea.—We have seen incidentally that many katabolic processes which it used to be thought were carried out by the direct action of protoplasm are now thought to be produced by ferments formed within the cell. It is perhaps premature to generalise and say that all katabolic processes are carried out by tissue ferments, but the discovery of proteolytic, lipolytic, glycolytic ferments, of various kinds of oxydases, and other ferments in the tissues, almost justifies such a view. The formation of urea from ammonia, however, cannot be looked upon as a process of breaking down but rather as a synthesis. According to Hofmeister's view it is an oxidative synthesis, and according to the views of Schmiedeberg and Drechsel it is a synthesis followed by dehydration. At one time it was thought that ferments could not produce a synthesis; this is now known not to be the case. The kidney, for instance, contains a ferment which synthesises glycin and benzoic acid with the loss of water into hippuric acid. But even more important was the discovery that some zymolyses are reversible, and that one and the same ferment could both break down and build up. Thus Croft Hill showed that yeast maltase could both hydrolyse maltose to glucose and condense glucose back to maltose. Similarly a lipase has been formed capable of converting fat into fatty acid and fatty acid back into fat. It is therefore, *a priori*, probable that one and the same ferment in the liver hydrolyses glycogen into dextrose and condenses dextrose into glycogen. It would certainly be premature to say that it is probable that in the body all the hydrolyses are reversible, but it is possible that a very large number of them are. Jacoby has shown that liver juice contains a ferment which can hydrolyse urea into some ammonium salt, just as urease, the ferment of the Bacillus ureæ, converts urea into ammonium carbonate in the urine. We have no experimental evidence that the action

of this liver ferment is reversible, however possible and probable this may be. It must be remembered that the net result of the action of a ferment capable of both katabolism and synthesis is determined by the exact conditions under which it works. It would not be surprising if we failed to reproduce outside the body the necessary renewal of material to be acted upon and removal of the product of activity, also the possible interaction of various ferments necessary to produce a certain result.

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CHAPTER XX

THE SECRETION OF URINE

The two facts which strike us at once when considering how the kidney produces urine from the blood supplied to it, are the differences in chemical composition between the two fluids and the extraordinary structural complexity of the kidney tubule. The main chemical difference between blood plasma and urine is in the percentage composition of the two fluids, and not in the presence or absence of substances special to each. Thus the urea, sugar, and other crystalloids of the blood are found also in the urine, but in wholly different percentage quantities. In fact, apart from percentage composition the differences between the two fluids are few. They differ, however, strikingly in reaction, in the absence of the blood proteids from the urine and in the presence in it of hippuric acid. The power of the kidney to secrete acid urine from the alkaline blood is an important point in any theory of urinary secretion, and will be considered later. The ability of the kidney to keep back the blood proteids is in health apparently perfect. Minute traces of proteid may be found in healthy urine, but it is not one of the blood proteids, and is probably derived from the urinary passages. On the other hand, structural and circulatory changes in the kidney soon cause one or more of the blood proteids to appear in the urine. Such albuminuria is of the greatest medical importance; but, until we know more of the mechanism by which the plasma proteids are kept back by the normal kidney, it is impossible to understand the true significance of many forms of albuminuria. Hippuric acid is interesting as being the only urinary constituent which is known to be manufactured in the kidney. We know that the kidney possesses a ferment which can synthesise benzoic acid and glycin into hippuric acid, but, as we do not know the site in the kidney of this synthesis, the fact does not help us to formulate a theory of urinary secretion. Structurally the kidney bears little

resemblance to other glands; not only is the glomerulus a structure without analogy elsewhere in the body, except perhaps in the choroid plexus of the brain, but the extreme length and complexity of the tubule is a feature peculiar amongst glands to the kidney.

It has become an axiom of physiology that difference of function and structure go hand in hand. This idea was clearly appreciated by Bowman, who, in 1842, published his anatomical researches on the structure of the kidney, and at the same time a theory of urinary secretion founded on those results alone. He pointed out the striking structural peculiarities of the kidney as compared with other glands, and suggested that in the glomerulus the watery portion of the urine was separated off from the blood, and that in the tubule urea, uric acid, and other solid constituents of the urine were secreted.

Two years later Ludwig published a mechanical theory of urinary formation founded on experimental work. He supposed that the capillary blood pressure in the glomerulus filtered off a dilute fluid which was concentrated in its passage down the tubule. This concentration of the glomerular filtrate in the tubules was due to the passage of water by diffusion to the more concentrated lymph on the other side of the epithelial cells. Heidenhain has pointed out that Ludwig's view contains three propositions: (*a*) That the secretion of water in the glomerulus is a mechanical filtration depending on blood pressure; (*b*) that all the solid constituents of the urine are passed out through the glomerulus with the water in dilute solution; (*c*) that this dilute urine is concentrated in the tubules.

Both of these views, which are in the main the two held to-day, have undergone some modification from the form in which they were originally stated. Ludwig's view had to be altered when it was demonstrated that the osmotic pressure of urine was generally greater than that of the blood. For, it was impossible to explain how by a process of diffusion the urine might finally be turned out from the tubule with a concentration four or more times greater than that of the blood. It was necessary to believe that the concentration of the glomerular filtrate was brought about by the active intervention of the tubule cells. The view as amended has, therefore, ceased to be purely mechanical, and consists now of mechanical filtration and physiological absorption.

Heidenhain revived Bowman's view, restating it with greater exactitude and placing it on an experimental basis. He has expressed his own views as follows—(1) In the kidney, as in all other glands the secretion depends upon the activity of special secreting cells; (2) the first kind of these cells is represented by the single layer of epithelium covering the glomerular capillaries. The function of these cells is to secrete water and such salts of the urine as are found in solution throughout the body, *e.g.* sodium chloride; (3) the second system of secreting cells is represented by the epithelial lining of the convoluted tubules and ascending loops of Henle. They secrete the specific constituents of the urine, and under certain circumstances water at the same time; (4) the degree of activity of both kinds of secreting cells is determined by (*a*) the amount of water or urinary constituent contained in the blood, and (*b*) by the velocity of blood-flow through the renal capillaries, since on this depends the supply to the cells both of substances to be secreted and of oxygen; (5) the great variations in the composition of urine are explained by differences in the secretory activity of both kinds of cell, either combined or relative to each other.

These two views represent the chief of those current at present, and those most diametrically opposed to each other. For, they differ greatly with regard to the function of the glomerulus, and in the case of the function of the tubule they are exactly opposite. It might be thought that it would have been easy to devise experiments which would have proved or disproved one of them. This has not proved to be the case. It is necessary to point out that these two views do not exhaust all the possibilities. Neither of them may be true in outline, much less in detail. In both views it is assumed that the function of the tubule is the same throughout, either excretory or absorptive. When we turn to the histology of the kidney tubule and see great variations in the structure of its different parts, we conclude that there must be corresponding differences of function. It is possible that in accordance with one or other of these two views the function of the different parts of the kidney tubule may be broadly the same, either absorptive or excretory; but it is, *a priori*, equally possible that one part of the tubule might absorb and another excrete.

The problem to be discussed resolves into two fundamental points—(1) How does the glomerulus act? Is it a mechanical

filter or do the cells of Bowman's capsule determine the kind of material and rate at which it shall pass through them? (2) Do the cells of the tubule secrete material from a glomerular filtrate back into the blood, or do they excrete material from the blood and add it to the glomerular secretion, or do they both absorb and excrete? It is theoretically impossible to discuss these two points apart, because we cannot, as a rule, experiment separately upon the glomeruli and tubules, nor are we able to obtain the glomerular fluid until it has passed down the tubules; but, as far as possible, the two points will be considered apart.

A large number of experiments have been performed on excised kidneys by various observers, and especially by Sollmann. He admits at once that the normal vitality of the kidney begins to disappear soon after excision, and that the "urine" obtained by the perfusion of such kidneys can be looked upon only as a physical filtrate and not as a vital secretion. Nevertheless, the experiments are of great value, for they do show which of the experimental results obtained on living kidneys are capable of a purely mechanical explanation; but of course they leave untouched the question whether in the living body the process is mechanical or vital. This being so, we shall refer to experiments on excised kidneys only incidentally.

Most of the experiments which have been performed on the secretion of urine have given results which are considered to be in favour either of Ludwig's or Heidenhain's view, and in order that the bearing of the various experiments to be described may be more readily understood, it will be well to point out in greater detail what these two views really imply. According to Ludwig's view a filtering force, derived ultimately from the heart, drives through the filtering membrane a fluid exactly similar to blood plasma, except that it contains no proteid. The glomerular filtrate will therefore be practically isotonic with the plasma, and the cells of Bowman's capsule will have done no work in separating the filtrate. Filtration may be assisted by an increased rate of blood-flow past the filter, but will depend fundamentally on the magnitude of the filtering force. The filtering membrane is impermeable to the blood proteids, but must be equally permeable to all other molecules, and any alteration in the permeability of the filter must affect equally all filterable substances. [illegible] kidney will be done entirely by the cells of t[illegible] the process

of concentrating the dilute glomerular filtrate. This process of concentration will be carried out by the tubule cells being readily permeable to some molecules, *e.g.* water, sugar, chlorides, &c., and relatively impermeable to others, *e.g.* urea, uric acid, pigments, &c. The quantity of urine passing away from the kidney will be determined by the difference between the rates of filtration and of absorption. The composition of the urine will depend on three factors—(*a*) the relative amounts of the various constituents in the blood, (*b*) the relative permeability of the tubule cells to them, and (*c*) the rate at which the glomerular filtrate passes down the tubule. For, this last factor will determine the amount of change which the tubule cells can produce in the glomerular filtrate, and we should expect that the greater the rate of flow through the tubule the more the urine would resemble the glomerular filtrate.

