CIHM
Microfiche
Series
(Monographs)

ICMH
Collection de
microfiches
(monographies)

Canadian Institute for Historical Microreproductions / Institut canadien de microreproductions historiques

Technical and Bibliographic Notes / Notes techniques et bibliographiques

The Institute has attempted to obtain the best original copy available for filming. Features of this copy which may be bibliographically unique, which may alter any of the images in the reproduction, or which may significantly change the usual method of filming are checked below.

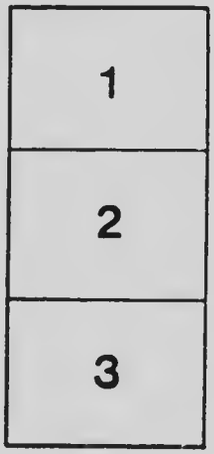
- ☐ Coloured covers / Couverture de couleur
- ☐ Covers damaged / Couverture endommagée
- ☐ Covers restored and/or laminated / Couverture restaurée et/ou pelliculée
- ☐ Cover title missing / Le titre de couverture manque
- ☐ Coloured maps / Cartes géographiques en couleur
- ☐ Coloured ink (i.e. other than blue or black) / Encre de couleur (i.e. autre que bleue ou noire)
- ☐ Coloured plates and/or illustrations / Planches et/ou illustrations en couleur
- ☐ Bound with other material / Relié avec d'autres documents
- ☐ Only edition available / Seule édition disponible
- ☐ Tight binding may cause shadows or distortion along interior margin / La reliure serrée peut causer de l'ombre ou de la distorsion le long de la marge intérieure.
- ☐ Blank leaves added during restorations may appear within the text. Whenever possible, these have been omitted from filming / Il se peut que certaines pages blanches ajoutées lors d'une restauration apparaissent dans le texte, mais, lorsque cela était possible, ces pages n'ont pas été filmées.
- ☐ Additional comments / Commentaires supplémentaires:

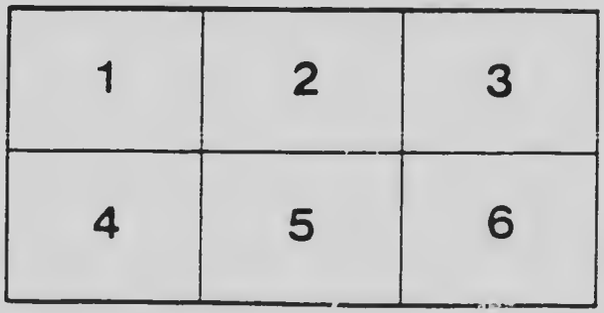
L'Institut a microfilmé le meilleur exemplaire qu'il lui a été possible de se procurer. Les détails de cet exemplaire qui sont peut-être uniques du point de vue bibliographique, qui peuvent modifier une image reproduite, ou qui peuvent exiger une modification dans la méthode normale de filmage sont indiqués ci-dessous.

- ☐ Coloured pages / Pages de couleur
- ☐ Pages damaged / Pages endommagées
- ☐ Pages restored and/or laminated / Pages restaurées et/ou pelliculées
- ☑ Pages discoloured, stained or foxed / Pages décolorées, tachetées ou piquées
- ☐ Pages detached / Pages détachées
- ☑ Showthrough / Transparence
- ☐ Quality of print varies / Qualité inégale de l'impression
- ☐ Includes supplementary material / Comprend du matériel supplémentaire
- ☐ Pages wholly or partially obscured by errata slips, tissues, etc., have been refilmed to ensure the best possible image / Les pages totalement ou partiellement obscurcies par un feuillet d'errata, une pelure, etc., ont été filmées à nouveau de façon à obtenir la meilleure image possible.
- ☐ Opposing pages with varying colouration or discolourations are filmed twice to ensure the best possible image / Les pages s'opposant ayant des colorations variables ou des décolorations sont filmées deux fois afin d'obtenir la meilleure image possible.

This item is filmed at the reduction ratio checked below / Ce document est filmé au taux de réduction indiqué ci-dessous.

10x 12x 14x 16x 18x 20x 22x 24x 26x 28x 30x 32x





A LABORATORY MANUAL

. . OF . .

EXPERIMENTAL PHYSIOLOGY

By

LOIS McPHEDRAN FRASER, M.A.

Lecturer in Physiology, The University of Toronto

F. A. HARTMAN, Ph.D.

Professor of Physiology, The University of Buffalo

J. J. R. MACLEOD, M.B.

Professor of Physiology, The University of Toronto

UNIVERSITY OF TORONTO PRESS

1919

PREFACE.

Experimental physiology is now recognized as a fundamental subject in the curriculum of the medical student and as one having a most important place in the training of the student of biology. In the medical course, the physiological laboratory serves as the portal to the clinic; as the testing ground where the student may try for himself in how far the known laws of physics and chemistry can be successfully employed to explain the normal working of the human machine. No more can theoretical study, or demonstration, by itself supply a correct understanding of the functions of the living body than could similar methods in training an engineer to understand an engine. Attempts to rectify, by operations or by drugs, functional derangement in the diseased animal without a practical knowledge of the normal working of the various organs, both isolated and as a whole must be as unjustifiable as attempting to repair a complicated piece of machinery would be by any other than a practical engineer.

In the training of the biologist, experimental physiology finds its value because it teaches how to interpret the relationship between structure and function. For the advancement of physiological knowledge it is essential that the functions of the lower animals should be more intensively investigated by those who have been trained in the methods of the experimental physiologist.

In the crowded curriculum, either of medicine or of biology, it is impossible to find an amount of time that is sufficient for the performance of more than a few of the fundamental experiments in each of the main subdivisions of the subject of physiology. It is the duty of the teacher, therefore, to select these experiments with the greatest care so that the experience which the student gains in their performance may guide him aright in building up his knowledge of this subject. The experience gained in the laboratory is to serve as the framework upon which the detailed construction is to be completed by fitting on to it in their proper relationship to one another the other facts of physiological knowledge, acquired by theoretical study and demonstration.

While admitting these principles some have averred that the technical difficulties of experimental physiology are such as to make it impossible for the average medical student to secure a sufficient number of results to justify the expenditure of time and energy required in the laboratory. This is a fallacious criticism for it assumes that every experiment to be of value should be crowned by results that are technically flawless, and it fails to recognize that the performance of the work, if carefully and intelligently done, affords that personal experience without which, in a highly practical science like medicine, theoretical knowledge by itself is valueless and without meaning.

There is, however, no subject of the medical curriculum in which the organization and arrangement of laboratory courses is more difficult for classes of average size than in physiology. In physics and chemistry, as well as the various morphological subjects, the material for the laboratory course is constantly available, and can be stored away for future use, whereas in physiology living material must be provided afresh for each experiment and there must be constant supervision of the practical work to see that the material is properly used. This requires that the student be adequately directed as to how he should proceed with the experiment without at the same time stifling originality on his part by requiring him explicitly to follow detailed directions in every step. The experiment is of no value unless it is performed with an inquiring attitude of mind, and laboratory directions and instruction should be no fuller than is necessary to guide the student in its general performance.

It is with these requirements in view that the present volume has been compiled, and while the authors realize full well its many shortcomings they hope that the practical instruction, not only in their own laboratories, but also in those of other institutions may be assisted by its publication.

The work is arranged in sections, each of which deals with some special part of the subject, this plan being adopted because it has been found the most practicable for courses designed for large elementary classes, as well as for smaller groups of more advanced students. The first section deals with the fundamental experiments in the physiology of isolated muscle and nerve, placed

in this position not because this is the simplest part of the subject of physiology to understand, but because it is the easiest to provide material for and therefore the best in which to permit of sufficient practice, so that the student may become familiar with the methods of physiological technique. The two following sections deal with essential experiments illustrating the principles of the heart beat and the circulation of the blood. In selecting experiments in the last mentioned group the endeavour has been made to apply them, as far as possible, to man. A certain number of experiments in which other mammalian material is used is, however, essential in order that the student may be in a position to appreciate the significance of results which will later be demonstrated to him. These demonstrations are described in the last sections of the manual in sufficient detail, so that the various steps may be followed by every student of the general course and the experiments may be performed by small groups of more advanced students.

In the experiments on the central nervous system the decerebrate or spinal animal is extensively made use of, as recommended by C. S. Sherrington whose work in this direction must be considered as the most important contribution that has recently been made to the advancement of the teaching of practical physiology. In the section on the special senses a relatively larger proportion of theoretical matter is given, along with the directions for the experiments, partly because the latter, being practically all subjective in nature, cannot be performed unless the student understands fully what he is looking for, and partly because most of them can, and should be performed in the study rather than the laboratory.

A great difficulty has always been felt by instructors in physiology in attempting to supply simple experiments bearing on the chemistry of respiration and yet there is no part of physiology in which practical experience is more important, if the student is to understand the principles of this difficult subject. A section of the manual is devoted to experiments in which simplified and inexpensive apparatus for gas analysis is employed.

Throughout the book, besides the directions for the experiments, a brief statement is given of the theoretical matter which bears on them. This is done to enable the student to appreciate the object of the experiment and to guide him in the interpretation

of the result; but the latter is not described in detail, this being left for the student to determine for himself.

While the general plan and arrangement of the book is the work of the authors jointly, each author is responsible for certain chapters as indicated by the initials given in the table of contents.

The authors wish to express their thanks to Miss Marion E. Armour for the care and patience which she bestowed in the preparation of the drawings for the illustrations, many of which are original while others are copied from other texts, acknowledgment for which is given in the legends.

They wish also to thank Miss Jean Halliday for her assistance in arranging the index and correcting the proof. They are indebted to Dr. J. P. Eisenberger for a final reading of the proof.

LOIS MCPHEDRAN FRASER.
F. A. HARTMAN.
J. J. R. MACLEOD.

The Universities of Buffalo
and Toronto.

CONTENTS.

Section I.

MUSCLE AND NERVE.

Section IIA.

CIRCULATION.

Section IIB.

CIRCULATION.

Section III.

REFLEX ACTION.

Section IV.

RESPIRATION.

Section V.

SPECIAL SENSES.

Section VI.

DEMONSTRATIONS.

CIRCULATION AND RESPIRATION.

Section VII.

DEMONSTRATIONS.

DIGESTIVE SYSTEM.

Section VIII.

DEMONSTRATIONS.

THE CENTRAL NERVOUS SYSTEM.

SECTION I.

CHAPTER I.

PRELIMINARY TECHNIQUE.

Electrical Stimulation.--In experimental physiology it is necessary in many instances to employ an artificial stimulus in order to cause action of the tissue studied. Many kinds of stimuli are capable of this, but most of them have their objections when used as stimuli of nerve or muscle. Mechanical, chemical and thermal stimuli are all more or less injurious. Moderate electrical stimulation on the other hand brings these tissues into action without appreciable injury. As this is the means by which the tissues are usually stimulated, we will discuss briefly the principles involved in the more important pieces of electrical apparatus employed.

For a source of current either a dry cell or a dynamo may be used. A dry cell is essentially a large zinc cup serving as container in which is suspended a carbon cylinder. A small amount of water containing ammonium chloride held by gelatin or some inert substance fills the rest of the container. A dry cell is essentially a wet cell, but with the water protected from spilling by the gelatin "sponge". Whenever the carbon and zinc are connected by a conductor, electrical energy is set free by the solution and is transmitted along the conductor. It has been found that if a live muscle is placed in the electrical circuit that the tissue will be stimulated at the time the circuit is made and again when it is broken provided the current is great enough.

Two important factors must be kept in mind in the use of an electric current, the resistance of the tissue and the "pressure" of the current. The unit of "pressure" is the volt. Therefore the amount of pressure is called the voltage. By increasing the voltage the penetrating power is increased and thus the physiological effect augmented. If the resistance of the tissue is high as in dry skin, a

greater voltage is necessary than if the skin had been moistened, because moist skin is a better conductor. Dry cells are made to give in the neighbourhood of one or two volts. Greater voltage in a circuit can be obtained by connecting two or more cells in series, i.e., zinc to carbon.

In most of your work the current generated by dynamos in the power plant will be used. At each working place two wires from the main line are connected with a metal ribbon of high resistance. Terminals are arranged so that different proportions of this ribbon can be used in the circuit, thus varying the amount of voltage. The amount of voltage obtained can be estimated by adding the total voltages between the two terminals employed. The following diagram gives the voltages between terminals. T represents the terminals as arranged on the table.

T 8 T 8 T 4 T 4 T 4 T 2 T 2 T
a b c d e f g h

To illustrate the method of finding voltage, connecting a to f gives 28 volts; f to h, 4 volts; d to e, 4 volts.

Besides the direct current one can use the induced current for stimulation. It is well known that if one wire is placed near another wire which carries a current, a brief current is induced in the first wire at the time of breaking the circuit in the second. Likewise a current is induced at the closing or making of the circuit. The wire carrying the direct current is called the primary circuit, while that in which the current is induced is called the secondary circuit. Greater effects can be produced by having a coil of well insulated wire surrounding a soft iron core as the primary circuit element and another coil of insulated wire as the secondary element. These two coils are arranged on a stand for convenience, the whole apparatus being termed an inductorium (Fig. 1). The chief advantages of the induced over the direct current for purposes of stimulation are; first, the voltage is very much greater so that high resistances can be overcome and, secondly, it can readily be altered in strength.

It has been found that the least induced current is produced when the secondary is at right angles to the primary; from this position the current is increased more and more as the secondary becomes more nearly parallel. In addition to the angular relation-

ship, the distance between the two coils determines the strength of current induced, the closer they are the greater the effect.

Experiment 1.—Try the following.—1. Connect the electrodes to two terminals on the table so as to obtain 2 volts. Touch the tongue with the electrodes and then make and break the circuit by means of the switch. Repeat with increasing voltage. Describe the effects at make, at break and during the passage of the current.

2. Connect the binding posts 1 and 2 (Fig. 1) of the primary coil to 4 volts on the table, with a simple key in the circuit.

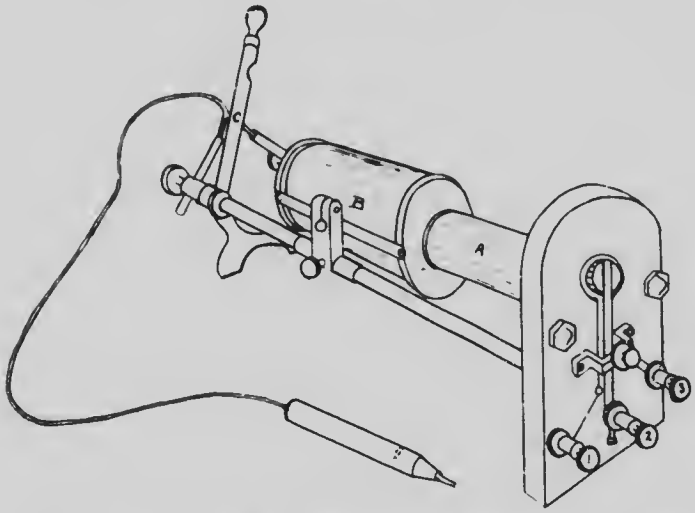


FIG. 1. Inductorium: (*a*) Primary Coil; (*b*) Secondary Coil; (*c*) Bar for short-circuiting the secondary terminals. Connect the source of supply to 1 and 2 for single shocks and to 1 and 3 for a succession of rapid shocks.

Connect the electrodes to the end of the rods upon which the secondary slides. With the secondary coil far out and parallel to the primary coil stimulate the tongue by making and breaking the primary circuit. Move the secondary closer and closer to the primary coil and note the effect on the current produced. Again move the secondary out into such a position that it just clears the primary coil. Now study the effect of tilting the secondary coil.