On the Bowman-Heidenhain view the cells covering the glomerulus are normally impermeable to all the constituents of the blood excepting water and inorganic salts, which are passed out by the vital activity of these cells and appear in the same proportion in the glomerular filtrate as in the urine. The glomerular filtrate will therefore be a very hypotonic fluid as compared with the blood plasma, and much work will have to be done by the cells of Bowman's capsule in effecting the separation of this extremely dilute fluid. The cells of the tubule will be permeable to and excrete urea, uric acid, &c., but practically impermeable to dextrose and most salts, and to a less extent to water. The tubule cells must also do much work, for, they have to pass out solid constituents in extremely concentrated solution, and so raise the osmotic pressure of the dilute glomerular filtrate up to that of the urine. The quantity and composition of the urine will depend upon (*a*) the quantity and proportion in the blood of substances which have to be excreted, and (*b*) the rate at which these substances are brought by the blood stream to the excreting cells.

Urine is secreted in order to get rid of material, and so help to keep the quantity and composition of the blood constant. On Heidenhain's view an excess or decrease of any constituent in the blood will lead automatically to its increased or diminished excretion. But on Ludwig's view, in which all materials are passed out through the glomerulus in the same relative proportion in

which they exist in the blood, it is necessary to suppose that the cells of the tubule can be influenced somehow to absorb selectively substances which it is not necessary to excrete, and so adapt the degree of absorption in the tubule to the needs of the organism.

General Relation of the Renal Circulation to the Quantity of Urine.—Both views admit an intimate relation between the formation of urine and the renal circulation. At first sight there would appear to be an obvious difference between the two; for, whereas on Ludwig's view the main relation is between quantity of urine and pressure in the glomerular capillaries, the influence of velocity of blood-flow on filtration being a comparatively minor point, on Heidenhain's view the relation is between quantity of urine and rate of blood-flow through the kidney. With one exception, however, any measure which raises the capillary blood pressure in the kidney will at the same time increase the velocity of blood-flow through the organ. Such measures are—(1) A rise in the general arterial blood pressure when caused either by increasing the force or rate of the heart beat by such a drug as digitalis, or by vaso-constriction in areas other than the kidney. This latter may be brought about by stimulating the lower end of the cut spinal cord or splanchnics after section of the renal nerves, which in the dog leave the central nervous system in the anterior roots of the 11th, 12th, and 13th dorsal nerves. Unless the renal nerves are cut the kidney shares in the general vaso-constriction, its volume shrinks, and in spite of the greatly raised arterial blood pressure no secretion of urine takes place. The same is true of the marked rise in arterial pressure seen during asphyxia. (2) Dilatation of the renal arterioles, the general arterial blood pressure remaining unaltered. This condition may be brought about by section of the vaso-constrictor fibres contained in the renal nerves, or by stimulation with slow rhythmical shocks of the vaso-dilator fibres for the kidney contained in the anterior roots of the 11th, 12th, and 13th dorsal nerves in the dog. In hydræmic plethora also, while the general arterial blood pressure is scarcely altered, there is a marked dilatation of the arterioles in the kidney, as well as in other abdominal viscera. Conversely both the pressure and rate of flow in the kidney vessels may be decreased in the following ways—(1) A fall in the general arterial blood pressure. This may be brought about by cardiac inhibition due to stimulation of the vagus in the neck. If the heart is

inhibited sufficiently to produce a fall in the arterial blood pressure, the kidney volume shrinks and the flow of urine is diminished. The vagus probably has no direct action upon the secretion of urine, as is shown by the fact that stimulation of the vagus below the diaphragm does not, in the hands of most observers, influence the secretion of urine. The necessary fall of general arterial blood pressure may also be brought about by widespread vaso-dilatation. Thus, section of the splanchnics in the rabbit may reduce the general arterial blood pressure sufficiently to counterbalance the effect of the dilatation of the renal arterioles, and may so prevent any increase in the urinary excretion. But section of the spinal cord in the cervical region produces such an enormous fall of arterial pressure that the kidney volume shrinks in spite of the renal vaso-dilatation, and the secretion of urine is greatly decreased or abolished. It has frequently been found that when the aortic blood pressure falls below about 40 mm. Hg, the flow of urine stops. (2) Constriction of the renal arterioles, the general arterial pressure remaining unaltered. This condition may be produced by stimulating the renal nerves, which causes a shrinkage of the kidney volume and a decreased flow of urine. The same result may be brought about by mechanically constricting the renal artery. It is then found that the flow of urine varies, after a certain point, with the degree of the constriction, and that when the artery is completely occluded, or even before, the flow of urine stops abruptly. It has been found that when the constriction is relieved after having lasted only a few minutes, there may be an interval of forty-five minutes before the secretion of urine begins again. At first sight this is a surprising result, and the explanation of it is by no means certain. It is possible that the temporary suppression of urine is in part due to an effect produced upon the cells of Bowman's capsule, and that they are affected seems to be shown by the albuminuria which accompanies the secretion. It would seem more probable that the explanation is to be found in the following series of events, namely, that arterial ischæmia is followed by arterial engorgement, which may be so severe and lead to such an increased formation of extravascular fluid that strangulation of the veins and cessation of the blood-flow through the part takes place.

Obstruction of the Renal Vein is the exception referred to above, for, while it raises the capillary blood pressure in the kidney, it

correspondingly decreases the rate of blood-flow through the organ. It would appear, therefore, to be a means which might help to decide between these two views. Complete obstruction of the vein necessarily stops the blood-flow through the kidney, and most observers have found that it has the same effect upon the secretion of urine, although it is supposed to increase greatly the pressure in the glomerular capillaries. Ludwig showed microscopically that the interlobular veins became so swollen as to obstruct mechanically the loops of Henle, and consequently the passage of fluid down the tubules. Heidenhain did not accept this explanation, and laid great stress on the experiment as showing that it is the rate of blood-flow through the capillaries rather than the pressure in them which determines the rate of secretion of urine. This conclusion of Heidenhain's was certainly not justified, for he never disproved the truth of Ludwig's observation, and further, it is obvious that complete cessation of blood-flow through the glomeruli must lead to such a concentration of the blood in their capillaries that filtration would soon become impossible. Since then, however, it has been shown by several observers that partial obstruction of the vein reduces the flow of urine, and De Sousa has measured the blood-flow through the kidney under these circumstances. He found that, other things being equal, the amount of urine secreted is directly proportional to the velocity of blood-flow through the kidneys, and that every degree of venous obstruction diminishes the secretion by slowing the blood-flow, and quite apart from any change in the aortic blood pressure. He was further able to disprove the statement of Schwarz that the cessation of urinary secretion is due, not to the venous obstruction as such, but to intravascular clotting, and that if a dog's blood is made uncoagulable by previous defibrination, obstruction of the vein then causes an increased secretion of urine.

We may safely conclude that obstruction of the renal vein, however slight, causes corresponding reductions in the flow of urine and the rate of blood-flow through the kidney. But, before accepting this as a powerful argument against Ludwig's view, it is necessary to consider the effect of partial venous obstruction on the filtering force, *i.e.* the difference between the pressures on the two sides of the filtering membrane. It is fair to assume that the capillary blood pressure would be raised; the only uncertainty on this point would be due to the fact that between the renal vein

and the glomerulus is the capillary plexus round the tubules. On the other, *i.e.* the tubule, side of the glomerular epithelium it is equally possible that the pressure would be increased and the available filtering force thereby reduced. For, if Ludwig's observations are true—and they have never been contradicted that obstruction of the renal vein begins to compress the tubule close to the glomerulus, then a rising pressure of glomerulus filtrate within the tubule becomes possible. Sollmann has demonstrated that this does take place in excised kidneys when perfused with 1 per cent. sodium chloride solution. Here the "urine" is a mechanical filtrate, and the fact that every degree of obstruction of the renal vein diminishes correspondingly the outflow of "urine" can be explained only by decreased filtration.

An *a priori* objective raised by Heidenhain to Ludwig's theory may be dealt with here. He calculated from the probable percentage of urea in human blood that, in order to account for the urea in a whole day's urine, 70 kilos of fluid would have to be filtered through the glomeruli, of which about 68 kilos would be reabsorbed. He estimated that the total circulation through both kidneys in twenty-four hours amounted to only 130 kilos of blood, and that it would therefore be necessary for the blood as it passed through the glomeruli to lose more than half its weight of fluid, a proposition which he considered absurd. De Sousa has given a very different calculation as the result of his experiments on the blood-flow through the kidneys. He assumes that blood may contain 0·1 per cent. urea. In order that a day's urine might contain 33 grm. of urea, 33 litres of fluid would have to be filtered. He calculates that the blood-flow through both kidneys together may amount to 882 litres in twenty-four hours. 33 litres is only 3·7 per cent. of this amount; consequently Ludwig's view need entail a concentration of the blood as it passes through the glomeruli by only 3·7 per cent. Such a concentration is a long way within the limits found by Barcroft to take place in the submaxillary gland during activity.

Obstruction of the Ureter.—It has been shown that when a mercury manometer is connected with the ureter above a complete obstruction, the pressure rises at first rapidly to about 20 m.m. Hg. and then more slowly to about 60 m.m. Hg. at which level it remains stationary. The ureter and pelvis of the kidney become distended with urine, and the kidney and surrounding

tissue œdematous. The interpretation of these results is of importance.

Ludwig, in accordance with his view, pointed out that the filtering force in the glomerulus must be the difference between the capillary pressure, P, and the pressure of urine in the tubule, p. Since p under normal circumstances is probably zero, $P-p$ will be equal to the capillary blood pressure. After section of the spinal cord it was found that the secretion of urine ceased when the arterial pressure had fallen to about 40 or 50 mm. Hg. He considered, therefore, that the filtering force must be greater than about 40 mm. Hg in order to be effective, a point to which we shall have to return. But it was clear that, when P remained normal, the filtering force might be reduced below its effective limit equally well by increasing the value of p. He explained the stationary level of the manometer in an obstructed ureter as the point at which filtration ceased, because $P-p$ was reduced below its effective level. In other words, during obstruction the ureter pressure represents the filtration pressure of the kidney, and must therefore bear a constant relation to the arterial blood pressure. Starling has given an experiment to show this relation. He injected diuretin into a dog, and thereby raised the arterial blood pressure from 115 to about 130 mm. Hg, and at the same time the ureter pressure to about 90 mm. Hg. Gottlieb and Magnus have, however, been unable to confirm this constant relation between the arterial and ureter pressures. They find that diuretics generally, and especially the salines, increase the ureter pressure, but quite independently of the general blood pressure, so that the actual difference between the two pressures may be either decreased or increased; and that, as the diuresis passes off, there is a fall of ureter pressure, again independent of the general arterial blood pressure. They use this as an argument against the filtration theory. But, as Sollmann has pointed out, they overlooked the fact that the hydræmia, by reducing the viscosity of the blood, would increase the glomerular, and so the ureter, pressure without raising the general blood pressure; and he has demonstrated on excised kidneys that reduction of the viscosity of the blood does raise the ureter pressure independently of blood pressure.

Heidenhain gave a different explanation of the effect of obstructing the ureter. He observed the same phenomenon when

a manometer was connected with an obstructed bile-duct. In this case it is generally allowed that the stage of equilibrium marks the point at which secretion and absorption exactly balance each other. He suggested that the same is true for the kidney. According to Heidenhain, the manometer does not register the filtration pressure of the kidney, but the pressure at which a wholly artificial and abnormal reabsorption balances secretion. And considering that Ludwig's own view supposes constant absorption by the tubules, it would seem that Heidenhain's explanation at any rate might be possible.

It is an assumption to conclude, as Ludwig did, that, when the ureter is distended with fluid under pressure, the same fluid pressure must exist right up the tubule to the glomerulus. It has been found impossible to inject fluid backwards from the ureter up the tubules, and according to Ludwig's view the glomerular filtrate has under normal circumstances two alternative routes of escape. The bulk of it passes back into the blood, and only a small fraction passes down the tubule into the ureter. We should therefore expect that obstruction of the ureter would not dam back fluid under pressure in the tubule, but would determine the escape of a greater proportion into the blood, as long as that route was open. Obstruction of the ureter might close this route into two ways—either by producing changes in the absorbing epithelium, or by the accumulation of urea and other crystalloids in the tubules until their osmotic pressure was equal to the absorbing power of the epithelium. For, according to Ludwig's view, urea and other specific urinary constituents are not reabsorbed after being filtered off from the blood. An examination of the urine which is found in the distended ureter and pelvis does not throw any light on this question. It is of low specific gravity and contains but little urea: a result which may be explained equally well by actual reabsorption or failure of excretion under the abnormal conditions. That obstruction of the ureter may alter the properties of the renal epithelium seems to be suggested by the character of the urine secreted after a temporary obstruction of the ureter. This urine is very copious, has a molecular concentration much below normal, and the reduction affects the inorganic constituents far more than the urea. But it is impossible to say whether this result indicates increased absorption of certain constituents or their decreased excretion.

The interpretation of the results of total obstruction of the ureter is further complicated by the fact that the blood flow through the kidney is at the same time interfered with. This may well account for the observed œdema of the perinephric tissues. It has been supposed that the distension of the renal tubules causes them to press upon the venules in the kidney. Whatever the explanation is, experiments have shown that when the ureter pressure is raised to 100 mm. Hg the kidney becomes dark in colour, the oncometer shows a great expansion of the kidney volume, and the outflow from the renal vein and pressure in its interior are considerably reduced. This interference with the blood flow through the kidney might affect filtration by the glomeruli or excretion and absorption by the tubules. In fact, we can only conclude that experiments dealing with the total occlusion of the ureter throw no light at present on the mode of action of the glomerulus or tubule.

Action of Saline Diuretics.—In no part of the subject dealing with the secretion of urine has more work been done recently than upon the action of the saline diuretics. They include all crystalloids which can be injected intravenously in large quantities, such as NaCl, Na_2HPO, Na_2SO_4, urea, dextrose, &c., and therefore correspond to Heidenhain's second class of lymphagogues.

It was known that the injection of one of these substances would cause a secretion after the flow of urine had been stopped by section of the spinal cord, and that at the time of the secretion the arterial blood pressure was raised. But Heidenhain considered that the action of these substances could not be accounted for by a vascular change, because their injection might under normal circumstances increase the secretion of urine without at the same time altering the aortic blood pressure. He therefore considered that these substances acted as specific stimulants to the secretory activity of the renal cells.

It was shown, however, that the diuretic effect of different solutions corresponded roughly to their osmotic pressure, and further that their injection produced hydræmic plethora. The vascular changes accompanying hydræmic plethora have been shown by Starling to consist of a very slight rise in aortic pressure, a dilatation of the visceral vessels, a general rise in the venous and capillary pressures, and an increased rapidity of blood flow. Starling investigated the action of dextrose on the renal circula-

tion, and found that the diuresis and increase in kidney volume were coterminous, and that both might considerably outlast the plethora. It was, therefore, clear that besides the plethora there was some local action on the kidney causing both dilatation of its blood-vessels and diuresis. In order to decide whether the diuresis was caused by the local vaso-dilatation or was independent of it, he injected dextrose and allowed blood to escape from the carotid artery at a rate sufficient to keep the kidney volume from expanding; he then found that no diuresis was produced. He concluded that the diuretic effect of these substances was due to the vascular changes induced by them, and was not due to their stimulating secretory cells; a conclusion which is further supported by the fact that the diuresis ceases before the excretion of the injected substance has come to an end. He ascribed the diuresis to two factors, (*a*) hydræmic plethora, and a consequent rise in a pressure and velocity of blood in the kidneys; and (*b*) a direct dilator effect of the injected substance on the renal blood-vessels which helped to increase capillary blood pressure and so filtration. This conclusion seems to be supported by two other observations. Saline diuresis is promptly stopped by the vaso-constriction of the renal arterioles, which is caused by an injection of suprarenal extract. Also caffein diuresis only takes place when the kidney volume is increased. The first effect of an injection of caffein is to decrease the kidney volume by vaso-constriction of peripheral origin, and during this stage little or no urine is excreted.

Gootlieb and Magnus subsequently investigated the subject, and agreed with Heidenhain's explanation. They found, like Starling, that an increased kidney volume generally accompanies, but denied that it caused the diuresis. For they observed that the diuresis outlasted the increase in volume, and might even take place with a shrinkage of the kidney. And Thompson from his experiments came to the same conclusion. They further objected that in Starling's bleeding experiment the loss of blood necessary to keep the kidney volume from expanding was such that the aortic pressure was thereby reduced to about 60 mm. Hg., and therefore invalidates the conclusion which Starling drew from the experiment. For, with such an aortic pressure, even on Heidenhain's view it could not be expected that the diuretic would have anything like the effect which it would have when the

pressure was normal. Cushny, however, has confirmed Starling's interpretation by experiments conducted in a different way. He controlled the kidney volume by putting a screw clamp on the renal artery, and found that, when the kidney volume was kept down to the normal, the secretion of urine in spite of the diuretic was unaltered. This experiment is open to the obvious objection that the rate of blood flow through the kidney must have been reduced by clamping the artery.

Magnus has raised another objection to Starling's interpretation of the mode of action of saline diuretics. For, he maintains that it is not the plethora—the alteration in volume of the blood—but the hydræmia—its alteration in composition—which causes diuresis. He performed the following experiment to show that alteration in volume, without alteration in composition, causes no diuresis. He transfused blood from one dog into another until he had increased its blood volume by 84 per cent., and found that no diuresis was caused, although the same changes in kidney volume and vascular pressures were produced as when saline diuretics were injected. On the other hand, if the transfused blood were of abnormal composition, as when obtained from a dog which had received an injection of sodium sulphate, then diuresis resulted. Magnus noticed that much of the fluid of the transfused blood left the vascular system as lymph, and that the fluid remaining in blood-vessels was far more concentrated than normal. It follows that there must have been in this experiment an enormous excess of corpuscles and proteid in the circulating blood which would greatly increase its viscosity, and so might impede the rate of circulation through the kidneys. And that this actually does take place under these conditions has been shown by Sollmann. Cushny tried to avoid this objection by injecting normal serum instead of normal blood, and found that serum injections do cause a slight and slow diuresis. But, of course, it is open to any one to say that serum injections do alter the composition as well as the quantity of the blood. While Magnus's experiment indicates that all forms of plethora do not produce diuresis, it is impossible to deduce from it that saline diuresis is independent of plethora. In fact, it is impossible to say how far in saline diuresis the vascular changes cause the diuresis, or how far they are merely adjuvant, as in the secretion of any other gland.