The Graphic Record.—By the use of a lever it is possible to magnify muscle contraction. The movement of this lever is recorded on smoked paper. The kymograph is devised for carrying the smoked paper.

Glazed paper is wrapped around the drum of the kymograph so that the glaze is outward. The gummed end is fastened to the opposite end so that a tight fit is made and the paper will not slip. The drum is held in the smokiest part of a gas flame while being rotated rapidly on the brass tube which is maintained horizontally by hand. In this way a uniform coating of carbon can be obtained on the paper. A light chocolate colour is to be preferred to a deep black because there is less danger of burning the paper.

A small triangular piece of photographic film or waxed paper is fastened by a bit of wax to the end of the lever in order to serve as a writing point. The lever is always on the right hand side of the drum so that when the latter is rotated it pulls away from the writing point. The lever must be at a tangent to the curved surface of the cylinder in order to prevent the writing point leaving the smoked surface when it rises. The writing point may be bent inward to keep the rest of the lever free of the drum.

When ready to remove the paper from the drum, it is cut at the overlap by a sharp knife in such a way that the knife strikes the under paper and not the drum. A corner of the paper is grasped against the edge of the drum with the other hand so that it will not fall. At this stage the record may be placed on the table and lettering or other explanatory matter inscribed. It is well to place your name and date on each record. The record, with smoked side up, is passed once through a rosin-alcohol solution (120 gm. rosin to 1,000 c.c. of 95% ethyl alcohol) and then hung over the tray to drain. When dry the parts desired are to be carefully cut out and preserved in the note book.

Instruments for Dissection. Each student should supply himself with the following: Two pairs of scissors, one heavy the other fine; two pairs forceps, one heavy the other fine; one dental probe, one strabismus hook, one scalpel, one small serre fine and one haemostat. The fine scissors and fine forceps are to be used only in delicate dissection. In order to do the best work the cutting instruments must be kept sharp.

CHAPTER II.

VOLUNTARY MUSCLE.

Animal life differs essentially from plant life in the power of movement. Whatever the power which certain plants may have in this respect is extremely limited. Animals on the other hand not only contain organs which are more or less constantly moving, but also possess the extremely useful power of locomotion. This power to move resides in contractile tissue. Muscle is the most important type of this kind of tissue in the higher and many of the lower animals. What can an animal do without muscle? There would be no respiration, no circulation, no movement. Truly muscle is a master tissue.

A portion of the muscle of the body is under the control of the will. Upon this muscle we depend for locomotion and the major movement in the organism. Such muscle is composed of fibres with a characteristic cross striation. These fibres are by far the most active in the body, not only working rapidly, but doing the most work.

We could study muscle by watching it contract or by finding out how much it could do. The knowledge gained by such means would be very limited. We should find that a muscle shortens and thickens when it contracts; we might discover that heat is produced in the process, but many changes would escape observation. A simpler method and one by which more accurate information can be obtained is to isolate a single muscle and study its responses to artificial stimuli.

STIMULATION.

Various kinds of stimuli will cause muscle to contract, but before we decide upon the best form to use we must know where to apply the stimulus. Normally the muscle fibres are called into action by impulses passing along the nerve fibres. Artificial stimuli can

be effectively applied along the same path. Thus mechanical, thermal, chemical and electrical changes can cause a muscle to act through its nerve. Any of these changes to be effective must not only be of sufficient magnitude but must be abrupt. A nerve slowly compressed may be without effect, but if quickly pinched, a twitch of the muscle results.

Of all artificial stimuli, the electrical is not only the most effective, but as before stated, the least injurious to nerve. Mechanical stimulation produces more or less injury. Thermal change which is great enough to have an effect is likewise detrimental. Chemical stimulation if not harmful, is at least difficult to control. Moderate electrical changes have none of these objections.

Although we are most interested in the physiology of mammalian muscle, we employ muscles from cold-blooded animals because, while similar in function, they survive for a much longer period when deprived of their circulation.

Experiment 2. —Nerve-Muscle Preparation.—Kill a frog in the following manner: By bending the frog's head down, a depression between the skull and first vertebra can be felt on sliding a blunt-pointed seeker back along the mid-line of the top of the skull. Plunge either the seeker or a scalpel into this depression and by moving crosswise sever the cord. Turn the seeker forward into the skull cavity and by transverse movement destroy the brain. Next destroy the cord by passing the seeker down into the spinal cord. If the frog is properly pithed it becomes limp. A frog thus prepared is dead as an organism although the heart and other tissues may live for a few hours. There can be no pain whatsoever. This method of killing a frog is called "pithing".

In a pithed frog, cut across the body just behind the fore limbs, with strong scissors. Remove the viscera, being careful to avoid injury of the nerves lying along the dorsal wall of the body cavity. Now divide the preparation by cutting longitudinally through the middle of the vertebrae and pelvis. Lay one leg aside until needed. Strip the skin from the other leg, being sure to keep the outer surface of the skin from coming in contact with the muscle.

Lay the preparation on a clean glass plate and keep it moistened with isotonic salt solution. Carefully dissect out the sciatic nerve by separating the muscles on the dorsal aspect of the thigh and freeing the nerve from the surrounding tissues. Always lift the nerve with a glass hook and avoid stretching it. By means of the vertebrae raise the roots of the sciatic nerve and clip beneath it until it is free down to the knee. Cut away the other muscles and the bone below the knee and free the femur from all muscle except the gastrocnemius.

In making a nerve-muscle preparation one must always remember that slight injury to muscle or nerve may ruin it for experimental purposes. Such injury may be caused by stretching, pressing, drying or chemical agents. NaCl dissolved in water to the extent of 0.7% produces an isotonic solution somewhat similar to the tissue fluid of the frog, therefore useful in keeping the tissues moist without the injurious effect of pure water. Apply this solution to both the nerve and muscle frequently.

NERVE-MUSCLE PREPARATION.

Experiment 3. –Different Kinds of Stimuli. –Fix the femur of a nerve-muscle preparation in the flat-jawed clamp, after it has been fastened to the stand. Clamp the nerve holder to the stand just below and then lay the whole sciatic nerve on the glass plate. Fasten the muscle lever underneath. Attach the tendon, by means of a hooked pin to the lever. Now adjust the after-loading screw so that the muscle supports no weight but is fully extended when at rest. Place a ten gram weight upon the scale pan (Fig. 2). Arrange the lever so that it will write on the smoked paper of the kymograph and obtain records with the following types of stimuli:

MECHANICAL.—Pinch the nerve near its end with forceps. Can you get a response the second time from the same place?

CHEMICAL.—Place a few salt crystals on the fresh end of the nerve, the injured portion having been cut away. After a few contractions of the muscle thoroughly remove the salt with isotonic salt solution.

THERMAL. Touch the fresh end of the nerve with a hot glass rod.

ELECTRICAL. Connect the stimulating electrodes directly to the current supply on the table but with a simple key in the circuit. Try the effect of make and break beginning with 2 volts and then increasing the current.

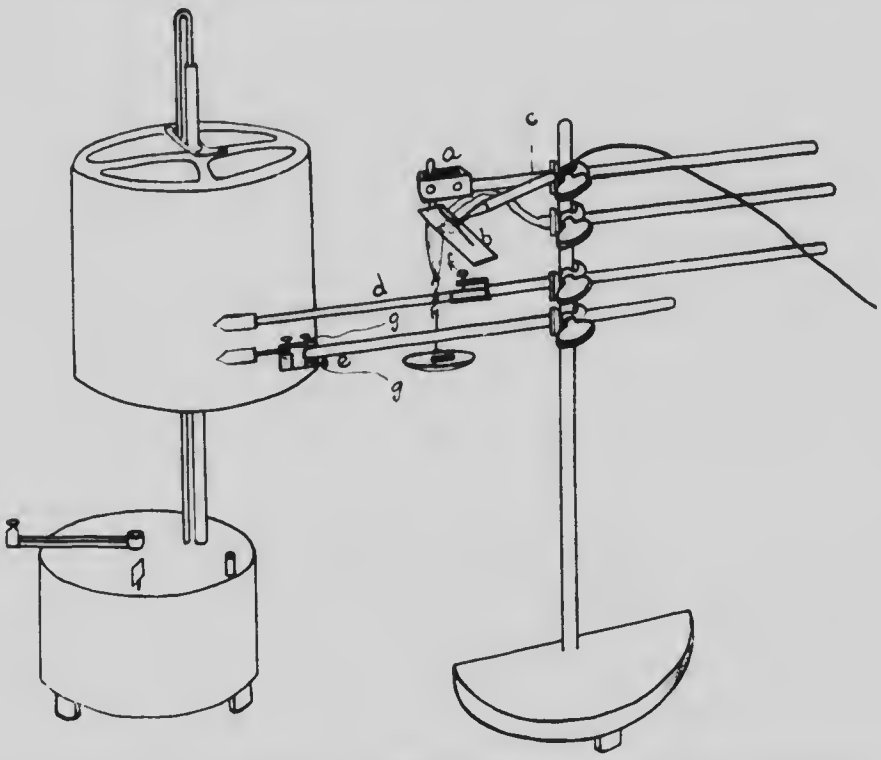


FIG. 2. Apparatus for recording muscle contraction: (*a*) femur clamp; (*b*) nerve hold , (*c*) electrodes; (*d*) muscle lever; (*e*) signal magnet; (*f*) after-loading screw; (*g*) wires connecte : in the primary circuit.

Next connect the electrodes to the secondary coil of the inductorium using a primary current of 2 volts; stimulate with make and break shocks of different intensities.

Compare the effects of make and break in direct and indirect currents.

Minimal and Maximal Stimulation.—Beginning with a stimulus too weak to cause contraction, then very gradually increasing its strength, a point is reached where a slight contraction

results. This is called the minimal stimulus. In other words it is the least stimulus which will cause a contraction. On the other hand the maximal stimulus is the least stimulus which will produce maximal contraction. When a series of stimuli of increasing strength, beginning with the minimal, is sent into a nerve or muscle, one obtains a graded response (Fig. 3). This is true no matter how finely graded is the increase of the stimulus. (See all-or-none principle, p. 55).

Experiment 4. Response to Stimuli of Different Intensities. —For this experiment employ a muscle which has been cut away from its nerve. Instead of using ordinary stimulating electrodes connect one terminal of the secondary coil to the

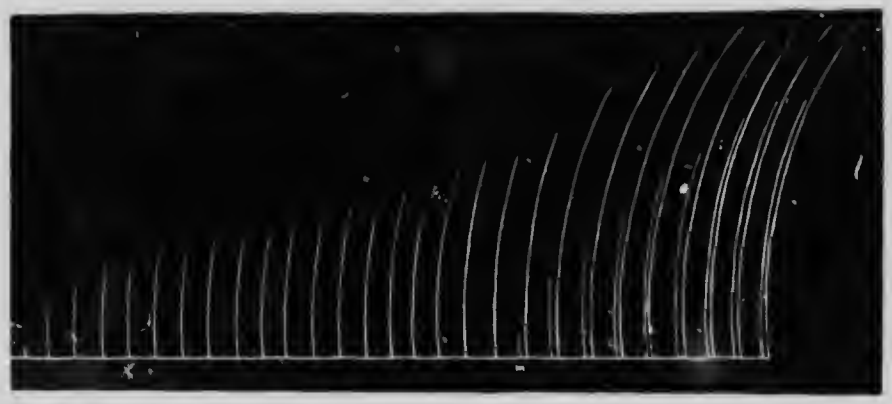


FIG. 3. Contractions produced by graded stimulation. Secondary coil moved 5 mm. at each step. Both make and break shocks employed. Make contraction begins at *m*.

femur end of the muscle by wire and in a similar way connect the other terminal to the binding post on the muscle lever. Then a bit of fine wire is wound around the tendon and finally fastened to the coarser wire at the binding post on the muscle lever. In this manner a current can be passed through the muscle.

Starting with the secondary as far as possible from the primary, cautiously move it forward by stages, each time stimulating the muscle with a make and break shock. When the first perceptible contraction is produced you have reached the threshold. Between each stimulation move the drum a

short distance by hand, not more than 1 mm. Now carefully move the coil toward the primary so that at each position there is produced a slightly greater contraction than before. Distinguish the "make" and "break" contractions on the record by the letters *m* and *b* placed beneath. Also designate the position of the secondary coil. It is possible by carefully increasing the stimulus to obtain a graduated series of contractions from both the make and break shocks (Fig. 3). When the series is complete, indicate the minimal and maximal stimuli.

Discuss the application of the all-or-none principle to your records.

INDEPENDENT EXCITABILITY OF MUSCLE.

Ordinarily the question, as to whether a muscle could respond if its nerves were absent, is of academic interest. But if we wish to test the power of a muscle which has lost its voluntary function by injury to the nerve, we find long after the nerve has degenerated, that the muscle responds to direct electrical stimulation. It can also be shown that muscle is directly excitable by paralyzing the motor end-plates, which are structures connecting motor nerve fibres with muscle fibres. This can be done by the injection of the poison "curare" into the circulation. After a few minutes has elapsed, stimulation of the nerve no longer affects the muscle, while stimulation of the muscle causes it to contract. From such experiments as these it appears that muscle can contract without the mediation of either nerve fibres or motor end plates.

The various types of stimuli can affect a muscle directly, perhaps not so readily as through the nerve fibres however. Amongst these stimuli there is one type which should be dwelt upon particularly in a certain connection. That is chemical change. Suppose you permit a fresh muscle, which has been dissected from the body, to be exposed to dry air. Moisture quickly evaporates from its surface. The solutions within the cells become more concentrated. Chemical stimulation may result, producing twitches. In any case the cells are injured if the water loss goes far.

The loss of water may be prevented by keeping the muscle in air saturated with water vapour or by frequently applying water to the exposed surface. But bathing the muscle with water produces consequences as serious, perhaps, as drying, because salts and other substances are drawn out of the cells. Now if we add the same salts in the same percentage as occurs in the tissue to the water which is used to bathe the muscle, such a solution can be used to prevent evaporation without taking salts from the cells. Any exposed tissue should be frequently bathed with such a solution.

When a muscle is stimulated by passing a current through it, we cannot say that its reaction is not brought about by the nerve fibres present. It is of considerable interest to be able to show that muscle tissue is independently irritable. This can be accomplished by the use of the drug curare.

Experiment 5. The sciatic nerves are exposed in each leg of a frog which has the brain destroyed. A ligature is passed under one sciatic and around the thigh and tied tightly so as to occlude circulation in that leg. A few drops of 1% curare solution are injected into the back of the frog. From time to time stimulate each sciatic nerve with induction shocks. In a few minutes, the leg with intact circulation refuses to respond or else responds very much less than the ligated leg. When this stage has been reached stimulate the muscles of the unligated leg directly. Their response demonstrates that the muscle is independently irritable for the nervous impulse has been ruled out. This has been accomplished by paralysis of the tissue connecting nerve fibres with muscle fibres, for it can be shown that impulses still travel along the nerve.

EXTENSIBILITY AND ELASTICITY OF MUSCLE.

In the body more or less tension affects each muscle. The weight of various parts and the antagonistic action of opposing muscles contribute to this tension. The result is a certain amount of elongation. This can be shown by fastening a weight to an isolated muscle. And then when the weight is removed the muscle soon regains its former length, that is, it exhibits elasticity.

The amount of elongation increases with increase in tension, but with each additional tension the elongation is proportionately less. To illustrate—suppose X grams cause an elongation of 2 mm. Then 2 X grams will not cause 4 mm. elongation but something less, perhaps 3 mm. 3 X grams would produce 4.5 mm. elongation, etc.