There can be no doubt, however, that vascular changes are not the whole explanation of saline diuresis. For, if they were, the diuresis ought to be equal for all solutions with the same osmotic pressure; but it is not. When different neutral salts are injected in such quantities that the blood becomes equally diluted, or when solutions isotonic with the blood are used, different degrees of diuresis are produced. Magnus, for instance, found that sodium sulphate is much more diuretic than sodium chloride, although the vascular changes in the two cases were much the same in kind and degree. He considered that the only possible explanation was that the sulphate stimulated the renal cells to greater activity than the chloride, and that the result was a strong confirmation of Heidenhain's view. Cushny has given an explanation of these results compatible with Ludwig's view. In this view it is clear that we have to consider as a possible cause of diuresis not only increased filtration, but also decreased absorption by the tubule. He injected equimolecular amounts of chloride and sulphate simultaneously into a rabbit, and found that at the height of the diuresis they were excreted in the urine in approximately the same proportion as they existed in the blood. He looked upon this urine as closely resembling the glomerular filtrate, which he considered was hurried through the tubules too fast for much absorption to take place. As the diuresis passed off he found that the sulphate in the urine predominated over the chloride, although in the blood the reverse was true. He explained this result on the assumption that chloride can permeate the tubule epithelium more readily than sulphate, and is therefore more readily absorbed, except when the glomerular filtrate is being passed with extreme rapidity down the tubule, as at the height of diuresis. The superior diuretic action of a sulphate is in his opinion due to its less ready absorption; consequently it opposes a greater osmotic resistance to the absorption of water by the tubule cells, and more fluid is retained in the tubule. He found that phosphates and urea were excreted in the same way as sulphate relatively to chloride, and explained their superior diuretic action accordingly. In order to test this explanation of the different degrees of diuresis produced by various crystalloids, he performed another series of experiments on rabbits. He compared the urine obtained from one ureter in which a resistance of 15 to 30 mm. Hg was introduced with the urine from the free

ureter. The object of the resistance was to slow down the flow through the tubule, and so afford greater opportunity for absorption. He found that the urine from the obstructed as compared with that from the free ureter contained less water and chloride relatively to the phosphate, sulphate, urea, and pigment. Filehne has been unable to confirm these results completely, and believes that slight obstruction of one ureter may bring about differences in the urine from the two sides either by reducing the amount of glomerular secretion or by affecting the secretion by the tubules on that side. Nevertheless Cushny's experiments as they stand, and others which will be considered later, suggest that the tubules can absorb. But it must be remembered that in order to bear this interpretation two assumptions are necessary. In the first place, that the urine secreted by the two kidneys under normal conditions is always the same. This has sometimes been found to be the case, but in dogs and man at any rate not always so. And in the second place, that moderate obstruction of a ureter brings about the observed change in the urine by favouring absorption from the tubule, and not by interfering with the blood supply of the kidney nor by altering the secretory activity of renal cells, nor by absorption in the ureter or pelvis of the kidney. It has been found by experiment that a ureter pressure of 50 mm. Hg reduces the venous outflow from the kidney by only 10 per cent. It does not seem likely, therefore, that the ureter pressures used by Cushny could have very materially interfered with the renal circulation. Schwarz has maintained that a slight obstruction of the ureter equal to 25 cm. of oil actually increases in dogs the flow of urine, while the obstruction lasts, and has suggested that it either interferes with absorption or stimulates secretion. And other observers have obtained similar results. It is clear, therefore, that the mode of action of partial obstruction of the ureter is still a matter of controversy.

We have seen that the injection of a salt solution alters both the volume and composition of the blood, and might produce diuresis either by stimulating secretory cells in accordance with Heidenhain's view, or by changes in the renal circulation according to Ludwig's view. But at present there is no evidence to prove which of these is right. The different degrees of diuresis produced by various crystalloids may be explained either by differences in their secreto-motor action or by different degrees

of their absorption. And again there is no experiment which proves finally which of the two explanations is correct.

The investigation of the action of diuretics other than saline has failed to throw much light on the secretion of urine. Phillips and Bradford found that when $\frac{1}{4}$ or $\frac{1}{2}$ grain of caffein citrate is injected into a vein the kidney volume shrinks for several minutes, and the flow of urine is checked entirely or in part. This constriction of the kidney is followed by a marked and persistent expansion, which is accompanied by a considerable diuresis. These vascular changes occur after section of the splanchnics, and seem to be of peripheral origin. The general arterial blood pressure undergoes after an initial fall lasting but a few seconds a rise to about normal; it therefore cannot cause the changes in kidney volume. Gottlieb and Magnus also, have found that the kidney volume and diuresis caused by the injection of caffein or diuretin run a more or less parallel course. Nevertheless it is probable that the changes in renal circulation do not cause the alterations in the secretion of urine. For, Phillips and Bradford noticed that after caffein injections the dilatation of the renal vessels might occur without any diuresis. And Gottlieb and Magnus were able to confirm Schroeder's statement that by the injection of chloral hydrate it is possible to replace the expansion of the kidney volume by an enormous shrinkage, and yet the diuretic effect of caffein may be observed. Caffein must therefore have a diuretic action quite apart from any changes it produces in the renal circulation, but whether it causes paralysis of absorption or a stimulation of secretory activity is unknown.

Phillips and Bradford investigated also the diuretic action of digitalin and strophanthin. They found that an injection of digitalin causes a slight but persistent rise in the general arterial blood pressure, due partly to increased force of the heart-beat and partly to peripheral vaso-constriction. The kidney volume undergoes a considerable diminution synchronous with the vaso-constriction, which may last half-an-hour; this shrinkage is not followed by an expansion, as it is after caffein. The flow of urine is not decreased, and may even be slightly increased during the period of renal vaso-constriction; this, again, is unlike the effect produced by caffein alone, but like the combined action of caffein and chloral. This slight diuresis after digitalin, in spite of renal vaso-constriction, might be explained by an increased arterial blood

pressure more than counteracting the local effect on the kidney. This explanation seems to be favoured by the observation of Marshall, that a combination of digitalin and a vaso-dilator, such as nitro-glycerine, increases the diuretic effect of digitalin. Unfortunately he did not record the kidney volume, which robs the experiments of value in this connection. On the other hand, the investigation of the action of strophanthin is strong evidence against this explanation of the action of digitalin. For an injection of strophanthin causes a marked rise in the arterial blood pressure, due to its action on the heart alone and without any peripheral vaso-constriction, the kidney volume is correspondingly increased, and the flow of urine is unaltered.

We again reach the same conclusion, that the vascular changes are not more a complete explanation of the action of these than of the same bodies; but we are unable to decide in what other way they act.

The Retention of Chlorides in the Body.—It is a well-known fact that in pneumonia and other fevers the percentage and total excretion of chlorides in the urine may be greatly reduced. The cause of this reduction is largely due to deficiency of chloride in the food, as Hatcher and Eyster have shown. The chloride content of the blood, however, is not reduced, and, further, large quantities of chloride given by the mouth may be retained in the body. The mechanism of this salt retention can be explained on either Ludwig's or Heidenhain's view. On either view we should have to suppose that, when the body cannot afford to lose chloride, this fact is communicated to the kidney cells, so that other chlorides are more fully absorbed from the glomerular filtrate, the cells of Bowman's capsule do not secrete them. In other words, the alteration would be due to the vital activity of the cells.

Koranyi has given a purely physical explanation of this phenomenon. He believes that crystalloids in the blood may exist in two states: a small part free and able to pass through the glomerular epithelium, and a larger part in combination with colloid and unable to be passed through the glomerulus. Consequently the glomerular filtrate would contain, not the entire ash, but only the salts of the plasma which were free, that is, beyond the combining power of the colloid present. This theory could be made to explain many of the ascertained facts in connection with the

cretion of urine. For instance, the fact that [illegible] does not appear in more than traces in the urine unless there is an excess of lextrose in the blood. Hyperglycæmia is present in all forms of glycosuria except phloridzin diabetes, and in this condition it is supposed by Loewi, Pavy [illegible] that sugar colloid compound is broken up in the kidneys and the dextrose so liberated is excreted. Indeed, the facts connected with phloridzin diabetes are capable at present of no other explanation. The theory can explain also why a diuretic leads to an increased excretion of sugar in all forms of glycosuria excepting phloridzin diabetes, and why in this last condition, in dogs at any rate, the diuresis produced by phloridzin is not accompanied by an increased excretion of chlorides. It readily explains why in some experiments and in individual animals no urine is excreted unless there is added to the blood in excess some substance capable of passing into the urine. For instance, Munk found that when the blood of a fasting dog was circulated through an excised kidney, no secretion of urine took place unless a chloride, sugar, urea, &c., was added. Nussbaum found that no urine was secreted by a frog after ligature of the renal arteries unless urea was injected. Magnus's experiment of transfusing whole blood has been referred to: he found no diuresis unless the composition of the blood [illegible] abnormal. Spiro in the same way found that fasting animals of solutions of gum or [illegible] even with the aid of caffein, although the circulation had been increased by 80 per cent. On the other hand, the animals had been fed and water diuresis resulted. The different degrees of diuresis produced by equimolecular solutions of different salts [illegible] the supposition that sodium chloride enters more readily into non-filterable combination with colloid than sodium sulphate, and therefore has a superior diuretic action. Most of these observations are, as we have seen, capable of other explanations. However simple Forster's theory may be, it lacks the necessary demonstration that compounds of crystalloids with colloids really do exist in the blood or elsewhere. For instance, it has been found that the freezing-point of a solution of sodium chloride is not altered by the addition of albumose or egg albumin to it. Further, Martin, Starling, and Waymouth Reid have found that by filtering serum through a gelatine membrane the bulk of the crystalloids

pass into the filtrate although the membrane is impermeable to proteids.

In connection with this subject, Sollmann has investigated in dogs the effect of different diuretics upon the excretion of chlorides in the urine; he divided them into four groups. (*a*) Diuretics which reduce greatly the percentage excretion of chloride, but which owing to the diuresis cause an increase in the total excretion, *e.g.* sodium sulphate, sodium phosphate, dextrose, urea, &c. (*b*) Those which decrease both the percentage and total excretion. *e.g.* water, and also chloride starvation, which is not accompanied of course by diuresis. (*c*) Those which do not affect the percentage excretion materially, *e.g.* caffein and allied compounds, phloridzin, oil of juniper, &c. These diuretics do not dilute the blood. (*d*) Those which increase both the percentage and total excretion in urines originally poor in this ion, *e.g.* sodium chloride, sodium iodide, sodium nitrate, &c. Comparing the first and second groups in order to find the common cause for the decreased percentage excretion, Sollmann concludes that the cause is neither diuresis, nor the presence of a foreign salt in the blood, nor dilution of the plasma, but must be a lowered percentage of chloride in the plasma. However, direct determinations of the chloric in the plasma do not show this to be the case. He, therefore, has recourse to Forster's theory, and considers that the essential factor in the production of a low percentage of chlorides in the urine is a lessened amount of unbound chloride in the plasma. He explains the power of a nitrate to increase the percentage of chloride in the urine by supposing that it can displace chloride from an unfilterable compound, and so liberate chloride for excretion. All observers who have worked with rabbits instead of dogs have obtained wholly different results. In rabbits the effect of all diuretics is to approximate the percentage of chloride in the urine to that of the blood as determined by direct estimation. Sollmann explains this difference between dogs and rabbits by the assumption that diuretics in the rabbit break down the resistance of the kidney cells to the excretion of combined chloride, but not in the case of the dog. It has been shown that the human kidney reacts like that of the dog.

The theory of the combination in the blood of crystalloids with colloid is interesting, and gives a possible physical explanation of many phenomena which have been considered by some to

be due to the vital activity of cells. But, it adds yet another possible explanation of some of the experiments directed to elucidate the action of the glomerulus, an explanation which was contemplated in neither Ludwig's nor Heidenhain's view, and which only adds to our difficulties in coming to any conclusion.

The Excretion of Dextrose.—On Ludwig's view, dextrose must always be passed out by the glomerulus like every other crystalloid in the plasma, and must then undergo almost complete absorption by the tubule. According to Heidenhain's view, dextrose is excreted only when it is in excess in the blood, and so stimulates the renal cells to secrete it. Loewi, Pavy, and Lusk believe that dextrose exists in the plasma as a non-filterable combination with colloid, and that only unbound dextrose can ever pass into the urine.

Numerous observers have found that the dextrose in the urine is materially increased during diuresis produced by caffein, theobromine, &c., and especially during saline diuresis. Sollmann and others have observed marked individual differences in animals in the ease with which diuresis can be produced in them, and have noted that many good diuretic animals have normally a pronounced glycosuria which is increased during diuresis. Such observations suggest that diuresis may cause increased glycosuria in normal animals and tend to give support to Ludwig's view. But the experiments of Brown seem to show that the diuresis and glycosuria do not stand in the relation of cause and effect to each other. Mathews and also MacCallum have shown that solutions of sodium chloride, sulphate, acetate, and citrate, individually or combined, produce diuresis, and that solutions of calcium, strontium, or gold chloride could prevent this diuresis, and might even produce complete anuria. Brown showed in rabbits that the same solutions which produce diuresis also produce marked glycosuria; that small doses of calcium or strontium chloride will stop the glycosuria without affecting the diuresis, and that these same salts will also prevent the glycosuria caused by phloridzin. Many of Brown's experiments show a phenomenon which was noted by Thompson, namely, that quite a small injection of solutions of various anions of sodium lead to a diuresis out of all proportion to the quantity of fluid injected. Brown has assumed that in his experiments the glycosuria as well as the diuresis is produced by the action of ions on renal cells. It is extremely likely that this

is the case, but he has not proved the point. It has been shown that the rate of conversion of liver glycogen into dextrose is retarded by calcium salts and favoured by the ions which cause diuresis. Until we know that the glycosuria in Brown's experiments is not accompanied by, and therefore possibly due to hyperglycæmia, we cannot exclude the possibility that the glycosuria may be of hepatic and not of renal origin. At present, however, we may assume with Brown that this glycosuria is due to the effect of ions on the kidney. This independence of the diuresis and glycosuria suggests several possible explanations; either that the dextrose and water are secreted by different parts of the kidney, and this is possible on Heidenhain's view; or that salts may influence separately the secretion by the glomerulus of water and dextrose, which again is possible on Heidenhain's view, but quite impossible on Ludwig's; or that the same salt may increase the absorption of dextrose by the tubule and not that of water. The data which we have for deciding between these possibilities is meagre. We do not know for certain the site in the kidney of the excretion of dextrose. Nussbaum's experiments placed it in the glomerulus, and although they are inconclusive, as we shall see, they do afford strong evidence that this is so. In phloridzin glycosuria we know that the kidney volume passively follows the arterial blood pressure and shows no independent expansion, that the diuresis is not associated with an increased percentage excretion of chlorides, and that repeated injections of phloridzin lead to changes in the cells of the convoluted tubes, leaving the glomeruli unaltered. This evidence suggests that the glycosuria of phloridzin at any rate is independent of the glomerulus, and caused by an excretion of dextrose by the tubules, and that the site of action of the calcium chloride is the tubule. Of course it does not follow that dextrose is normally excreted by the tubule, and that when calcium chloride stops the glycosuria of a saline diuresis it is not acting upon the glomerulus. The experiments of Mosberg and others, in fact, seem to show that the path of excretion of sugar after phloridzin is abnormal. For he found in frogs after ligature of the renal arteries that injection of dextrose led to no secretion of urine, but that phloridzin caused a flow of urine containing sugar. Thus in phloridzin glycosuria we seem to have a strong indication that the tubule can excrete and not simply absorb. The fact that calcium

chloride can stop the glycosuria of a saline diuresis without affecting the diuresis can, on Ludwig's view, not be explained by any action on the glomerulus, but must be due to the same ion producing a selective influence on the absorbent power of the tubule cells. Such a thing is conceivable. For, while it has been found generally that anions increase and kations retard the activity of various cells and ferments, it has also been found that the same ion may so influence the properties of a given cell as for instance, to give it a selective permeability which it did not previously possess.

The Properties of the Cells of Bowman's Capsule. The bare possibility of Ludwig's view depends upon the properties of this membrane; for it must under all conditions in which any filtration takes place through it at all be equally permeable to all crystalloids. The difficulties of determining the permeability of this membrane are obvious; it is impossible either to experiment with it directly or to obtain the glomerular filtrate as it leaves the membrane. There is no known artificial membrane which has properties exactly similar to those which on Ludwig's view the glomerular epithelium must possess. For we know of no membrane for which the concentration of crystalloids in the filtrate is that of the original solution. Important analogies have been drawn from a study of gelatine membranes; but these membranes are equally impermeable to the blood proteids and egg albumin, while the cells of Bowman's capsule keep back the blood proteids and let through egg albumin. We know nothing about what determines the permeability of dead membranes for different substances in solution; permeability may be a chemical phenomenon, a physical one, or both. We know nothing, of course, about the permeability of living cells; whether it has a physical and chemical basis, or depends upon some other property of the bioplasm which is associated with its living activity and must be called vital, we do not know.

There can be no doubt that the glomeruli do produce at least the major part of the water of the urine; Nussbaum's experiments of ligaturing the renal arteries in the frog show this. But when we look for other experiments to indicate the rôle of the glomerulus in the production of urine, we find that there are only very few which give us the slightest hint of the properties of the glomerular epithelium. Adami thought that he had definitely

proved the selective secretory activity of these cells in the following way. He injected laky blood or egg albumin into frogs whose secretion of urine had been stopped by ligature of the renal arteries, and into dogs after section of the spinal cord. He found in the glomeruli menisci of hæmoglobin or proteid, which, he argued, must have been secreted by the glomerular epithelium, because no urine had been formed, and therefore no filtration could have taken place. But it has been shown by Beddard that Adami's ligature of the renal arteries did not put all the glomeruli out of circulation, as he imagined, and although no flow of urine was observed, it is impossible to say that no glomerular filtrate whatever had been formed. In the same way with the experiments in dogs, a flow of urine has been observed with arterial pressures far lower than those recorded in Adami's experiments, and although no urine left the kidney, some filtration through the glomeruli may still have taken place.