Beyond a certain tension the reverse is true; the elongations per unit increase being greater and greater with each step. Where this reversal begins the so-called elastic limit has been passed. Finally there comes a time when the muscle fibers are ruptured.

Needless to say a muscle should not be extended beyond its elastic limit.

In conclusion, tension is very useful in keeping a muscle prepared for contraction, for we find that a muscle will not only respond more quickly but more energetically if it is under a certain amount of tension. This condition holds in the body.

Muscle can be stretched and again regain its resting length when the force producing the change is removed. It differs somewhat from a rubber band in that it does not respond by equal extension for equal increments of weight as the total weight increases nor does immediate recovery occur when the weights are removed.

Experiment 6. Isolate the two large muscles (gracilis and semimembranosus, Fig. 4) on the inside of the thigh of a frog; cut through the tibia below their insertion and through the femur above the knee. Remove them with the bone from which they arise. You should have a preparation with bone at each end. Suspend the muscles by clamping one bone in the flat-jawed clamp. Fasten the other end to a muscle lever to which a large scale pan is attached. Obtain a record of the comparative length of the muscle without weight and with weights increasing by 10 gms. at each trial. The drum should be moved by hand between each increment. Continue until the point of rupture is reached. Plot a curve of these results.

In a second preparation, add 10 gm. increments, but stop before the muscle is injured. Now remove 10 gms. at a time, securing a record in each case. Plot a curve of the recovery.

In a similar manner obtain records from a rubber band, both for extension and recovery.

FIG. 1. *A* ventral view of frog.- 1, M. Gastrocnemius. 2, M. Tibialis anticus. 3, M. Gracilis. 4, M. Adductor magnus. 5, M. Sartorius. 6, M. Adductor longus. 7, M. Vastus internus. 8, N. Vagus. 9, N. Glosso-pharyngeal. 10, N. Hypoglossal. 11, N. Superior laryngeal.

B. Dorsal aspect of hind leg. 12, M. Rectus anterior. 13, M. Vastus externus. 14, M. Semimembranosus. 15, M. Peroneus. (After Jackson).

Study the extensibility of a contracted muscle as compared to the same muscle at rest. Arrange a gracilis-semimembranosus preparation as before. Obtain short records of the muscle at

rest and when stimulated by a single maximal break shock without any weight. Continue to do this for 10 gram increments as far as possible. What happens when a load is reached which the muscle cannot lift?

Plot extension curves of the contracted muscle and the resting muscle so that they may be easily compared.

THE SIMPLE CONTRACTION.

Although muscle seldom responds by a simple twitch when called into action in the body, its behaviour can be conveniently studied when it gives such twitches. Simple contractions can be caused by single electrical stimuli. In order to observe the action of a single muscle it is isolated from its neighbours and arranged as described in the experimental work so that when contracting a record is made upon the surface of a rapidly rotated cylinder (Fig. 5). By means of a signal magnet and a tuning fork the time required, for the initiation and completion of the action, can be determined. The time elapsing between the application of the stimulus and the first visible contraction is about 0.01 sec. for a frog's gastrocnemius. With the more quickly responding mirror method it is 0.0065 sec. The time consumed in the contraction averages 0.04 sec. while that used in relaxation is slightly more, 0.05 sec. Roughly the total time involved in the various changes is 0.1 sec. for the frog's gastrocnemius (Fig. 6).

The duration of the simple twitch varies in different animals. It is less in the rabbit, 0.07 sec., and considerably less in insects, 0.003 sec.

The real LATENT PERIOD is primarily due to the time consumed in inducing the changes in muscle leading up to contraction. Such changes vary with the onset of fatigue, modification of temperature, and intensity of the stimulus. Besides this, other factors are added. If a load is attached to the muscle, the preliminary effect of attempted contraction is a slight elongation on account of the inertia of the load. With the ordinary apparatus friction also slows the response.

Contraction Period. A muscle requires time for the development of the maximum contraction because each individual muscle

FIG. 5. Rapid kymograph. (*a*) Terminals to be connected in primary circuit; (*b*) Movable ring for second stimulus; (*c*) Release which starts drum.

fibre is not stimulated in all parts simultaneously, but receives its impulse at the motor end-plate located near the middle of the fibre. Hence the contraction spreads outward at the rate of 3 to 4 meters per sec. In human muscle the velocity of such a wave is 10 to 13 meters per sec. Therefore, even though each muscle fibre of a mass may be stimulated simultaneously through a nerve trunk, time is required for the spread of the contraction throughout the fibres.

This can be shown experimentally in a muscle with parallel fibres such as the sartorius. Levers are arranged resting on each end of the muscle so that when the corresponding part of the muscle contracts a record will be made on a drum. These levers are so adjusted that they write directly one above the other. If both parts of the muscle contracted simultaneously the levers would

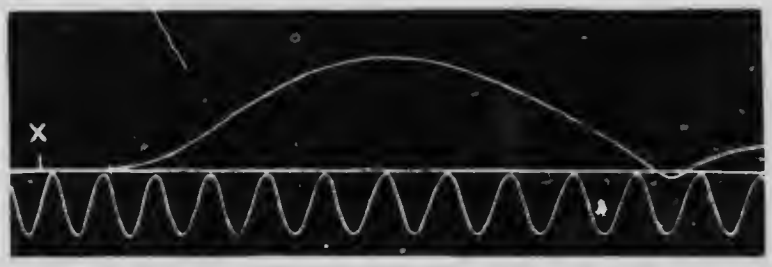


FIG. 6. Record of an isotonic contraction of a frog's gastrocnemius muscle. Stimulated at *X*. Tuning fork tracing, 1 100 sec.

move simultaneously. One end of the muscle is stimulated with a single shock. That end contracts some time before the other, as shown by the records upon the kymograph. By measuring the length of muscle between the levers and determining the time with a tuning fork on the drum, the rate in meters per sec. can be ascertained.

Relaxation is an active process and is not entirely due to the effect of tension upon a muscle which has ceased to contract.

If a frog's muscle is carefully isolated and dipped in olive oil to reduce friction, it will actively relax after contraction, provided it is floated on mercury.

Volume of a Contracting Muscle.—The contraction of a muscle does not change its volume as the following experiment will show.

*__Experiment 7.__ —Two well insulated wires from the secondary terminals of an inductorium are passed through one hole of a rubber stopper. The bare ends of the wire are connected to opposite ends of a frog's gastrocnemius, which is then suspended within a wide-mouthed bottle. The hole through which the wires passes is made water-tight. A glass tube drawn out to a capillary is inserted in the other hole after the bottle has been completely filled with isotonic NaCl solution. The solution should extend a short distance into the capillary portion of the tube. The inductorium is arranged to produce a tetanizing current. In spite of the short-circuiting through the solution, if a sufficiently strong current is used, the muscle can be made to contract.

Determine whether or not any change in volume takes place due to contraction.

__Isotonic Contraction.__ —When a muscle lifts a light load it shortens with little opposition, the tension remaining nearly constant throughout. Such a contraction is literally one with constant or iso-tension, hence called isotonic.

__Experiment 8.—Isotonic Muscle Contraction.__ —The time elements in the contraction and relaxation of a muscle can best be studied by obtaining a record of the muscle on a rapidly moving drum. A gastrocnemius preparation in the moist chamber is prepared for direct stimulation by connecting each end of the muscle to a binding post (Fig. 5). These binding posts are connected in turn to the terminals of the secondary coil. Moist filter paper in the chamber will prevent drying of the muscle.

The muscle lever must be in such a position that it will continue to write on the drum when the muscle contracts. Weight the scale pan sufficiently to permit an appropriate excursion of the lever. Adjust the after-loading screw so that the resting muscle does not support this weight. The electromagnetic signal should be supported on the same stand as the muscle lever and its writing point should be close to the writing point of the muscle lever, in line vertically with it. Both the

electromagnetic signal and the kymograph should be introduced into the primary circuit of the inductorium.

After determining a suitable strength for the stimulating current, record a contraction, using the spring-driven drum. By means of the lever at rest, record below this a base line. Now draw an arc with the lever from the point of maximal contraction to the base line. Obtain a tuning fork record between the base line and signal line.

In this manner secure three or more good records. After the paper has been removed from the drum and the surface fixed with rosin, determine the time for the latent period, contraction period and relaxation period.

If you have time obtain additional records in which the after-loading screw is changed, so that the load is picked up earlier or later than at first.

Discuss the factors which might be involved in production of the latent period.

Isometric Contraction.—Sometimes a muscle tries to lift a load which it cannot budge. The muscle tugs at the weight with an increasing effort, but it cannot shorten, although the tension increases with the effort. Such a muscle maintains a constant length. It is said to undergo an isometric contraction.

In the preceding experiment the force which opposed the contraction of the muscle was essentially a constant one, so that the muscle tension remained nearly the same. In the present experiment the tension is made to increase as contraction proceeds and the change in muscle length is small. It is true that the muscle does not maintain a constant length, but it approaches that condition.

***Experiment 9.** —Fasten the femur of a gastrocnemius preparation in a flat-jawed clamp which has been fixed in the upper part of the tripod. Connect the tendon by a hook to the spring lever, the latter being supplied with a writing point. Connect the secondary terminals of the inductorium with the binding posts of the clamp and the lever. To avoid poor contacts you should connect the binding post of the lever to the tendon by means of a fine wire.

With the muscle under some tension secure a record of a single contraction upon the drum of the spring-driven kymograph.

Secure a second record with an increase in tension. Record an isotonic curve by means of the other lever on the tripod. Stimulation signals and tuning fork records must be shown under each record.

In order to obtain the tension or resistance overcome by the muscle in the isometric contraction, turn the spring over and attach the large scale-pan (weight 20 grams). Then add sufficient weights to stretch the spring to the same extent as occurred in the isometric contractions. Turn the drum by hand to make short abscissae. Mark these lines with the weights.

Compare the latent periods, contractions and relaxations in the isometric and isotonic records.

It is never possible to obtain a purely isotonic contraction, for in . . contractions of this kind, tension does change, though ever s . e. Nor does the purely isometric type exist, in spite of the fact that the muscle does not lift its load and thus appears to be free from any shortening of fibres. Watch such a muscle at work. Some of the fibres do thicken at least in part of their course, therefore they must shorten. They probably do so at the expense of a slight extension of the tendon or of other fibres.

Throughout the activity of the muscles of the body we may have contractions predominantly isotonic or predominantly isometric or these may be combined in various proportions.

EFFECT OF TEMPERATURE ON CONTRACTION.

Chemical changes underlie muscle activity. All chemical processes are influenced by temperature, an increase speeding them, a decrease checking them. Within certain limits we find this true in muscle. There is this difference from inorganic chemical processes—the maximum reaction is soon reached in muscle, increase beyond that temperature reduces more and more the response of the muscle until at about 38° C. in the frog the irritability of the muscle is lost. Muscle is not alone in possessing an optimum temperature for its activities. All tissues have an optimum temperature. This optimum varies in different individual animals, especially cold-blooded animals like the frog. Winter frogs possess a lower optimum than summer frogs.

FIG. 7. Influence of temperature upon contraction. Frog's gastrocnemius muscle. The muscle had been exposed for three minutes in a beaker of isotonic NaCl solution at each temperature. Stimulation at X.

Not only is the height of contraction changed by temperature rise or fall, but the duration of its phases as well. At low temperatures the latent period and duration of the contraction may be several times the corresponding stages at higher temperatures (Fig. 7).

Mammals differ from frogs in maintaining a more or less optimum temperature for their tissues independent of external changes. That is one reason that a mammal is physiologically much more efficient than a frog.

Experiment 10. — Fasten, by means of thread or fine wire, the femur of a gastrocnemius preparation to the short arm of the L-shaped glass rod which has previously been fixed by its long arm in the flat-jawed clamp (Fig. 8). Connect the tendon by means of a fine wire to the pulley of the muscle lever, suitably weighted. Connect the secondary terminals of the inductorium arranged for single shocks with the muscle lever and with the femur. On a rapidly moving surface secure records of twitches immediately after the muscle has been immersed in isotonic salt solution at the following temperatures, each for three minutes: 4° C., 10° C., 15° C., 25° C., 30° C., 35° C., and 40° C. Use the

same strength of stimulus in each case. The records can be superimposed as in the "load and work" experiment.

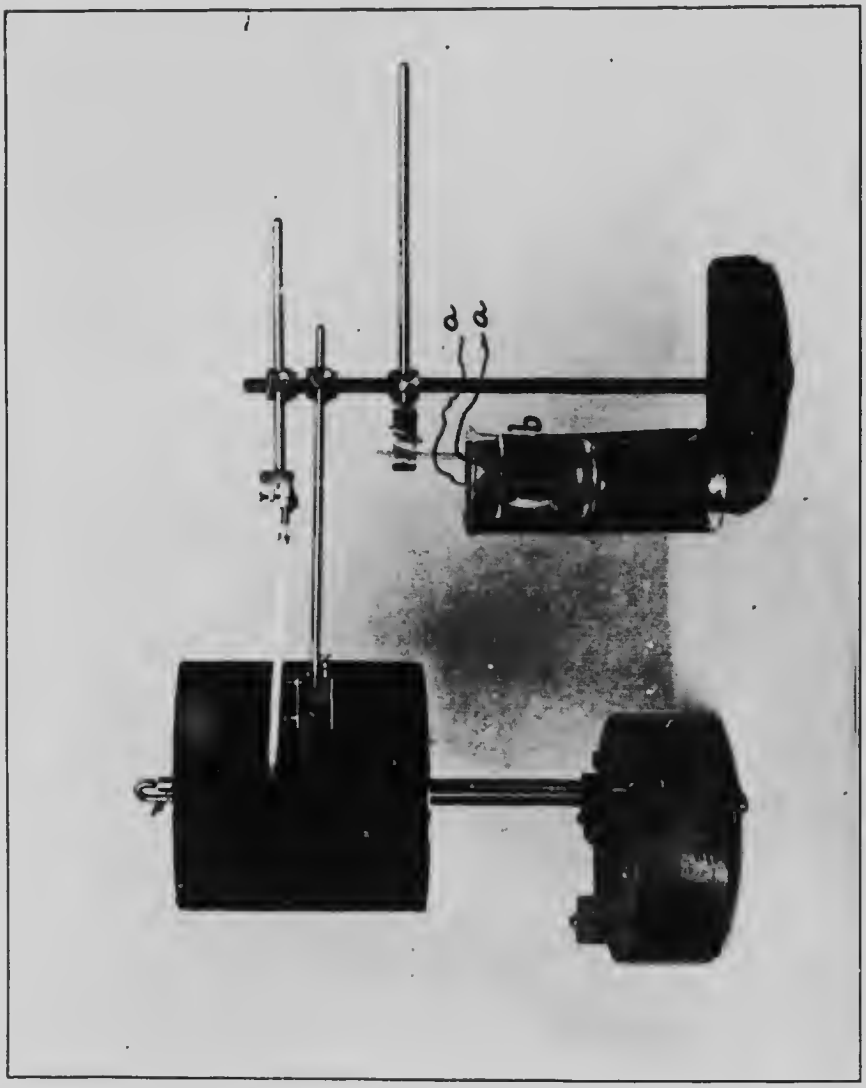


FIG. 8. Apparatus arranged to demonstrate the influence of temperature upon contraction. (*a*) Fine wires to the secondary coil. Beaker (*b*) containing isotonic NaCl solution removed during stimulation.

A tuning fork record is made and the latent period is also shown by the signal magnet.

Discuss the variations in rate and magnitude of the phases of the single contractions at the different temperatures.