There can be little doubt that alterations in the glomerular epithelium apart from change in the arterial blood pressure can cause anuria. We have already seen that injections of calcium, strontium, or gold chloride will bring this about. Injections of a certain albumose may lead to the same suppression of urine. Thompson has shown that the various constituents of Witte's peptone injected intravenously cause a great fall of arterial pressure due to vaso-dilatation in many areas; but that the renal vessels are not caused to dilate, and hence the kidney volume follows passively the arterial blood pressure. He further found that proteose dissolved in normal salt solution caused diuresis, but subsequently showed that the effect could be explained entirely by the injection of the solvent without the proteose. Chittenden, Mendel, and Henderson found that an injection of hetero-albumose in normal salt solution might stop the secretion of urine completely for half-an-hour or more, although the fall in arterial blood pressure was slight and transient. It is difficult to see how such anuria can have been brought about except by some effect on the glomerulus. And, as the filtering force appears to have been unreduced, we are obliged to believe that the anuria is due to some change in Bowman's membrane. The change in the cells might be a purely physical one; but it seems unlikely, for Sollmann has shown that, when this membrane has undergone complete chemical or *post-mortem* coagulation, the ordinary capillary pressure is able to

filter fluid. We are therefore driven to think that the anuria is due to some change in the vital properties of the membrane. And, if it is once admitted that the living cells can resist successfully all filtration through them, it certainly seems likely that they must under normal conditions have the power of regulating independently of the filtering force the material which passes through them.

On the whole, the little knowledge we have of the properties of Bowman's membrane is in favour of Heidenhain's view. But as it is impossible to come to a definite conclusion, we may still inquire whether, granting the properties to this membrane necessary to Ludwig's view, the filtering force is sufficient to produce filtration under all the conditions in which urine is formed.

Adequacy of Blood Pressure as the Filtering Force.[1]—According to Ludwig's view the only force available for filtration in the glomerulus is that of the capillary blood pressure, a force which we unfortunately have no means of measuring directly, but which is certainly small. Consequently, no decrease in the concentration of crystalloids in the glomerular filtrate as compared with the blood would be possible; for any such decrease would produce an osmotic resistance to filtration which the available filtering force would be inadequate to overcome.

Starling has attempted to obtain evidence in favour of Ludwig's view by the following line of research. He determined the osmotic pressure of the serum proteids, and from the value so obtained calculated that the osmotic pressure of the plasma proteids would correspond to about 40 mm. Hg. From this it follows that when the filtering force falls to 40 mm. Hg filtration must cease. And in accordance with this it has been often found that, when the arterial blood pressure falls to about this figure, the secretion of urine stops. Starling tried to prove the same point in another way. According to Ludwig's view ureter pressure is a derivative of blood pressure, and, when the ureter is completely obstructed, will reach a height which is equal to the capillary pressure minus the osmotic pressure of the plasma proteid. From this it follows that the ureter pressure will always remain about 40 mm. Hg below the blood pressure, and that when it has reached this level the formation of urine must cease. Starling injected diuretin into a dog and found

[1] See Editor's note, page 618.

that the ureter pressure rose with a rise in the arterial blood pressure, and tended to become stationary at a pressure of about 40 mm. Hg below that in the carotid artery. Gottlieb and Magnus were unable to confirm Starling's observations. They found that urine was still secreted when the difference between the arterial and ureter pressures was only a few mm. Hg, and further that urine was still formed when the carotid blood pressure had fallen as low as 16 mm. Hg. These observations would invalidate Ludwig's view only if the osmotic pressure of the plasma proteids were anything approaching 40 mm. Hg. Starling's method of estimating their osmotic pressure was as follows. He freed serum of proteid by filtering it under pressure through a gelatinised Chamberland filter. This filtrate was placed in an osmometer and separated from the original serum by a gelatine membrane supported on peritoneum, and values were obtained in mm. Hg. Waymouth Reid has thrown doubt on the correctness of Starling's conclusions. For he has shown that, when serum is filtered through gelatine, the filtrate is not the original serum minus proteid only; for, the Δ of the filtrate, instead of being within ·005° C. of that of the original serum, differs from it by ·035° C. Although Reid has demonstrated that pure crystalline proteids have no osmotic pressure, he admits that blood serum does give a readable but variable osmotic pressure on a gelatine membrane due to substances other than proteids. The question, therefore, resolves itself into how far the cells of Bowman's capsule have properties anything like those of the gelatine membrane, and this, as we have already seen, it is impossible to answer.

It is also necessary on the filtration theory that the osmotic pressure of the glomerular filtrate should never be less than that of the blood plasma by more than the osmotic pressure of the plasma proteids. As we have seen, unless the proteid osmotic pressure is extremely low it is impossible to explain filtration with very low arterial blood pressures. Assuming, then, that the osmotic pressure of these proteids is something much less than 40 mm. Hg, it is clear that the osmotic pressure of the glomerular filtrate must be but little below that of the plasma. Unfortunately we cannot obtain the glomerular filtrate until it has already passed down the tubules and been converted into urine, and our ideas as to its osmotic pressure are derived en-

tirely by inference. Dreser found that a urine whose $\Delta = 2{\cdot}3°$ C. was formed from blood with a $\Delta = {\cdot}56°$ C. This difference represents an enormous amount of work done by the kidney cells, and can be explained equally well as brought about by the absorption of water on Ludwig's view or the excretion of solids by the tubules on Heidenhain's view. Starling found that during the diuresis following intravenous injections of normal saline and of strong salt or dextrose solutions the Δ of the urine was rapidly reduced until it approximated but always remained larger than that of the blood. In this he sees a strong argument in favour of Ludwig's view, for, the less time the tubule has to concentrate the glomerular filtrate, the more nearly should we expect the osmotic pressure of the urine to be like that of the blood plasma. Dreser, however, has found that the Δ of the urine in diabetes insipidus, during caffein diuresis or after drinking 1·5 litres of beer, may be as small as ·16° C., and even smaller values have been found in man by other observers. Assuming that in these cases the Δ of the blood was as low as 46° C., there would be a difference between the Δ of blood and urine of ·3° C., which would correspond to an osmotic pressure of many hundred mm. Hg. It is manifestly impossible for the blood pressure to have filtered against such an osmotic resistance as this. Dreser considered that these results could be explained only by imagining that the glomerular epithelium had done work and acted as a secreting membrane in Heidenhain's sense. To explain these results on the filtration hypothesis we should have to imagine that in these cases the glomerular filtrate in passing down the tubules became actually more dilute instead of undergoing the more usual concentration. Starling has suggested that this might take place in one of two ways: either by the tubule cells secreting extra water practically without solids, or by their absorbing solids out of all proportion to water. The first suggestion supposes a mechanism which is contemplated in neither Heidenhain's nor Ludwig's view, and is opposed to Starling's explanation of diuresis in general. The second suggestion is that natural to Ludwig's view. As has already been pointed out, it is necessary with Ludwig's view to believe that absorption by the tubules can be regulated to the needs of the organism. In diabetes insipidus the daily excretion of urinary solids is normal, and the only abnormality is the enormous quantity of water

secreted. If the glomerular filtrate is practically isotonic with the plasma, then the observed result could be brought about only by the absorption of solids out of all proportion to water. But it may be doubted whether the approximation of the osmotic pressure of the urine to that of the blood during diuresis is really a point in favour of Ludwig's view, as Starling thinks. In hydræmic plethora it is the clear duty of the kidney to get rid of the excess of water in the blood, and if it does the Δ of the urine must fall. If the Δ of the urine in diuresis always approximated closely to that of the blood, it might be considered a point in favour of Ludwig, as showing that the urine always closely resembles the glomerular filtrate, which according to Ludwig's view must have a definite Δ in relation to the blood. But Dreser's observations show that in some cases of diuresis this is not the case; consequently Starling's observations lose to a considerable extent the significance which he attributed to them. Galeotti has followed in detail the changes in the quantity and composition of urine and in the work of the kidney, which follow the production of hydræmic plethora. After injecting hypertonic solutions of sodium chloride or dextrose into dogs, he found that the osmotic pressure of the blood rose immediately, and that the kidney at once started to correct this and the hydræmic plethora with the least possible work to itself. At first the kidney passes out very large quantities of a fluid which is nearly isotonic with the blood plasma and contains large quantities of the injected molecules. In this way the maximal amount of water and of the substance injected are eliminated with the least possible work to the kidney. This urine, which has a Δ only just greater than that of the blood, is presumably in the main the glomerular filtrate. At the end of two hours the osmotic pressure of the blood has been greatly reduced and at the same time the diuresis begins to pass off. For, it would be clearly impossible for the body to go on parting with the quantities of water necessary for the production of this nearly isotonic urine, unless it received fresh supplies, because the kidney is secreting not only the injected substance, but also the normal organic solids at the usual rate. Consequently, the diuresis begins to pass off, the molecular concentration of the urine steadily rises, and with it the work done by the kidney. Galeotti found that after injecting 150 c.c. of 10 per cent. sodium chloride solution into an unwatered dog, the

kidney took forty-six hours to reduce the osmotic pressure of the blood to normal, and that in so doing the kidney performed 10 million grm. cms. of work; but if the dog was given water to drink the same result was produced in half the time with half the expenditure of work. There is really nothing in these experiments which shows by what mechanism the glomerulus brings about the early diuresis, nor whether the subsequent rise in the molecular concentration of the urine is caused by the reabsorption of water to meet the needs of the body or by the excretion of solids together with a minimal quantity of water.

From all the above considerations we can conclude only that there is no evidence which decides whether or not the capillary pressure is a force adequate to produce urine by filtration, but on the whole the evidence would seem to be against such an idea.

The Function of the Tubules.—We have incidentally dealt with the problem whether the tubules are an excretory or absorbent mechanism, but we must now turn to the experiments which have been specially directed to the solution of this problem.

Injection of Dyes.—Efforts have been made to decide this question directly by tracing the course through the kidney of some substance which could be recognised microscopically. Since none of the normal constituents of the urine can with any certainty be recognised within the renal cells, Heidenhain had recourse to indigo-carmine, which after intravenous injection rapidly appears in the urine. His method of experiment is as follows. The spinal cord is cut in the cervical region in order to stop the flow of urine, and so prevent the pigment from being washed at once down the tubules; then 5 c.c. of a saturated solution of indigo-carmine are injected into a vein; ten minutes later the animal is killed and absolute alcohol is rapidly forced through the renal artery in order to precipitate the pigment *in situ*. On splitting the kidney open it is seen that the cortex is deep blue but the medulla is unstained. Microscopical sections show pigment granules in the lumen of the tubules, in the cells of the convoluted tubules and the ascending loops of Henle, but none in the glomeruli or the cells of other parts of the tubule. Heidenhain concluded from this result that the cells of the convoluted tubules and the ascending loops of Henle excreted indigo-carmine from the blood, and had, therefore, normally an excretory function. This experiment, however, is inconclusive

and unsatisfactory in many ways. For, unless Heidenhain's directions are followed exactly, pigment may be seen also in the cells of Bowman's capsule, especially if more than 5 c.c. of the solution are injected. The use of indigo-carmine at all is open to the serious objection that it readily undergoes reduction in the living tissues into a colourless compound; and this, coupled with the diluteness of the glomerular filtrate, which would keep the pigment in solution until it had undergone concentration in the tubules, makes the microscopical appearances explicable also in accordance with Ludwig's view. Carmine also is excreted by the kidney, and has the advantage of undergoing no reduction in the tissues; but the injection of this dye by various observers has led to contradictory results and equally equivocal microscopical appearances. V. Sobieranski found the glomerular epithelium stained, and granules of the dye in the cells of the convoluted tubules. He pointed out that, as in the case of indigo-carmine, the granules are always found in the portion of the cell bordering upon the lumen of the tubule and never in the outer portion; he advanced this as evidence that the pigment is being absorbed and not excreted by these cells. Other observers have pointed to the same appearance as evidence of excretion; and the granules in secretory glands during activity do have this distribution. Ribbert denied that carmine is to be found in the glomeruli, and stated that the same result was obtained when laked blood, which does pass out by the glomeruli, was injected at the same time. On the other hand, Grützner found that, when a 2 per cent. solution of gum was injected with indigo-carmine, the dye could always be found in the glomeruli.

The Anatomical Separation of the Glomeruli and Tubules. – Several attempts have been made to separate the activities of these two structures by various operative procedures. Ribbert made a bold attempt to separate the functions of the cortex and medulla of the mammalian kidney by complete removal of its medulla. He used rabbits for this purpose because they possess but one pyramid. His method was to gouge away the whole medulla of one kidney, leaving its cortex intact, and then to remove completely the other kidney. The animals survived the operation about two days, and secreted about twice as much of a more dilute urine than the controls. He considered that his experiments supported Ludwig's view. Boyd repeated these

experiments, but obtained a different result. He found that it was impossible to remove the whole medulla without interfering with the blood supply of the cortex, and so causing its necrosis. With partial excision of the medulla he observed no increased secretion of urine. He found that the relation of the intake of water and its output as urine was normal, and that there was no change in the animal's metabolism except such as could be accounted for by deficient food.

The occurrence of diuresis following excision of one kidney and a portion of the other has been confirmed by Bradford, who used a different method. He excised a large wedge from a dog's kidney, and after the wound had healed removed the other kidney whole. He obtained one of two results according to the amount of kidney substance ultimately left. If as much as one-third of the total kidney weight was left, the operation was not followed by death. But the urine excreted was altered in quantity and composition. The daily quantity of urine passed was increased twofold or more, and the animal was extremely thirsty; the urine was of low specific gravity, and the total daily excretion of urea was not increased. The blood was hydræmic and contained a slightly increased percentage of urea. An increased quantity of proteid food caused the total daily quantity of urea and urine to rise, but the urine was of the same character as before. In fact, the animal seemed to have lost the power of excreting a concentrated urine with a high percentage of urea in it. On the other hand, when the proportion of total kidney weight remaining was only a quarter or less, death occurred in from one to six weeks, and appeared to be due to inanition associated with great muscular wasting and thirst. The blood showed the same hydræmia, but a much greater increase in the percentage of urea. The urine was of the same character, but the total excretion of water and urea was greater. Increase of nitrogenous food had the same effect as before. The increased daily output of urea was due to the breaking down of the muscles and other nitrogenous tissues, as shown by the inanition and wasting. But the increased nitrogenous excretion was unable to keep pace with the breaking down of the tissues, for the percentage of nitrogenous extractives in the blood and tissues rose considerably. This failure of the kidney fragment to excrete sufficient nitrogen was not due to circulatory failure, because the arterial blood pressure remained

high. The only point in these experiments which concerns us here is the relation between the quantity of kidney substance and the changes in the blood and urine. In Bradford's experiments the reduction in the number of glomeruli and tubules must have been about the same; and as the urine was similar to that secreted in Ribbert's experiments, it seems likely that the change in the urine observed by Ribbert was due to the reduction in total kidney substance rather than to deficiency of tubules alone. The change in the character of the urine observed in these experiments is probably to be explained in the light of Caleotti's results as the effort of the reduced kidney substance to excrete the maximal amount of material with a minimal expenditure of energy. But they do not help us to understand the mechanism by which the kidney brings about this altered secretion.

Of all the experiments dealing with the functional separation of the glomeruli and tubules, those of Nussbaum are the most important; in fact, they appeared to be the crucial experiments which settled once and for all the whole question which we are discussing. His method was based on the anatomical fact that the amphibian kidney has a double blood supply, from the renal artery and from the reno-portal vein. The renal arteries alone give off the vasa afferentia to the glomeruli, from which the vasa efferentia pass to the capillary network round the tubules, and are there joined by the branches of the reno-portal vessel. Nussbaum satisfied himself that, when the renal arteries had been ligatured, the glomeruli were completely and permanently out of the circulation, and that no collateral or backward circulation through the glomerular capillaries was possible. He found that the effect of ligature was to prevent any spontaneous secretion of urine, but that a flow of urine was set up by injecting urea. From this he concluded that the tubules normally excrete urea in solution. He found further that dextrose, carmine, egg albumin, and peptone which pass into the urine after injection into normal frogs, failed to do so in ligatured frogs, even when a flow of urine had been set up by injections of urea. He concluded from these experiments that the glomerulus normally passes out water, salts, and dextrose, and that the tubules are definitely excretory, and secrete urea and similar substances in solution. A repetition by Adami of these experiments threw doubt on the accuracy of their anatomical basis. He found that after ligature of the renal arteries a collateral circulation was set up through the

glomeruli by way of a small vessel connecting the capillary plexus round the tubules and the vas afferens of the glomerulus, so that within a short time of ligature 50 per cent. of the glomeruli might be in circulation again.

Owing to the crucial importance of these experiments, further investigation has been carried out by several observers, all of whom appear to have been satisfied with the accuracy of their anatomical basis; but the grounds of their satisfaction have not been in all cases convincing. Beddard agreed with Nussbaum that, when all the arteries supplying the kidneys were ligatured, the glomeruli were permanently out of circulation and no spontaneous secretion of urine took place. He found that it was a matter of some difficulty to ligature the whole arterial supply of the kidneys, and that by merely observing that a given frog secreted no urine spontaneously, it is impossible to say that the ligature was complete and all the glomeruli out of circulation; in fact, that it was impossible to say this unless the kidneys had been injected and serial sections cut. He did not confirm Nussbaum's other results. For, he never found that injections of urea could bring about a secretion of urine after a complete ligature. But this result is not necessarily in opposition to Nussbaum, for none of Beddard's injections were made until two days after the ligature, at which time he found that a rapid degeneration of the tubule cells set in. And there is nothing to show whether the failure of urea in his experiments to set up a secretion was due to the ligature being really complete or to the tubules having begun to degenerate. Mosberg obtained results which resemble closely those of Nussbaum. He found that the ligature prevents a spontaneous secretion of urine; that in ligatured frogs injections of dextrose cause no secretion, but simultaneous injection of dextrose and urea produce a flow of urine which does not contain sugar, while injections of phloridzin alone lead to glycosuria. The injections were made directly after the operation, but the experiments are not conclusive in that he did not control the completeness of the ligature by a microscopical examination of the kidneys. Halsey has confirmed Nussbaum's results even more fully. He found that ligatured frogs excreted urea and indigo-carmine in their urine, but not dextrose, egg albumin, peptone, and carmine. He showed, like Mosberg, that phloridzin can still produce glycosuria after the ligature, and added that the same was true of simultaneous injections of theobromine and dextrose. Halsey does not mention in

the account of his experiments, which we have seen, at what interval after ligature the injections were made, but presumably following Nussbaum they were made soon after the operation. He controlled his ligature by injection of a pigment and a microscopical examination of the kidneys, and found in every case glomeruli in circulation, but he adds that their number was so small as to be a physiologically negligible factor. The conclusion to be drawn from these repetitions of Nussbaum's experiments seems to be that, while they do not afford a final proof that the tubules excrete, they bring forward strong evidence in favour of it.