The temperature of cold-blooded animals depends very much upon the temperature of the surrounding medium, therefore, it is much more variable than that of the warm-blooded animal. Whatever can be said regarding an optimum temperature for frog's muscle can be applied in a modified form to mammalian muscle. The latter, however, is scarcely ever subjected to as great a range of temperature and the optimum is higher.

If one goes beyond the temperature at which the muscle remains irritable certain changes will take place due to irreversible modifications in the muscle cells. The shortening of the muscle fibre due to coagulation of the muscle proteins can be shown by securing a record, upon a very slowly moving drum, of the effect of a gradual increase of temperature. Describe the appearance of a muscle so treated.

Chemical changes can also be shown by comparing the reaction to litmus paper of the cut surface of the muscle with that of a fresh muscle.

LOAD AND WORK.

The amount of work which a muscle can do depends upon the load which it carries. If a load is light the muscle does not accomplish a great deal, even though it contracts to its full extent. On the other hand too great a load overtaxes the muscle so that the load can scarcely be lifted and thus little work performed. There is then for every muscle an optimum load. With this the muscle is able to do the greatest amount of work at each contraction.

Experiment 11.—Arrange for direct stimulation of a gastrocnemius muscle fastened to the lever of the tripod. Adjust the after-loading screw so that the muscle picks up the load in the large scale pan as it starts to contract. Choose a stimulus which is approximately maximal. The records are to be superimposed upon a rapid kymograph, so that after once starting to record contractions, do not move the relative position of the apparatus. A signal magnet is used to indicate the latent period. One tuning fork record does for the whole set. Using increments of ten or twenty grams, depending upon the

size of the muscle, increase the load until the muscle can no longer lift it. Obtain a record at each increase in the load.

Compare the latent period, contraction period and relaxation period as the load increases.

Calculate the work done in gram-millimeters at each contraction by multiplying the load by the vertical distance through which the load was moved. The latter is to be determined by dividing the height of contraction as recorded on the drum by the magnification of the lever. Now plot a curve of work and load, with work as ordinate and load as abscissa.

What is the relation of the magnitude of the load to the work accomplished?

INFLUENCE OF VERATRINE UPON CONTRACTION.

It is possible with certain drugs to affect one phase of the contraction in a muscle without materially modifying the other. Veratrine and to some extent glycerol and nicotine produce this effect.

*__Experiment 12.__—Prepare one gastrocnemius muscle of a frog without injury to the circulation of the other gastrocnemius. Inject a few drops of 0.1% veratrine acetate solution into the dorsal lymph sac of the frog as soon as the first muscle has been prepared and removed so that the other gastrocnemius may get a sufficient amount of the drug by the time that it is wanted.

Secure a record of the contraction of the muscle first prepared, using the spring-driven kymograph. Now prepare the second gastrocnemius and obtain a contraction in a similar fashion. From time to time take other records to discover when the veratrine effect disappears.

Compare the contractions of the normal and veratrinized muscles.

FATIGUE.

It is well known that excessive use of a muscle reduces its efficiency. In fact all phases of contraction are affected. A careful study of the subject shows the following changes. Repeated contraction at first causes an increase in the response, called the

FIG. 9. Effect of fatigue upon contraction in the frog's gastrocnemius muscle. Every twenty-sixth contraction recorded. 1 is the twenty-sixth, 3 is the fifty-first, etc. Tuning fork, 1/100 sec.

"staircase" effect. A maximum is soon reached and persists for a time. Then the contractions gradually decrease in magnitude until a point is reached where the muscle fails to respond (Fig. 9). In the latter stage of fatigue the muscle may fail to relax entirely between twitches so that it persists in a state of contracture.

It has been shown that certain substances are produced during muscular activity such as carbon-dioxide, mono-potassium phosphate and para-lactic acid. If these substances are injected into a fresh muscle the first effect is to augment the contractile response. Injection of larger amounts reverses the effect so that the response is reduced. In other words it is possible to induce in a fresh muscle the various stages of stimulation and fatigue by means of the waste substances produced in muscle, the effect depending upon the quantity of these substances. These observations lead one to conclude that the accumulation of waste substances in a muscle is a large factor in producing these conditions. This is further substantiated by the fact that increasing the circulation to a muscle delays the onset of fatigue and prolongs the staircase effect.

In frog's muscle fatigue is accompanied by a lengthening of the time for contraction and relaxation, while in mammalian muscle the contractions merely become shorter, with little effect upon the duration. The

using-up of materials stored in muscle also contributes to fatigue, for in starvation fatigue is induced more readily while the feeding of glucose helps to prevent it.

Effect of Fatigue upon Contraction.—Fatigue is partially due to the accumulation of waste substances, therefore any reduction in the circulation will hasten its onset. In the ordinary muscle preparation which has been removed from the circulation, fatigue develops quickly and is easily studied.

Experiment 13.—Prepare a gastrocnemius muscle in a moist chamber. Arrange apparatus for obtaining latent period, contraction period, and relaxation period upon a rapidly moving drum. Place a simple key in the primary circuit so that the muscle can be stimulated either by the key or by the contact on the drum. After-load the muscle with 5 to 20 grams, depending upon its size.

First obtain a record of a normal contraction, then move the stand slightly so that the writing points do not touch the drum, noticing the exact place where they left the paper. Stimulate the muscle with 25 shocks. Immediately move the writing points to their original position and secure a record of a single contraction. Shift the stand again, stimulate 25 times and then make another record. Continue this process until the muscle has become greatly fatigued.*

Allow the muscle to rest 10 minutes. Is there recovery? If so, how do you account for it without circulation?

Make a careful study of the changes in the muscle curve at different stages of fatigue. Each curve should be numbered and identified as 1, 26, 51, 76, etc.

The first effect of the accumulation of fatigue products is an increase in irritability so that greater contractions from the same stimulus may be obtained.

TETANUS.

Voluntary muscular action is caused by a rapid succession of impulses travelling outward along a motor nerve. The muscle

*This movement of the stand must be made with every precaution to see that when the writing point is reapplied to the drum it writes at precisely the same point each time.

therefore does not respond by a simple twitch, but by a more or less sustained contraction. Even the quickest voluntary movements may be a fusion of several simple contractions.

Several proofs have been obtained of the composite nature of such contraction. First it has been found by the use of a sensitive galvanometer that several impulses pass along a nerve which is exciting a muscle even to brief contraction.

Second, a sound can be heard through a stethoscope applied to a muscle contracting voluntarily. This sound is no doubt due to the contractions occurring so close together and thus causing

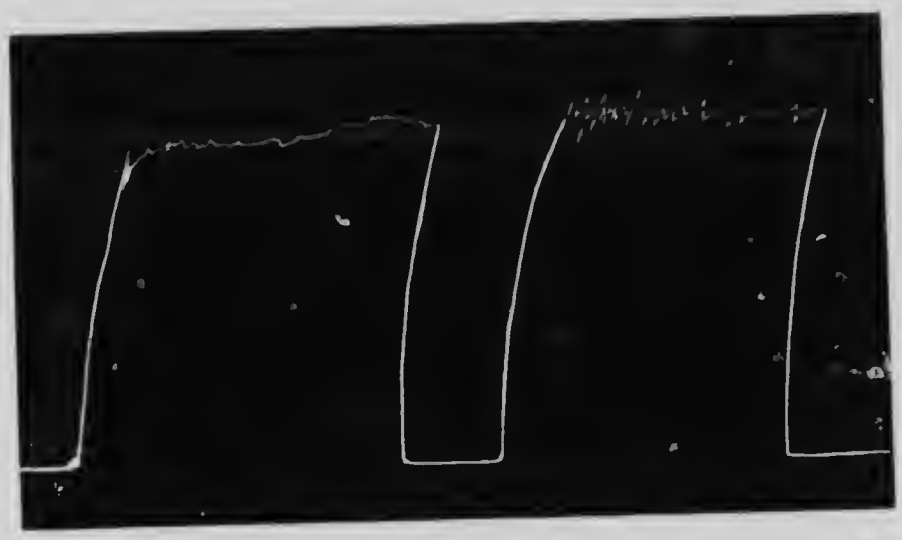


FIG. 10. Graphic record of two voluntary contractions showing their tetanic nature. Made by pull of the index finger against a stiff spring at the end of which a writing point is attached.

vibration of the air. The sound heard is usually an overtone because the actual vibration rate is so near the limit of audibility.

Third, a magnified record obtained by means of a lever, of a muscle, under sustained voluntary contraction, is not perfectly smooth, but contains many fine regularly occurring contractions superimposed upon the main contraction (Fig. 10).

Finally, in order to produce a sustained artificially stimulated contraction resembling a voluntary contraction, it is necessary to send into the muscle a rapid succession of stimuli. Such a contraction is called tetanus.

Summation.—If two stimuli are sent into a muscle in close succession so that the muscle has not completely relaxed before the second stimulus is effective, the latter contraction will be higher than the first. The second contraction adds its effect to that of the first, therefore the greatest summation will occur when

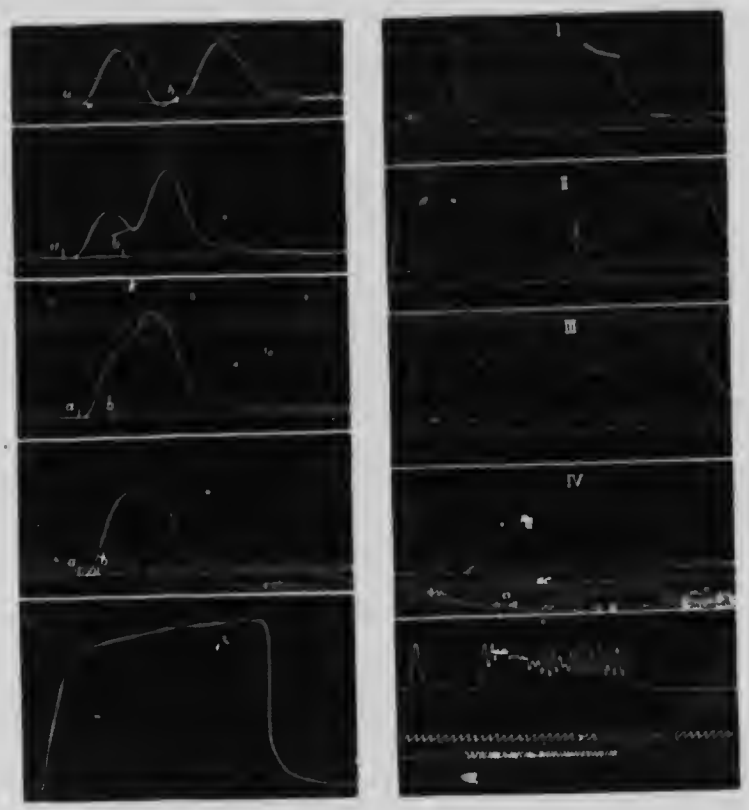


FIG. 11. The effects of successive stimuli on skeletal muscle and cardiac muscle. The vertical marks show where stimuli were introduced. Tracings at bottom are the result of stimuli sufficiently close together to produce tetanus. Notice that the heart shows neither summation nor tetanus. (Compiled from tracings published by T. G. Brodie and Leonard Hill).

the second stimulus is effective during the greatest height of the first contraction (Fig. 11). A certain amount of summation results even though a maximal stimulus is used. A series of rapidly repeated stimuli on account of the summation effects, will cause a much higher contraction than is obtained from a single stimulus.

Analysis of Tetanus.—Voluntary muscle contraction is really a series of twitches occurring so rapidly that relaxation is incomplete or fails to develop. This action can be analyzed best by a study of two simple twitches occurring close together.

Experiment 14.—Arrange a muscle in the moist chamber for stimulation by the inductorium. Set an electro-magnetic signal close to and in line with the writing point of the muscle lever. Connect the signal and the kymograph in the primary circuit. Adjust both contacts upon the disk of the kymograph so that two stimuli are sent into the muscle with each revolution of the drum. With the contacts far apart obtain a record of the twitches when the drum is released. Make a series of records in which the stimuli are closer and closer together, by adjusting the sliding contact. Continue this series to the point of complete summation.

In order to observe the effect of more than two successive stimuli, replace the kymograph by the wheel interrupter in the circuit. Make records on a slower drum, turning the interrupter at different rates. With each new trial increase the rate until complete fusion is obtained.

Next replace the wheel interrupter by the vibrating spring on the inductorium. In this way tetanus can be produced automatically.

Discuss summation and the development of tetanus.

RED AND PALE MUSCLE FIBRES.

Voluntary muscle fibres may be of two kinds, one pale in aspect, the other red. The red fibres are thinner and possess a larger amount of sarcoplasm. They possess a greater number of nuclei and a larger blood supply. Many of the capillaries to red fibres are furnished with dilatations.

Red fibres contract more slowly and fatigue less readily than do pale fibres. The former appear to be adapted for heavy work, while the latter are for rapidity of action.

Some muscles are composed largely of red fibers, such as the soleus in the rabbit. The gastrocnemius on the other hand contains mostly pale fibres. Quite often both types of fibres are present in a muscle, but one predominating.

HEAT PRODUCTION.

A muscle gives up a considerable amount of energy in the form of heat when it contracts. Everyone is familiar with the increased amount of heat after vigorous exercise. Sustained contraction in a large muscle may raise the temperature 1° C. or more, but due to the circulation and the control mechanism, excess heat is distributed and eventually lost.

It has been estimated that 60 to 75 per cent. of the energy used in contraction produces heat, the balance being converted into work. Unpractised movements result in a greater proportion of heat production. Training, therefore, is highly important in getting the greatest work from a muscle.

There are two stages of heat production. The first is immediate or "explosive" in character and will take place in the absence of free oxygen. For example, a muscle which contracts in Ringer's solution from which free oxygen has been removed or in nitrogen, releases heat of the first stage. The second stage develops slowly and is postponed if oxygen is absent. The evolution of heat in this stage continues for a long time after the mechanical response. The amount of heat evolved is as great as that of the first stage.

Heat is extremely important in the maintenance of a constant temperature in mammals. It is this maintenance of a more or less optimum temperature, independent of the external temperature which makes mammals more efficient than the lower animals. Without muscular activity this would be impossible.

RIGOR MORTIS.

Within a few hours after an animal dies, its muscles undergo a pronounced irreversible change. They contract and lose their extensibility. The contraction is not vigorous as can be shown by resisting it with slight tension.

The chemical changes which seem to underlie the process, are the production of carbon dioxide and lactic acid, which by causing coagulation of the muscle proteins, myosinogen and paramyosinogen, bring about the contraction. Incomplete oxidation seems to favour the development of rigor, for if plenty of oxygen is supplied neither rigor mortis nor lactic acid is present.

Heat is also produced as rigidity comes on, which accounts for the warmth which may be noticed sometimes hours after death.

Rigor mortis being due to chemical changes, anything which hastens these causes an earlier development of the condition. Thus the onset is much earlier at high temperatures; low temperatures, conversely, postpone. Fatigue, by speeding up the production of lactic acid and other substances, causes an earlier appearance of rigor.

This coagulation of the muscle in rigor mortis may last for two or three days, when it is terminated by autolysis.

Coagulation of muscle proteins may be caused by heat. If a muscle is gradually heated two stages are found to be present in the development of rigor, the first at 39° C. in the frog and 47° C. in the mammal, the second at 50° C. in the frog and 62° C. in the mammal. The first is due to the muscle proteins proper and the second to the connective tissue substances.

Heat rigor is more complete and does not disappear when autolysis supervenes.

Similarly, clotting of the muscle proteins may be produced by alcohol, chloroform or other substances.