Gurwitsch experimented on frogs by a method which is the converse of Nussbaum's. Having introduced urea into the intestine and tied a canula into each ureter, he ligatured the reno-portal vein and its tributaries to the kidney on one side. In this the kidney circulation through the glomeruli would be undisturbed, but that round the tubules must have been reduced. In two experiments he found that the kidney on the ligatured side secreted 0·5 c.c. urine, and on the unligatured side ·8 or 1 c.c. This result affords evidence that the function of the tubule is not absorbent, unless we imagine that the tubule had been stimulated to absorb more water owing to the reduction of its blood supply. He experimented also with indigo-carmine and other dyes, and found that the tubule cells on the ligatured side were nearly free from pigment, but on the unligatured side were deeply pigmented. He admitted that small quantities of pigment were passed out through the glomeruli, but concluded that pigments were passed out chiefly by the tubules.

The Reaction of the Urine.—No fact in the secretion of urine is more striking than the power which the kidney has of producing an acid urine from alkaline blood. Numerous experiments have been devised to discover where this change in reaction takes place and the means by which it is brought about.

The site of this change in reaction has been settled by Dreser's experiments. He injected into frogs a large variety of indicators and examined their kidneys microscopically. His most successful results were obtained with acid fuchsin, which is nearly colourless in alkaline solutions and red in acid ones. After a single injection of the dye into the dorsal lymph sac, he found that the urine was red in an hour or two, and that the red colour was confined to the fluid in the collecting tubules. After repeated injections the glomeruli still remained colourless, but in the convoluted

tubules the red colour appeared both in the lumen and in the cells lining it. There can be no doubt, therefore, that the glomerular filtrate is alkaline and becomes acid in the convoluted tubules. And this conclusion is supported by the observation that diuresis decreases the acidity of the urine, and that this decrease is proportional to the degree of diuresis, no matter how the increased flow of urine is brought about.

We have now to inquire into the mechanism by which this change in reaction is brought about. It is clear that the alkaline glomerular filtrate might be made acid in the tubule either by the absorption of bases or by the excretion of acids. Dreser tried to prove that the change in reaction was brought about by a process of excretion. He injected acid fuchsin into frogs ligatured by Nussbaum's method, and found that the red colour could still be seen in the convoluted tubules. But, in the absence of evidence that the glomeruli were wholly out of circulation, this conclusion cannot be accepted. Liebermann put forward a view to explain how the tubule cells could excrete acid salts although they receive only alkaline ones from the blood. He separated from the kidney a leeith-albumen capable of combining with bases, so that it would convert a neutral or alkaline phosphate into the acid salt. He found that the neutral combination of leeith-albumen with base would give up its base to CO_2 and become again capable of uniting with fresh base. He imagined that the following alternation of changes went on during the secretion of urine—firstly, base was taken from the blood phosphates and the acid radicles passed into the urine, and then the base remaining behind in the cell, in combination with the leeith-albumen, was taken up by CO_2 and passed into the venous blood as carbonate. Such a transference of base from phosphate to carbonate could not take place unless the mass influence of the two acids were rapidly alternating backwards and forwards, but there is no obvious reason why they should.

Before attempting to explain the change in reaction between the blood and urine, it is necessary to inquire into the real extent of the change. This can be done by comparing the reactions of both fluids with indicators whose acid radicle or anion have different strengths of affinity for base or cation. Cushny has pointed out that both blood and urine are acid to phenol-phthalein and alkaline to methyl-orange, but to litmus blood is alkaline and normal urine acid. The alkalinity of the blood to litmus

means that the blood contains some salt so weak that litmus can take away its cation from it; but phenol-phthalein is weaker than litmus, and unable to take away the cation from any salt in the blood. Normal urine is acid to litmus because litmus can no longer successfully compete with any anion for its base, owing to the great reduction in the relative number of cations to anions present in urine. The stronger methyl-orange, however, is still able to compete, and urine appears to be alkaline to it. It has been found that in a large variety of conditions the acidity of urine becomes so reduced that it may become alkaline to litmus. Such conditions are diuresis, digestion, after the introduction of a chloride or bromide into the alimentary canal or their injection into the blood. But under no conditions does urine become alkaline to phenol-phthalein, not even after the intravenous injection of sodium carbonate. On the other hand, urine never becomes acid to methyl-orange. Therefore the extreme variations in the reaction of urine correspond roughly to the interval between Na_2HPO_4 and NaH_2PO_4. Cushny has investigated the mechanism by which this change in reaction is brought about by the kidney. His method was to compare under different conditions the urine from one ureter with that obtained from the other, in which the flow of urine was reduced by the introduction of a resistance equal to 15–30 mm. Hg. The object of the resistance, as in his experiments previously referred to, was to favour any absorption which might take place in the tubules. He injected intravenously into dogs normal solutions of sodium chloride or sulphate, and 10 per cent. solutions of dextrose or sodium bromide, nitrate, malate, or tartrate. After the injection of any of these solutions he found that the percentage acidity always fell, so that the urine was alkaline to litmus and almost neutral to phenol-phthalein, and was more or less equal on the two sides. This result suggested that the fall in acidity was due not so much to a too rapid flow through the tubules as to an absence of acid-forming salts in the blood. He therefore injected Na_2HPO_4, together with sodium chloride or dextrose, and found that the urine in spite of the diuresis became much more acid to phenol-phthalein, and might become acid even to litmus. In the urine secreted under these conditions he found that the percentage of phosphates and the percentage acidity to phenol-phthalein ran more or less parallel to each other, and were greater on the obstructed than the free side; this difference could be accounted for

simply by the greater absorption of water on the obstructed side. But he showed that on the obstructed side the percentage acidity to litmus rose out of all proportion to the percentage increase in phosphates, and, as the absolute quantity of phosphate was reduced on the obstructed side, there must have been an even greater reduction in the sodium present. In other words, the effect of obstruction was to decrease the absolute excretion both of phosphate and sodium, but the decrease in the excretion of sodium was the greater. From these results he concludes that the greater acidity on the obstructed side is due not to an increased excretion of acids, but to an increased absorption of base as compared to the phosphate. He therefore considers that the normal difference between the reaction of blood and urine is brought about, not by the addition of $=HPO_4$ anions, but to the absorption of Na− cations in combination with $-OH$ or $-HCO_3$.

Although these experiments by Cushny, like others of his already considered, are suggestive of absorption by the tubules, they can hardly be considered to prove it. For, the essence of the experiments is the differences in the urine from an obstructed and free ureter. It is quite possible that partial obstruction does favour absorption by the tubules, but it is conceivable that it might affect and alter their excretory powers.

Conclusions.—We have seen that there are as yet no experiments which prove how the kidney produces urine from the blood. We are therefore reduced to weigh the evidence for and against the various theories in order to find a working hypothesis. And in doing so, it must be remembered that if we knew the function either of the tubule or of the glomerulus, we could deduce roughly the function of the other. For, if the tubules were shown to excrete, it would follow that the glomerulus could not act as the mechanical filter supposed on Ludwig's view; and conversely, if the glomerulus is a mechanical filter, the tubules must absorb. But, on the other hand, if the tubules were shown to absorb, it would still be possible for them also to excrete, because, on Heidenhain's view of the function of the glomerulus, there is room for absorption as well as for excretion by the tubule. We have seen that the evidence that the tubule can excrete is certainly stronger than that it can absorb; it follows that the glomerulus does not act as a mechanical filter, and this again coincides with the balance of the experimental evidence on this point. But when we come to the question whether the

tubule can absorb as well as excrete, it is impossible to give a definite answer. There is evidence for this, and several observers have put forward this view of the double function of the tubules. They have considered, from the results of experiments with dyes and from the histological appearances of the tubule, that while excretion takes place from the convoluted tubules and ascending loops of Henle, absorption is carried on by the descending loops of Henle, the junctional and collecting tubules.

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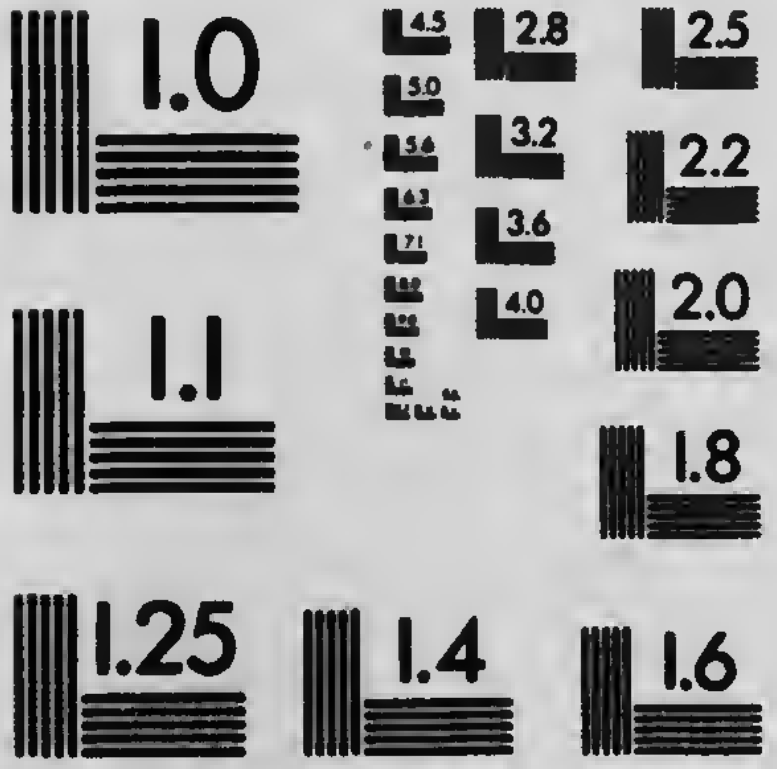

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