ELECTRICAL CHANGES IN MUSCLE.

Electrical changes accompany the contraction of muscle. This can be shown by connecting a sensitive galvanometer to a muscle which is caused to contract voluntarily or through artificial stimulation. A wave of negative variation travels from the point stimulated, whether it be the motor end plate or one end of the fibre in contact with stimulating electrodes. All other parts of the muscle are positive in relation to this wave. Suppose as in Fig. 12 the galvanometer G is connected to the muscle at 2 and 3 and the stimulating electrodes are at 1. A single contraction is initiated at 1. This contraction is preceded by a negative electrical condition which first reaches 2 (A), at that moment 2 will be negative in relation to 3, causing a deflection of the galvanometer. The wave continues to move onward, quickly passing 2 and soon reaching 3. When 3 is reached (B) it will become negative to 2. There will therefore be a reversal of the galvanometer. The gal-

vanometer moves first in one direction and then in the other. This is called a diphasic variation. The electrical change attending the contraction of muscle is designated the ACTION CURRENT.

When a muscle is cut or injured, that particular region becomes electronegative to other parts of the muscle which are uninjured. If connected by a conductor a slight current will be set up, called the CURRENT OF INJURY.

THE NERVE-MUSCLE AS A RHEOSCOPE.

The electrical changes set up in muscle can be detected by a vigorous nerve-muscle preparation. The nerve is permitted to touch the muscle at a negative region and a positive region. In

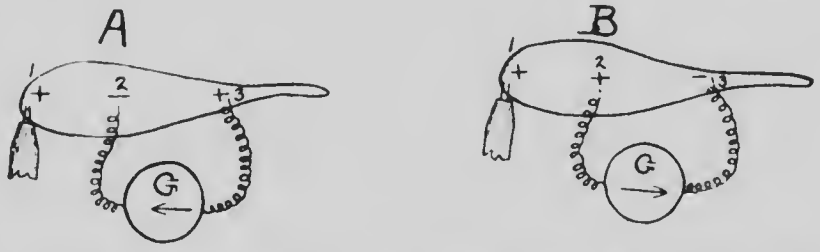


FIG. 12. Diagram to show the passage of an electrical change over muscle. When a muscle is stimulated at one end (*1*) a negative electrical change is started, passing along the muscle, just preceding the contraction. A galvanometer (G) is connected with the muscle by two leading off electrodes, *2* and *3*. As the negative change reaches *2* (A) the current will pass through the galvanometer from *3* to *2*. But when the negative variation reaches *3* (B) the current is reversed. This is called the diphasic variation.

a freshly cut muscle at the time of making the second contact, the rheoscopic muscle will twitch. In a muscle stimulated through its nerve the rheoscopic muscle will twitch at each twitch of the tested muscle. The explanation is that the electrical changes in the tested muscle are strong enough to initiate impulses in the nerve of the rheoscopic preparation. However, due to the poor condition of the rheoscopic nerve-muscle, the experiment frequently fails.

The sciatic-gastrocnemius preparation to be used as a rheoscope should be taken from a very healthy frog and dissected out with the greatest care. Abuse of the nerve may destroy its sensitiveness so that a new preparation must be made.

Experiment 15.—Current of Injury. One end of a gastrocnemius muscle is cut across transversely. This and the rheoscopic preparation are placed side by side on a clean glass plate. The rheoscopic nerve, near its muscle, is placed in contact with the uninjured surface of the muscle to be tested, the rest of the nerve being held apart by means of a glass hook. Now the tip of the rheoscopic nerve is allowed to touch the injured surface of the muscle to be tested. A contraction of the rheoscopic muscle should result at the moment of the nerve's contact with the injured surface.

Current of Action.—Two nerve-muscle preparations are laid side by side on a glass plate. The nerve of the one which is to serve as the rheoscope is placed lengthwise against the muscle to be tested and is then lifted by a glass rod at its middle so that it is in contact with the muscle only at both ends. The nerve of the muscle to be treated is next stimulated by single shocks. With each twitch the rheoscopic muscle should respond. If the test muscle is tetanized, the rheoscopic muscle should be tetanized.

A secondary twitch can also be obtained by laying a freshly prepared nerve upon a beating frog heart. The two regions of contact for the nerves are the base and the apex, the middle part of the nerve being held away from the contracting muscle as before. Each beat should produce a twitch of the gastrocnemius.

CHAPTER III.

SMOOTH MUSCLE AND CILIATED CELLS.

SMOOTH MUSCLE.

There are two types of muscle which are independent of voluntary action, cardiac muscle which is striated and smooth muscle which lacks transverse striations. Smooth muscle fibres are elongated and pointed at both ends. They are generally collected into bundles. These bundles are attached at their ends to the membranous parts where they occur.

The importance of plain or smooth muscle will be realized when its wide distribution is considered. It is found in the lower half of the gullet, the stomach, and intestines. In the alimentary canal it occurs not only in the muscular coat, but as a layer in the mucous membrane and in the villi. It is present likewise in the trachea, bronchial tubes, bladder, ureters, uterus, glandular ducts, genital organs, spleen, ciliary muscle and iris. The contractile element in blood vessels consists of plain muscle. Thus it is by means of smooth muscle that blood is shifted from one part to another and emergencies are met. This adjustment is controlled largely through the nervous system.

Movements of the alimentary canal, mixing of the digestive fluids with the food and onward propulsion of the contents, are brought about by smooth muscle. Its function in regulating blood supply and the mechanics of digestion are alone sufficient to demonstrate its necessity in the organism.

Extensibility and Elasticity.—The extensibility and elasticity are similar to those properties in striated muscle. However in the stomach, bladder and uterus the extensibility is much greater. The capacity of these organs depends upon the power of extension, for when empty they are contracted to a small size.

Irritability.—Electrical stimulation is relatively ineffective

with plain muscle, strong currents being required. Induction shocks are less effective than galvanic.

Mechanical stimulation in the form of stretching is by far the most adequate form of stimulus, at least among artificial stimuli.

Contraction.—The LATENT PERIOD for smooth muscle response may be as much as one hundred to five hundred times that of striated muscle. In the frog's stomach it is from one to ten seconds; for the cat's bladder, 0.25 sec., and for vascular muscles, 0.3 to 0.5 sec.

The CONTRACTION PERIOD may be as much as 15 to 20 seconds for the frog's stomach. The amount and duration of the contraction depends upon the stimulus. In the frog's stomach a single contraction may decrease the stomach by 45%, while tetanus may reduce it 59%. Although the contraction may be large, it is much gentler than that of voluntary muscle.

RELAXATION PERIOD.—The time consumed in relaxation is much longer than that for contraction. In the frog's stomach it is said to be from 60 to 80 sec.

Summation and Tetanus.—Two successive stimuli properly spaced will cause summation. A series of stimuli will cause tetanus. To do this they need not be very frequent. A stimulus every five seconds is sufficient in the frog's stomach.

Tone.—Smooth muscle possesses the power of remaining in a condition of persistent shortening for long periods. Thus hollow viscera can adapt themselves to their contents. Tone can be varied in different ways. Cooling increases it, while heat decreases it. The most important means of controlling tone seems to be through the extrinsic nerve supply, e.g., stimulation of the vagus increases stomach tone, cutting the vagus produces flabbiness in the same organ. Throughout life the walls of the arteries resist a high pressure, this resistance being controlled by the nervous system.

Rhythmicity.—Smooth muscle in certain parts of the body is able to undergo rhythmical contraction. These are the alimentary canal, ureter, bladder, spleen and blood vessels. In the stomach and intestine rhythmical activity is induced by tension. The rate of these contractions is somewhat as follows: stomach, 3 per min., intestine 12 per min., spleen, 1 per min.

In this connection it is important to make it clear that these rhythmic movements are superimposed upon whatever condition of tone prevails.

The intrinsic nerve supply seems to preside over rhythmicity.

Experiment 16. Contraction of Smooth Muscle.—An L-shaped glass rod is fastened by a clamp so that it can be used to support a piece of intestine about 2 cm. long in a small beaker of Ringer's solution. The latter is maintained at a temperature of 37-38° C. by immersing the beaker in a large tin can used as a water-bath. Air is allowed to bubble slowly through the Ringer's or Locke's solution[1] by fixing a small glass tube connecting with the compressed air supply, so that its opening is near the bottom of the beaker. When all is ready one end of a piece of intestine, cut from a rabbit which has just been killed,[2] is attached to the hook of the glass rod and the other to a heart-lever which is arranged to record on a slowly-moving drum. Contractions of the longitudinal muscle will produce movement of the lever.

After recording several normal contractions try the effect of temporarily reducing the air supply. Later allow the temperature of the Ringer's fluid to fall to that of the room and note the changes in tone and rhythmic contractions. Finally study the effect of stimulation by electricity. When ready to stimulate remove the Ringer's solution for a short time in order to prevent short circuiting. Try make and break, direct and indirect currents. Later attempt to tetanize the segment. On account of the long latent period do not run the stimuli too close together when single shocks are used.

Observe the direction in which a contraction travels when a piece of intestine is pinched with forceps. In order to remember which is the oral end a ligature should be tied around that end when the segment is removed. Do the contractions always move in the same direction from mechanical stimulation? Is this type of stimulus more or less adequate than the electrical?

[1]The water with which these solutions is made up must be pure distilled. It has been our experience in this laboratory that chlorinated lake water cannot be used to distil from. We are compelled to use spring water from which to prepare the distilled water.

[2]The intestine, after removal from the rabbit, is preserved in cold Locke's solution through which oxygen or air is occasionally bubbled.

CILIATED CELLS.

In addition to the muscular tissue of the body there are other contractile elements, viz., ciliated cells.

The purpose of ciliated cells is to remove mucous and foreign bodies from passages. They are therefore found in the mucous membranes of the trachea, larynx, bronchi, nose, lachrymal duct, uterus, Fallopian tubes, tubules of the epididymis, Eustachian tubes and middle ear.

There is a bunch of cilia to each cell. Not only the cilia of the same cell, but those of neighbouring cells, move in a definite sequence, so that there is co-ordination.

The power of these cilia acting together is considerable. It is estimated that the cilia on 1 sq. cm. of the frog's oesophagus can move more than 300 grams.

Warmth increases the activity of cilia; cold decreases it. Drugs also influence their movement

Experiment 17.– Ciliary Action.—Remove the lower jaw of a frog and slit the oesophagus. Spread open the oesophagus with pins. Determine the time required for a bit of cork placed on the roof of the mouth to be moved one centimeter down the gullet. Keep the mucous membrane moistened with salt solution. Pour isotonic NaCl solution, warmed to 30° C. but no more, over the membrane, drain it off, then determine the rate of movement of a bit of cork. After a time blow ether vapour over the membrane and again determine the rate of ciliary action.

CHAPTER IV.

PHYSIOLOGY OF NERVE.

A nerve fibre is constructed for the conduction of impulses. These impulses may have their origin through voluntary acts or through external stimuli. It is largely through the office of nerve fibres that the various parts of complex organisms are correlated. Nerve fibres are similar to the wires of a telegraph system which correlate the different parts of a country. Cut the wires and unity of action is upset for a time. Cut the nerves to an organ and it loses its coordination with the rest of the body.

Just as in wire a nerve fibre can conduct in both directions. This can be shown by a galvanometer. Stimulation of a fibre in the middle of its course will cause a deflection of a galvanometer placed at either end. The following experiment also proves the truth of the statement.

FIG. 13. Dissection of the frog's gracilis muscle to demonstrate that a nerve conducts in both directions. Stimulation at (*a*) will cause contraction of both (*a*) and (*b*) or *vice versa*.

Experiment 18.—Expose the inner surface of a gracilis muscle in a frog. It can be seen that the two portions of the muscle are fed by branches of the same nerve. Separate the two parts of the muscle without injury to the nerves. It is possible to do this so that the nerve is the only connection between the two parts (Fig. 13).

Stimulation of either branch of the nerve even applied at the finer branches will cause contraction of both muscle masses. Conduction must travel in one direction in the stimulated branch and in the opposite direction in the other. Now if the opposite branch is stimulated, both muscle masses contract as before. In this case the impulse must pass in a direction

opposite to that in the first stimulation. Therefore an impulse can pass in either direction in a nerve.

Nerve fibres are classified into two groups, depending upon the direction in which the impulses usually travel. Those which carry toward the brain and cord are called afferent, while those carrying impulses in the opposite direction are designated as efferent. The latter activate muscles and glands. Either type of fibre could conduct in both directions, but in their normal location, conditions are so arranged that impulses start only at one end—in afferent nerves at a sensory ending, in efferent nerves at the central end. Even though you dissect out an efferent nerve so that it can be stimulated at its peripheral end, the impulse travels only as far as the synapse or connection with the next nerve cell or neurone. In other words the synapse allows the passage of impulses in one direction only—in sensory nerves, inward; in motor nerves, outward.

When we consider the necessity of careful insulation to prevent the spreading of current in contiguous wires, it is extremely interesting to know that there is complete isolation of impulses travelling along contiguous nerve fibres. If this were not the case, endless confusion would result where great numbers of both afferent and efferent fibres are closely bound up in the same nerve trunk.

Summation of Inadequate Stimuli.

An impulse passing along a nerve produces a change in the nerve whether it is sufficient to cause response in an end organ or not. Whatever the change may be it is in the direction of that which will evoke response. That this is true is shown by the fact that a stimulus which is insufficient when applied once, may bring the end-organ into action if applied several times in close succession.

Experiment 19.—Choose a stimulus which is just below the threshold of that necessary to cause contraction of a muscle through its nerve. Now apply this stimulus in rapid succession.

Velocity of the Nervous Impulse.

The time required for the passage of an impulse along a nerve trunk can be determined by means of a sensitive galvanometer or

by the method described in the experiment below where the response of an end organ is used.

Helmholtz, by means of a myogram of the thenar muscle when stimulated through the median nerve at the axilla and then at the wrist, estimated that the rate in man was 30-35 meters per second.

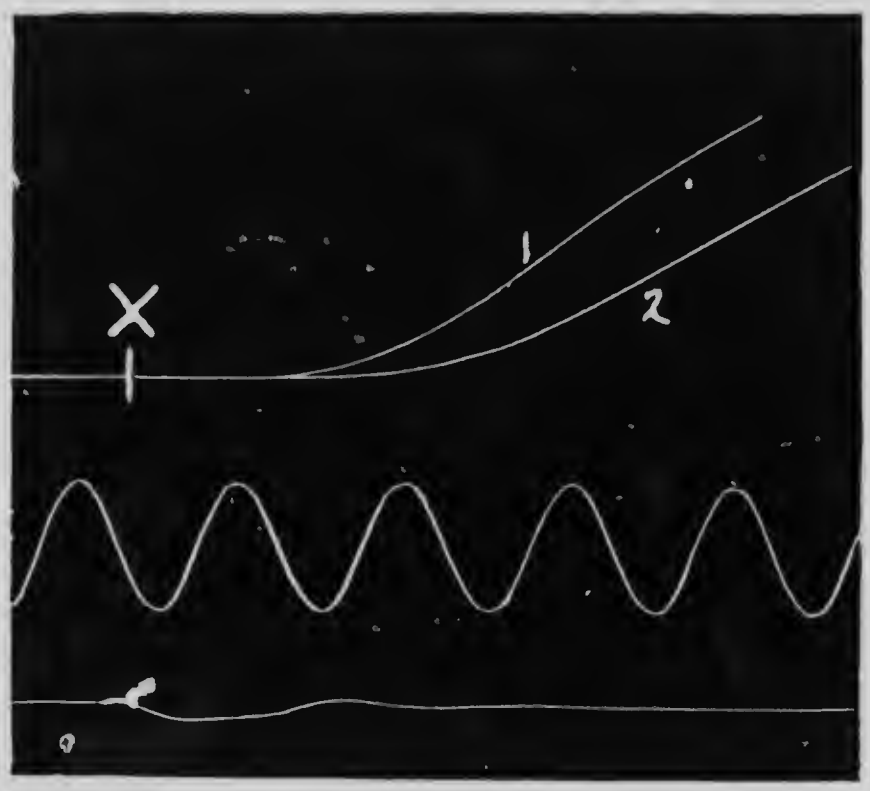


FIG. 14. Tracing to show rate of conduction of the nervous impulse. (1) Contraction of the gastrocnemius when the electrodes are near the muscle; (2) contraction resulting from stimulation at the far position. Stimulation at X.

Piper, by means of the string-galvanometer, has studied the same nerve and found the rate to be as high as 125 meters per second.

Non-medullated fibres seem to conduct more slowly, the rate being as low as 8 meters per second.

Experiment 20.—Although the rate of transmission of the nerve impulse is rapid, it can be determined by a high speed kymo-

graph. Prepare a gastrocnemius muscle with the nerve as long as possible. Clamp the femur in the moist chamber, then lay the nerve across two sets of cork-held electrodes. One pair should be close to the muscle and the other as far away as possible. Avoid stretching the nerve. The electromagnetic signal is placed in line with the point of the muscle lever. The signal and kymograph are connected in the primary circuit. Adjust the muscle lever so that only the beginning of the contraction is recorded.

Using the base line each time, record the interval of the latent period, first for the far position of the electrodes and then for the near position. This can be done by changing the wires of the secondary over to the electrode desired in each case. Estimate the duration of the latent period by means of a tuning fork tracing (Fig. 14).

The time required for the passage of the impulse along the nerve is the difference between the latent periods resulting from the far and near stimulations. The length of nerve traversed may be taken as the distance between the nearer points of the two electrodes.

Determine the velocity of the nerve impulse in meters per second. Make four or more sets of records. Tabulate the results.

FACTORS INFLUENCING NERVE FUNCTION.

Mechanical.—If a nerve is stretched or compressed its power to conduct may be lost. When the compression is neither too severe nor too prolonged conductivity may be re-established. Compression over a broad area requires much greater force to paralyze than when over a narrow region. The first effect of compression, frequently, is to increase the excitability. This stage, however, is quickly passed.

Thermal.—Beginning with 0° C. the rate of conduction increases as the temperature is raised. Below 0° C. conduction is suspended. At the higher limit of conduction, about 47° C. in the mammal, due to coagulation of the proteins the nerve may become permanently paralyzed.

Chemical. Many drugs lower or suspend conductivity when applied locally. Ether, chloroform, chloral, cocaine, phenol and alcohol have this power. Conduction will return in time if the drug does not act too long. Lack of oxygen will also destroy conductivity in time.

*__Experiment 21. The Action of Carbon Dioxide, Ether, and Chloroform upon Nerve.__ A gastrocnemius muscle with its nerve attached is fastened to a lever so that its contraction can be recorded. The nerve is passed through the two openings in the bottom of the gas chamber. These holes are made air-tight with a paste made of kaolin and isotonic NaCl solution. One pair of electrodes is set so that the nerve within the chamber can be stimulated, another touches the nerve at the end farthest from the muscle. Minimal break shocks are to be used for stimulation. Carbon dioxide is passed through the chamber, stimuli being applied from time to time at both electrodes in order to discover changes in the response.

Wash out the carbon dioxide by passing air through the chamber. When the nerve is again normal blow ether vapour through until it affects the nerve, or if necessary paint the nerve with ether. Do not prolong anaesthesia too long or it will be difficult for the nerve to recover. In a like manner study the influence of chloroform.

Electrical. When a direct current is passed through a nerve the excitability around the kathode is increased, while around the anode it is decreased. The changed condition produced by the direct current is called electrotonus—katelectrotonus in the first case and anelectrotonus in the second case. Midway between the two electrodes there is a point where the excitability of the nerve has not been changed. This indifferent point moves toward the kathode as the current is increased and in the opposite direction as the current is decreased. This can be shown by stimulating the nerve along its course during the passing of the direct current.

Experiment 22. Electrotonus in Nerve.—When a direct current is to be passed through a tissue for any length of time non-polarizable electrodes must be used as the ordinary electrodes are easily polarized, thus altering the efficiency of the current. The former consists of two boot-shaped porous

receptacles which are partly filled with a 10% $ZnSO_4$ solution. A piece of pure zinc attached to the source of current is inserted into each boot. The boot is kept moist on the outside by

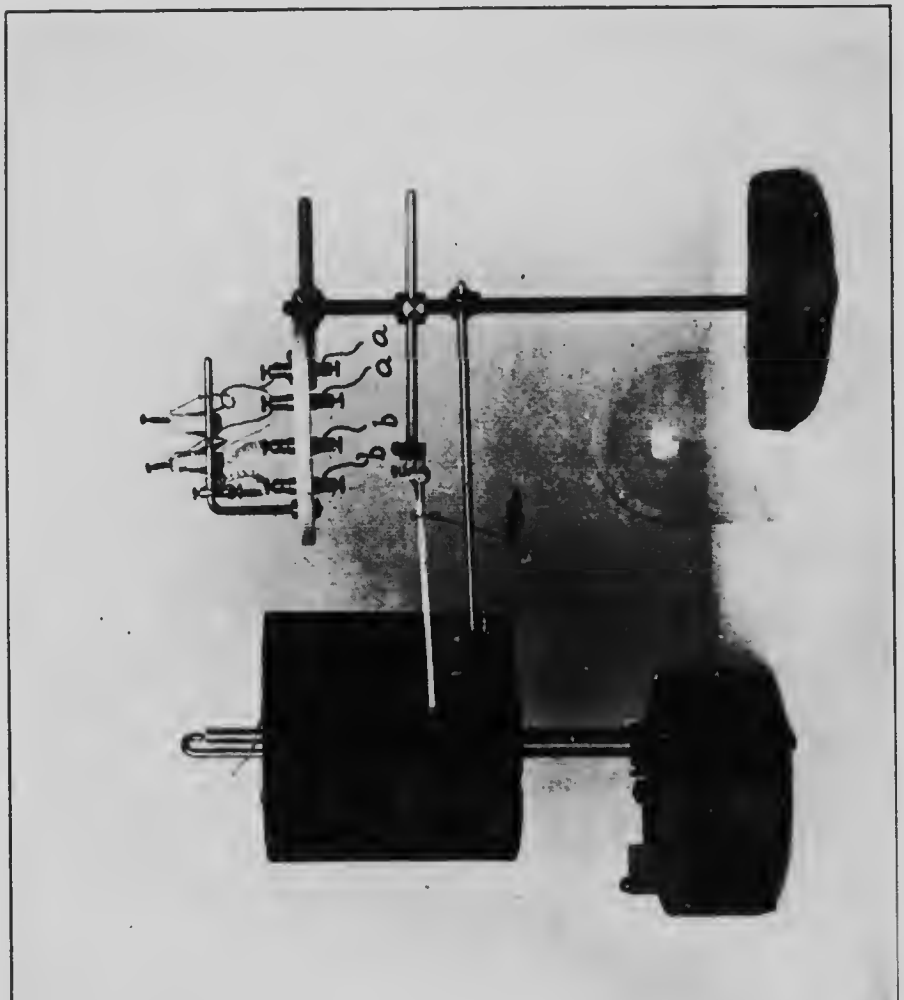


FIG. 15. Apparatus for demonstrating electrotonus in nerve: (*a*) wires connected with the direct current; (*b*) connections to the secondary coil, the primary circuit of which is independent of the circuit fed at *a*.

isotonic NaCl solution, the porous material acting as a barrier to the passage of zinc sulphate during the time usually consumed in an experiment.

The boots must be kept clean. Each time after using they should be thoroughly washed with water and then soaked in isotonic NaCl solution. When in use the $ZnSO_4$ must not be allowed to spill over as it is injurious to the nerve.

The boots are held in position by the clips on the horizontal rod of the moist chamber base (Figs. 15 and 16).

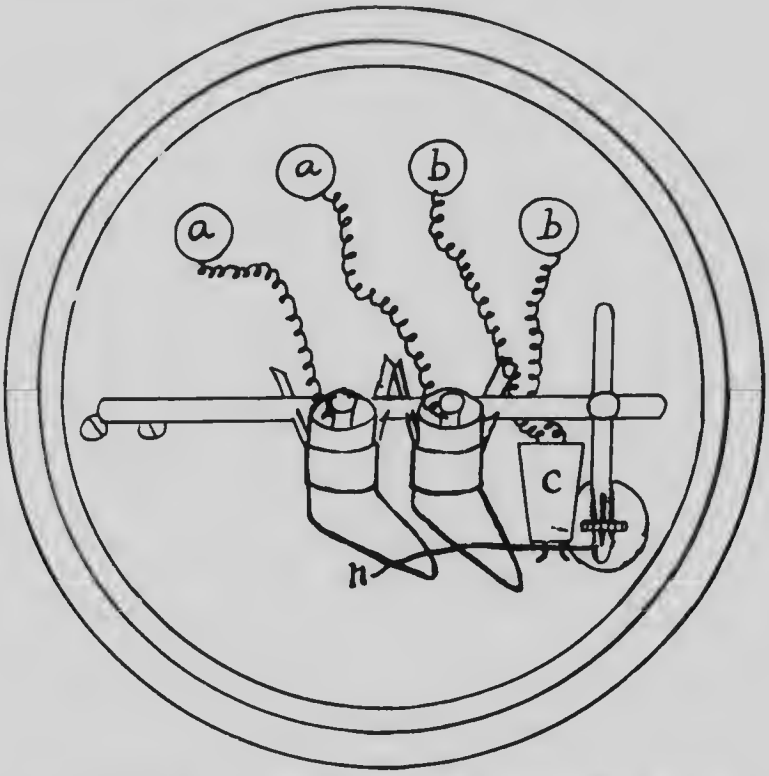


FIG. 16. Top view of moist chamber in the preceding figure, showing the arrangement of the electrodes (*c*) from the secondary coil in relation to the boot electrodes. Nerve (*h*) is shown across the two sets of electrodes. (*b*) connects with secondary. (*a*) connects with direct current supply.

A gastrocnemius muscle, with a long fresh nerve attached, is arranged in a moist chamber with the nerve lying on a pair of electrodes in cork near the muscle and a pair of boot electrodes farther out. Adjust the induction coil so that a break shock just causes contraction of the muscle through the electrodes in cork. Record the height of this contraction either on a

slow or stationary drum. Next connect the boot electrodes to the current from the rheostat so that the kathode is nearer the muscle. Try two volts at first. Determine which boot is kathode and which is anode by dipping the two wires into NaCl solution; bubbles of hydrogen will issue from the kathode. While the constant current is flowing through the nerve by way of the boot electrodes, stimulate the nerve with the same break induced shock as before. Then change the wires to the boots so that the anode is nearer the muscle, and stimulate with the same break induction shock.

Increase the direct current and test the condition at the kathode and anode as before. A certain strength of current is necessary to produce marked changes in the region of the kathode and anode. It is possible to completely block the passage of impulses by means of a direct current in the anodal region. The reverse effect is obtained in the kathodal region.

These electrotonic changes in the nerve only last for a short time after which the reverse effect may occur. By reversal it is meant that, e.g., around the kathode the stage of increased excitability finally gives way to a condition of lowered excitability. This reversal developes more quickly with a strong current.

DIRECT CURRENT STIMULATION.

A direct current will produce stimulation of a nerve or muscle when there is a quick change in intensity. This occurs at the make or break, but not during the passage of the current.

It can be shown that the stimulus caused by making a direct current arises at the kathode, while that caused at the break arises at the anode. If the kathode and anode are placed far apart on a motor nerve the latent period for contraction of the muscle is greater at make when the kathode is in the far position, than when it is in the near position. Likewise at the break the latent period is greater when the anode is in the far position than when in the near position. Therefore the galvanic make stimulus is kathodal and the break stimulus anodal.

Electrotonus, Kathodal and Anodal Contractions in the Heart. Because of the regularly occurring contractions the heart

is an admirable muscle for the study of electrotonic changes as well as the effect of kathodal and anodal stimulation.

***Experiment 22a.** Destroy the brain of a frog and expose the heart with as little hemorrhage as possible. Two boot electrodes are prepared by attaching a tiny strip of cotton wool to each at the toe. The cotton is soaked with isotonic NaCl solution and serves as a contact. The kathode is made to touch the frog's mouth while the anode touches the ventricle. Now if the current is closed during contraction of the ventricle, it will be seen that at the moment of closing the latter fails to contract in the region of the anode. This is due to the anelectrotonus set up around the anode. Of course only a limited area will be affected, so that very close observation will be necessary.*

Now if the current is broken during diastole the ventricle will contract slightly in the neighbourhood of the anode. This is the anodal opening contraction.

The condition produced in muscle by the kathode can be studied by changing the electrodes so that the kathode touches the ventricle and the anode the mouth. At the closing of the circuit the ventricle contracts in the region of the kathode, if it is in diastole. This is called the kathodal closing contraction. If the current is passing during systole there may be a small area of heightened contraction around the kathode. This is due to the katelectrotonus.

The response at make and break depends upon the strength of current and upon whether the kathode or the anode is next to the muscle.

With the KATHODE NEAR THE MUSCLE a contraction is obtained at the make whether the current be weak, medium or strong. But with this position of the kathode, contraction is obtained at the break when only a medium current is used. The break stimulus always being less effective than the make, its effect, when a very weak current is used, will be below the threshold of stimulation. On the other hand when very strong currents are used, the break stimulus being anodal, is blocked in the nerve by the development of electrotonic changes, so that the impulse does not reach the muscle.

*The local contraction or failure of contraction is revealed by a change in color due to variations in the blood content of the heart tissue at the affected part.

When the ANODE IS NEAR THE MUSCLE, a weak current causes a contraction at the make, but has no effect at break, not having reached the threshold. With medium currents both make and break are effective. In the case of strong currents, the break is effective because it occurs at the anode, which is near the muscle with nothing to block it. The make occurring at the kathode has to traverse the whole region of the anode at the time when the anode produces its greatest block to conduction, i.e., immediately after making the current.

We do not know the nature of the nerve impulse, but whatever it is, an electrical change always accompanies it. This can be shown by connecting a sensitive galvanometer to the nerve. For the same reasons as in the muscle, the galvanometer will show a DIPHASIC VARIATION, that is a deflection, first in one direction and then in the opposite direction.

METABOLISM IN NERVE.

The metabolism in nerve is so slight that it is very difficult to obtain indications that such changes are going on.

So far it has been impossible to demonstrate the production of heat in nerve. Hill has used a thermo-electric couple sensitive to $\frac{1}{100{,}000{,}000}$ °C. without a positive result.

It is possible to show that living nerve produces CO_2. Tashiro has found that the quantity of CO_2 can be more than doubled in active nerve. This alone indicates metabolic changes. The necessity of oxygen is a further proof of metabolism.

However nerve must use an infinitesimal amount of energy in its work, not only in view of the above considerations, but because it is very difficult to fatigue.

A nerve has been stimulated continuously for ten hours and still transmitted impulses, as could be shown by a galvanometer or by preventing the fatigue of the muscle which is done by blocking the contraction with curare, cold or a narcotic. Then at the end of the time, the block being removed, the muscle contracted.

Experiment 23. The Resistance of Nerve to Fatigue.—Arrange two nerve muscle preparations so that they support levers in such a position that they can write one above the other upon the same drum. Both nerves are to lie side by side

on the same pair of electrodes. Abolish the conductivity of one nerve by keeping it moistened with ether. Keep the nerve moist by improvising a moist chamber from filter paper. Stimulate the nerves by means of a tetanizing current and obtain a record of the contraction upon a slowly moving drum. If sufficient ether is used the muscle on that side should not contract. As soon as this stage is reached, tetanize both nerves until the active muscle is exhausted. When this stage is reached, wash away the ether, with isotonic NaCl solution, continuing the stimulation. The muscle upon the treated side should soon begin to contract.

It can be shown further that the fatigued muscle will respond by direct stimulation. Therefore if nerve fibres are still carrying impulses as is shown by response of the other muscle, the seat of fatigue must be between nerve fibre and muscle fibre, or at the end plate. The end plate according to this becomes fatigued much earlier than the muscle fibre and thus saves the fibre from excessive fatigue.

THE ALL-OR-NONE PRINCIPLE IN NERVE AND MUSCLE.

Stimulation of a muscle either directly or through its nerve produces a step-like increase in response, when successively increasing stimuli are used. When once a step has been established further increase of the stimulus causes no increase of contraction until a new step is reached. The maximum contraction of the whole muscle is frequently reached in a few definite steps. The number of steps is never greater than the number of motor nerve fibres supplying the muscle; the most plausible explanation is that each nerve-fibre when excited causes the contraction of all the muscle fibres which it innervates. A nerve fibre supplies a large number of muscle fibres, 150 to 200 in the CUTANEUS DORSI of the frog.

From a study of the CUTANEUS DORSI of the frog Lucas reached the conclusion that as a rule the passage from one step to another takes place without any contractions of intermediate size. The fibres usually contract either not at all or to an extent which is nearly maximal. This is the all-or-none principle. According to this when a muscle mass is giving a minimal or sub-maximal contraction only a proportionate number of fibres is being called into action, the rest remaining inactive.

SECTION II A.

CHAPTER V.

CARDIAC MUSCLE.

Cardiac muscle resembles voluntary muscle in its cross striation. The individual cells, however, are joined end to end and many of them interconnected by fork-like projections. Anastomosis is so complete that there is continuity throughout the myocardium of both auricles and ventricles. Even in mammals there is evidence of a complete syncytium in the form of muscle-fibrils passing from cell to cell. The connection between the auricles and ventricles consists of a narrow bundle of somewhat modified muscular tissue.

Cardiac muscle contracts much more rapidly than smooth muscle, but more slowly than voluntary muscle.

The heart is not only involuntary in action, but undergoes rhythmic contraction throughout life. This rhythmicity is so well established that it is impossible to tetanize a heart by artificial stimulation. Herein lies the most essential difference between cardiac and skeletal muscles. If a stimulus is sent into the heart during systole there is no result, but when stimulated during diastole an extra contraction follows. The whole period of systole is refractory to stimulation. In case an extra contraction is induced during diastole, the relaxation which follows is prolonged to compensate for the pause which was missed.

All-or-none Principle. The all-or-none principle applies to cardiac muscle as well as to voluntary muscle, in fact it was first observed in the former. Due perhaps to the syncytium-like structure of the heart muscle, all of its fibres have the same threshold of stimulation, so if one contracts all contract. Moreover as in voluntary muscle, those fibres which contract, do so to their full extent if at all. Therefore the whole heart contracts to the maximum or not at all.

Action of Cations on Cardiac Muscle. The ions found in the blood play a very important role in the maintenance of the normal heart beat. Remove one of these essential ions and the heart finally stops beating.

The SODIUM IONS not only play a large part in the maintenance of the normal osmotic pressure but when present alone, they produce relaxation of the heart.

POTASSIUM IONS are present in much smaller quantity than sodium and are not so essential. They tend to produce relaxation and when increased above normal the heart rate is reduced, then finally the heart stops in extreme diastole.

CALCIUM IONS are absolutely essential. Excess of calcium produces calcium rigor, that is the heart finally stops in systole. Calcium is supposed to promote contraction, while sodium and potassium aid in relaxation.

Temperature Effects. Although the mammalian heart is so protected in an organism with a relatively constant temperature, it is influenced by thermal changes if subjected to them. In fact it acts like the heart of a frog or turtle, except that it does not withstand the effects of a lowered temperature long. The rate increases with increase of temperature up to a certain point. Below 17° C. the mammalian heart does not beat, likewise above 44° C. contraction soon ceases. The heart of cold-blooded animals responds at lower temperatures.

NERVOUS CONTROL OF THE HEART BEAT.

Inhibition. It can be shown by stimulation that the vagus inhibits the activity of the heart. Weak stimuli may merely decrease the magnitude of the beat. Stronger stimuli slow or even stop the contractions. Even with stimulation intense enough to stop the heart, after a time the beats begin to break through or escape. In the frog, because the vagus and sympathetic fibres run together in the same trunk, both are stimulated. The stimulus must be chosen so that the vagus is affected more than the accelerator or else there will be either no change or an increase in rate. This is done by gradually increasing the stimulation until vagal effects predominate.

Normally in the frog the vagus carries very few impulses to the heart, therefore cutting the vagi has little influence upon the rate. But in mammals the vagi continually hold the heart in check so that cutting of these nerves permits an increase in rate.

The vagus is reflexly called into action by sensory stimulation. A good example of this is obtained by sharply striking the abdomen of a frog with a blunt instrument. Frequently the heart may be stopped or slowed in this way.

An increased blood pressure will also reflexly act along the vagus to slow the heart. This is due to the stimulation of afferent fibres ending in the arch of the aorta. High pressures may also directly stimulate the vagus centre.

The vagus seems to exercise a beneficial influence on the heart, tending to oppose acceleration and overwork of the heart. The vagus is most effective in animals which are accustomed to strenuous muscular exercise and therefore great activity of the heart.

Acceleration. Certain fibres, which are called accelerator fibres, oppose the action of the vagus. They are found in the cervical sympathetic below the inferior cervical ganglion. Stimulation of these fibres increases the rate of the heart and sometimes the amplitude as well. On the other hand cutting of these fibres permits the vagus to have full sway (in the mammal) and thus the heart beat becomes slower. The accelerators are called into action reflexly, although it is difficult to say just what stimuli will affect the accelerators and what will call the vagi into action.

The arrangement of opposed fibres, i.e., accelerator and inhibitor, renders quick adjustment to changing requirements easier.

Experiment 24. Frog's Heart.—Pith a frog, destroying the brain only. Expose the heart by slitting the upper part of the abdomen lengthwise. Avoid injury to the anterior abdominal vein by cutting a little to one side of the mid-line. Observe the beating of the heart, especially the rate. Then open the pericardium and pass a thread under the fraenum and tie. The fraenum is a slender band of tissue which connects the posterior surface of the heart with the pericardium. Has this operation influenced the rate? By means of the thread raise the heart and notice the large veins, the sinus, the white cres-

centic line at the sino-auricular junction and the auricles. What is the sequence in the beat?

Prepare for dissection of the vagus by stretching the neck tissues over a large glass rod inserted into the oesophagus. On clearing away the connective tissue near the angle of the jaw three nerves can be found running from below the angle diagonally downward (Fig. 5). The upper one glosso-pharyngeal, soon turns and passes forward; the lower one also passes forward in a similar manner. Lying between these nerves before they bend upward can be found the third nerve or vago-sympathetic lying under the edge of a fine muscle which connects the angle of the jaw with the hyoid bone. Stimulate the vagus

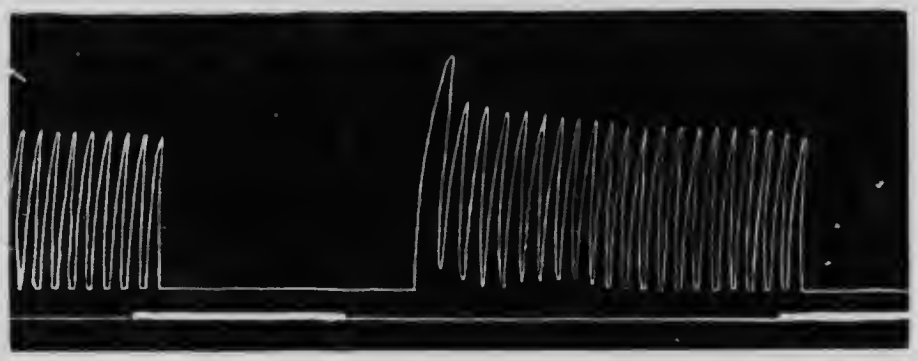


FIG. 17. Effect of vagus stimulation upon the heart of the turtle. Note the increased amplitude following the inhibition.

to make sure that the nerve is effective in modifying the heart rate (Fig. 17). Use the tetanizing current, beginning with a weak current and gradually increasing if there is no effect. Bear in mind that the sympathetic is being stimulated at the same time and therefore its stimulation may counteract the effects of vagus stimulation. That is the reason that it is necessary to find a current which will affect the vagus more than the sympathetic. Obtain a record of the contracting heart before and after vagal stimulation, using the suspension method as shown in Fig. 18. Use the magnetic signal to mark the time of stimulation. If you fail to obtain effects from one vagus, try the other.

Next stimulate the white crescentic line with the electrodes. Inhibition should be obtained.

Apply a few drops of 1% nicotine solution to the heart and again try the effects of vagus and crescentic stimulation, from time to time, recording the results on the kymograph. Nicotine will paralyze the ganglia. Explain.

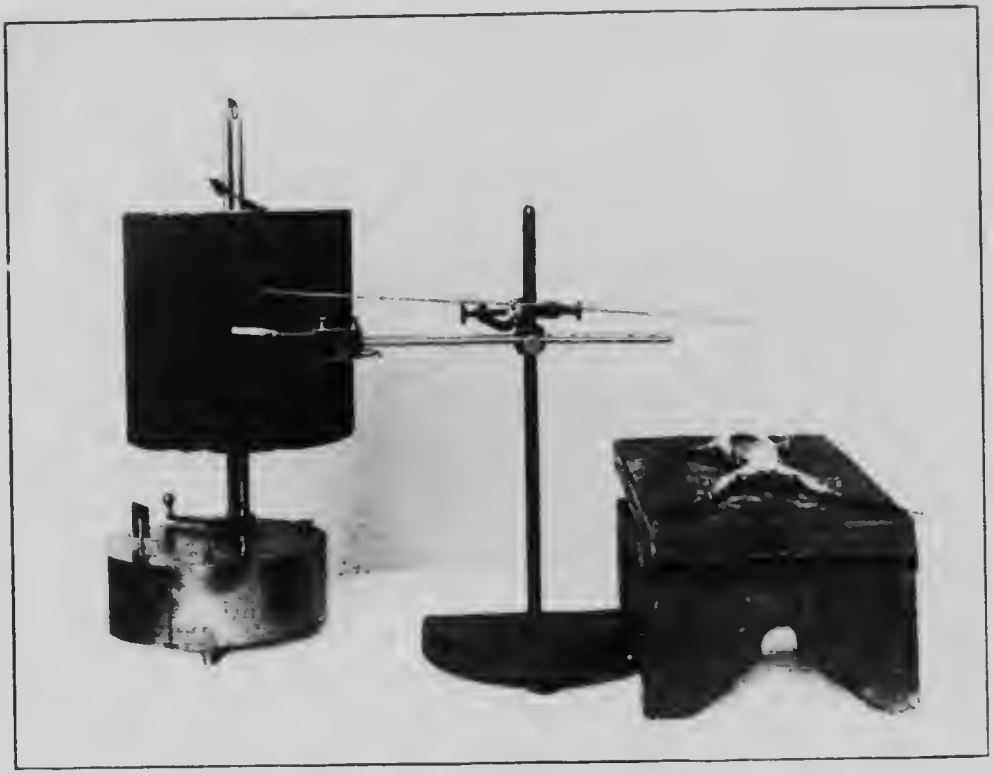


FIG. 18.—Suspension method for heart contraction.

***Experiment 25. Stannius Ligature.**—Expose the heart of a pithed frog in the manner already described. Pass a thread behind the aorta. Lift up the apex and tie the thread so that the knot lies on the crescent, thus separating the sinus from the auricles. If properly done, the auricles and ventricles soon cease to beat; if not, tie a second thread nearer the auricles. This is the first Stannius ligature. Certain properties of cardiac muscle can be studied with quiescent muscle which cannot be studied in a heart beating normally.

Attach the ventricle to the heart lever by a fine wire. Fasten a dissecting needle in a clamp and arrange it so that the point touches the sino-auricular junction. The secondary coil is connected with this needle and with the suspension wire. Study the result of stimulating the heart directly with induced shocks beginning with the weakest possible and increasing to the maximal. Record on a stationary drum, moved 2 mm. after each stimulation. Observe carefully and note results. Allow 15 secs. rest between each stimulation. Compare with your observations on skeletal muscle.

Staircase. Arrange a drum to rotate slowly, and record the contraction produced by stimulating every 5 secs. Record in this way about 20 contractions. Explain.

Next determine whether it is possible to tetanize cardiac muscle by stimulating with the tetanizing current. Explain your results.

Experiment 26. Study of the Turtle Heart. A turtle is killed by striking it on the head. The carapace or ventral portion of the shell is removed by the use of a saw and scalpel. Operate so that hemorrhage is reduced to a minimum.

Study the action of the heart and arteries. Examine the pericardium, then remove it. Where does the contraction seem to originate? What path does it follow in passing over the heart? Is there any delay in the passage between auricle and ventricle?

A-V INTERVAL. In order to observe the time relations between the contractions of the auricle and ventricle arrange to take simultaneous records of the two chambers one directly above the other, using heart levers. Attach the levers to the tip of each chamber by means of tiny hooked pins on the ends of threads, but take care not to puncture the wall. By using a very rapid drum the interval between the contraction of auricle and ventricle can be determined (Fig. 19).

STIMULATION OF THE VAGUS. Dissect out the right vagus. It can be located by stretching the neck and finding the carotid artery. It lies close to this artery. Pass a thread under the vagus so that it can be lifted when applying the electrodes.

Study the effect of stimulating the vagus by tetanizing currents of different strengths. Secure records to demonstrate the latent period and the duration of the after-effect. Note other effects such as changes in rate, and escapement. (Fig. 17.)

INFLUENCE OF TEMPERATURE.—Continuing with the same preparation obtain records with the heart at the following temperatures: 4°, 10°, 20°, and 30° C. These temperatures can be obtained by pouring Ringer's solution of the corresponding temperature over the heart. How does temperature influence the rate and amplitude of the beat? What modification takes place in the A-V interval?

EXTRA SYSTOLE.—Remove the heart by cutting widely around the base. Drop it in Ringer's solution. After studying it

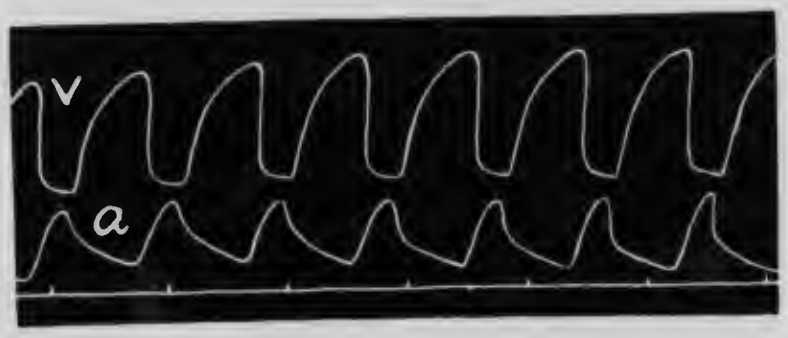


FIG. 19. Simultaneous tracings of the contraction of the auricle (*a*) and ventricle (*v*) in the turtle. Time interval 5 sec. (contractions slower than normal).

there for a short time, pin the base to a frog board and attach the apex to a heart lever. Frequently pour Ringer's solution over the preparation. The writing-point of a magnetic signal in the primary circuit is exactly aligned with the writing point of the heart lever. The secondary coil is connected by fine wires to the base and apex of the heart. A record of the normal heart is made upon a fairly rapid clock-work drum. Then single break shocks are sent into the heart during different phases of the cardiac cycle in an attempt to secure an extra contraction (Fig. 20).

During what phase can an extra contraction be produced? What follows an extra contraction?

When you consider that the heart obtains its rest only between beats, what do you think of this arrangement as a safeguard against encroachment upon the rest period?

Next, cut the ventricle across near its junction with the auricles. If the ventricle does not stop beating, cut still closer until the beats disappear. Now pin the cut surface of the apex

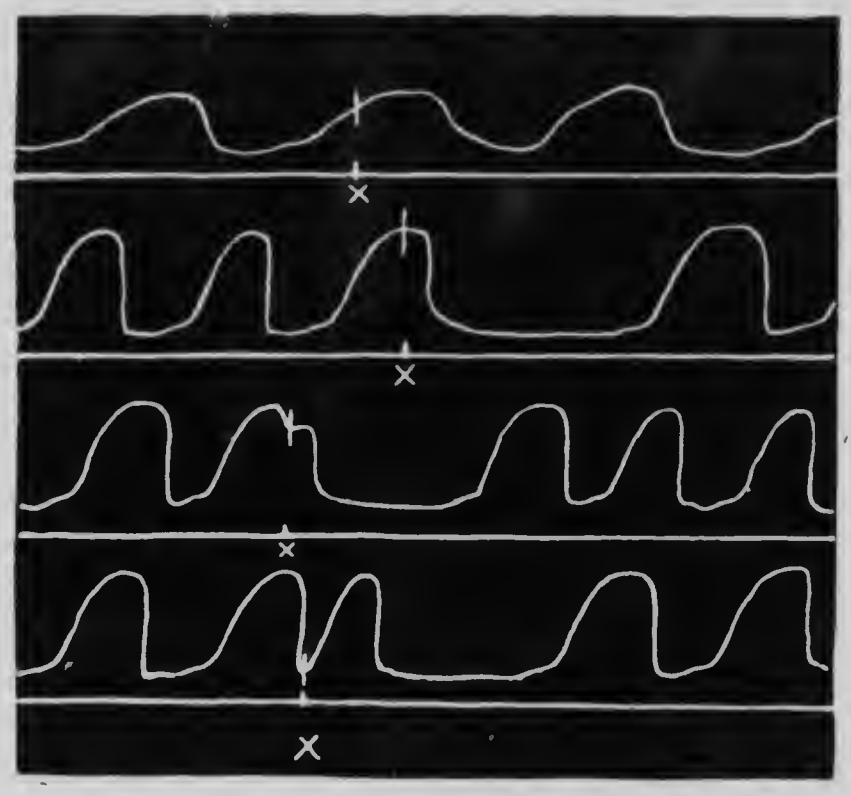


FIG. 20. Refractory period and compensatory pause in the heart. Stimulation at *X*.

to the board and apply the wire which formerly connected the base of the heart. Study the effects of a series of graded single shocks. How does this agree with the all-or-none principle? Also attempt to get the "staircase" effect. Try the effect of two successive stimuli in order to discover whether there is a refractory period.

Experiment 27. Perfusion of the Turtle Heart.—The difference of different inorganic salts upon the activity of cardiac muscle can best be shown by perfusion experiments.

In order that the experiment may have the greatest chance of success you must understand and prepare the mechanical arrangements before beginning the operation. A small beaker containing Ringer's solution is placed on a shelf or stand so that pressure is obtained for a siphon connecting the contents with a rubber tube which is long enough to be manipulated with ease when the time comes to insert the cannula. A small glass cannula fits into the lower end of the rubber tube, the flow of solution being controlled by a clamp. Make sure that the system of tube and cannula is absolutely free from air. Expose the heart; then carefully free a short length of the inferior vena cava without puncturing it. Place a fine ligature around this. Also pass ligatures around the other veins which supply the sinus venosus. Now with small forceps pick up the lower end of the freed portion of the vena cava. With small sharp scissors cut obliquely downward and forward not quite halfway through the vein. Holding the vein flap with forceps insert the cannula and tie the ligature securely about the constriction. Air must not be allowed in the vein or cannula. Next start a small flow of Ringer's fluid through the heart, immediately opening the aorta, and then tie off all the other veins entering the sinus. Obtain tracings of the ventricle by means of a heart lever.

Next determine the effect of the following solutions upon the heart beat. Between each solution, Ringer's fluid must be perfused until the heart beats normally. When changing the siphon from one beaker to another keep air from entering by placing the tip of the finger over the opening.

Solutions to be used are as follows:

1. 0.75% NaCl in distilled water.

2. A solution containing 0.5% NaCl and 0.25% KCl in distilled water.

3. 0.75% NaCl in distilled water to which a few drops of a 5% $CaCl_2$ solution has been added.

4. Distilled water.

CHAPTER VI.

BLOOD PRESSURE.

EFFECT OF CHEMICAL SUBSTANCES ON THE BLOOD VESSELS AND HEART.

The blood stream must be maintained at a certain pressure in order to furnish the tissues a sufficientty rapid supply of oxygen and nutrition as well as to carry away waste products. The heart is directly responsible for the movement of blood, so that any increase in its output will increase the movement. Such an increase may be obtained either by a greater rate or a greater amplitude. Both means are used.

The blood pressure would tend to fall to zero between the contractions of the heart if it were not for the elasticity of the artery walls. With each beat of the heart the arteries are expanded. As soon as the force of the heart is spent, the arteries contract, thus preventing a fall to zero. Fluctuations in pressure are partly prevented by the resistance offered by the capillaries.

The lowest pressure which is maintained is called the diastolic pressure, because it occurs during the diastole of the heart. The pulse pressure is the difference between the systolic and diastolic pressures. The more elastic the blood vessels, the smaller is the pulse pressure.

The blood supply to different regions of the body may be increased by a greater output, or by a shifting of the blood from one region to another. The latter method is made possible by the presence of vasomotor nerves. Certain nerves cause constriction, while others cause dilatation. Thus the supply to the muscles can be increased by constriction of the vessels in the abdominal organs and dilatation of those in the muscles.

Experiment 28.—The Measurement of Blood Pressure in Man.—A sleeve containing a rubber bag, which connects with a mercury manometer as well as with a valved rubber bulb, is

wrapped snugly about the arm and fastened. The sleeve should be placed high enough to permit application of the stethoscope over the brachial artery (Fig. 21). While palpating

FIG. 21. Sleeve in place for measuring blood pressure. It should be high enough to allow ample room for the stethoscope.

the radial artery the pressure in the rubber bag is raised above that necessary to produce obliteration of the pulse. Now no sound can be heard over the brachial artery at the elbow. By gently releasing the valve the pressure is gradually lowered. Just as soon as the pressure becomes less than systolic pressure, the blood is forced through the occluded arteries and a distinct thud is heard at each heart beat. At the same time or often after the pressure has been lowered 5-10 mm. more, the pulse is felt at the wrist. The first sound is never below the tactile indication; if it is, some error has been made in the application of the apparatus, usually the stethoscope. The pressure read at the time that the first sound is heard is systolic pressure.

As the armlet pressure is further lowered, the sound becomes progressively louder and sometimes resembles a murmur in character, and then, rather suddenly, it becomes feebler and at the same time duller. At this point, the manometer indicates the diastolic pressure. After this sudden sound, the sound may continue to be heard for a longer or shorter time as the armlet pressure continues to fall.

The difference between systolic and diastolic pressure is the pulse pressure.

Determine the heart rate, systolic and diastolic pressures under the following conditions in as many different subjects as possible:

Supine position, sitting, standing and immediately after vigorous exercise as running up and down stairs. How does the pulse pressure change under these conditions?

PRINCIPLES OF HAEMODYNAMICS.

The blood propelled by the heart, circulates in a closed system of tubes. Both the output of the heart and the response of the vessels affect the rate of flow. Increase the output of the heart either by a greater number of contractions per unit of time or by increasing the amplitude; either change will produce a greater flow of blood.

If the blood circulated in perfectly rigid vessels the pressure in the system would rise to a maximum during the contraction of the

heart and fall to zero during the relaxation. The flow in such a case would be spasmodic. On the other hand if the walls of the vessels are elastic, they are stretched by each spurt of blood from the heart, and then as the heart begins to relax the distended vessel wall begins to contract, thus forcing the blood onward in spite of the inactivity of the heart. The elastic wall therefore keeps the blood pressure from falling to zero during diastole.

If the blood meets with resistance somewhere in the path the vessels on the near side are distended more than they would be if

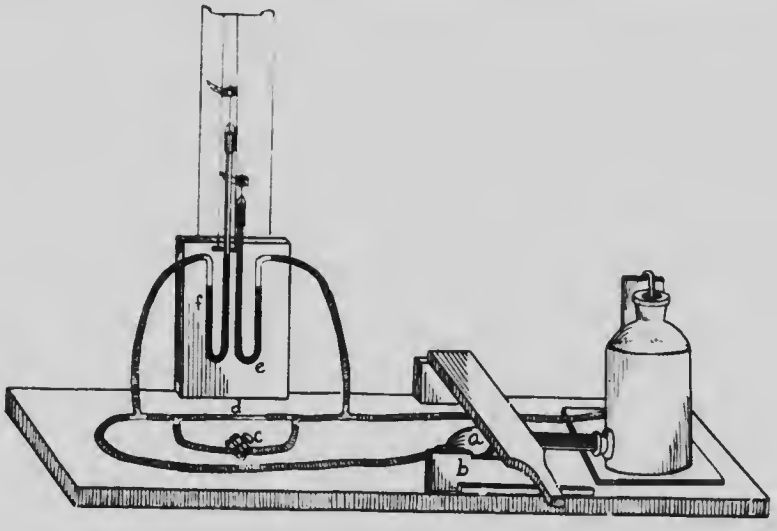


FIG. 22. Circulation scheme. The bulb (*a*) which represents the heart can have its output varied by either a change in rate of compresssion or by sliding the block (*b*) and thus changing the amplitude. The openings to the bulb are provided with valves so that flow can take place only in one direction. The resistance which corresponds to the capillaries is the part (*c*), (*d*). This may be varied by means of the clamp (*c*). The elasticity of the rubber tubing corresponds to the elasticity of the arterial wall. The "venous" and "arterial" pressures are measured by manometers (*e*) and (*f*) respectively.

the resistance were decreased or absent. The greater the distension of the tubes the longer they require to contract so that a steady flow would be kept up for a longer period. When the peripheral resistance is constricted venous flow is regular; great dilatation produces an intermittent venous flow.

Experiment 29.—Circulation Scheme.—Many of the foregoing principles can be demonstrated by a simple system of rubber tubes (Fig. 22). A rubber bulb represents the heart. The

rubber tubing nearest the heart takes the place of the arterial system, while the venous system is the tubing on the other side of the resistance.

1. With moderate capillary resistance determine a rate of bulb compression which will cause a pronounced rise in pressure as shown by the manometer. (If possible both arterial and venous manometers should record in line vertically with each other; to do this the arterial pressure zero should be higher than that for the venous pressure.) By means of the block vary the amplitude, but maintain a constant rate. What do the records of venous and arterial pressure show? What can you say in regard to the venous outflow?

2. With the same capillary resistance and with a moderate amplitude vary the rate. Obtain pressure records and note venous flow.

3. With the rate which gives the best results and with moderate amplitude vary the capillary resistance, recording pressure changes and noting venous outflow as before.

Compare diastolic and pulse pressures under the foregoing conditions.

What relation does capillary resistance bear to them?

How do amplitude and rate of the heart contraction affect diastolic and pulse pressures?

Response of Blood Vessels to Chemical Substances.

The amount of blood to any region can be increased by an increase in the output of the heart or by a dilatation of the blood vessels in that region. Dilatation may be brought about through action of the nerves to the vessels or by chemical substances in the blood. For example certain waste products such as lactic acid may cause dilatation. The blood vessels therefore not only by their elasticity help to maintain a steady blood flow, but by their active constriction and dilatation shunt the blood to regions most in need of it. That the blood vessels will respond to chemical substances, can be shown by the following experiment.

Experiment 30.—Perfusion of the Frog. Not only can the heart be kept beating for a considerable time by perfusion, but

circulation can be maintained for some time through the blood vessels as well. Arrange the beaker and siphon for perfusion as used in the turtle heart. After exposing the heart of a frog whose brain has been destroyed, tie off one aorta and fasten a cannula into the other so that it will lead away from the heart. Air must not be allowed to enter the system on any account. Hang the frog toes down and allow the fluid to escape from an incision in the sinus venosus. Determine the rate of flow in cc. per minute for Ringer's solution. This can be done either by measuring the inflow from a burette or by collecting the outflow in a graduated cylinder. Next perfuse with Ringer's solution containing 0.1% $NaNO_2$. What is the rate now? Wash out the latter fluid with Ringer's solution, then perfuse with Ringer's solution containing adrenalin.

Although adrenalin causes constriction of the blood vessels in the frog and under certain conditions in mammals, small quantities of adrenalin cause dilatation of the vessels supplying skeletal muscle in most mammals when injected into the general circulation.

SECTION IIB.

CHAPTER VII.

HAEMODYNAMICS.

Although many fundamental facts relating to the function of the cardiovascular system can be learned from experiments on cold-blooded animals, it is essential for a proper understanding of the physiology of the circulation in man that a certain number of experiments be performed by the student on mammals. In order that this may be done without causing any pain, the animal must be deeply anaesthetised by an expert assistant before starting the experiment, the anaesthesia must be maintained throughout, and when the experiment is finished, the animal must be painlessly killed.*

The following two experiments are selected to illustrate certain fundamental factors governing the circulation of the blood. Other fundamental experiments will be demonstrated before the class.

Each experiment is performed by a group containing from four to six students. Two of these act as operators and are responsible for the operative manipulations, one or two attend to the apparatus (technicians), and the remainder make detailed notes of every step in the experiment. After the experiment is completed, the group should review the results and each member of it prepare a report, embodying copies of the tracings secured. These reports are to be handed in for inspection.†

*For small classes it is practicable to have these essential mammalian experiments done on decerebrated preparations (see p. 225), but this is impossible when large numbers have to be provided for.

†For the experiments each student must provide himself with the following instruments: dissecting case, including 1 pair scissors and a blunt hook (aneurism needle or strabismus hook); 2 pairs, haemostats (Pean or Spencer Wells forceps); 2 pairs, bull dog forceps; a stethoscope.

Experiment 31.

BLOOD PRESSURE. NERVE CONTROL OF HEART BEAT AND PERIPHERAL RESISTANCE. HAEMORRHAGE AND TRANSFUSION. INSPECTION OF HEART.

1. PREPARATION OF ANIMAL.—Weigh the animal. In the case of dogs, inject morphine solution (sulphate or hydrochloride) subcutaneously (0.5 c.c. of 2% solution per kilo of body weight). In the case of cats, pass the stomach tube and pour the required amount of urethane solution into the stomach. (Give 1 gram of urethane per kilogram of body weight, dissolving it in water to make a 20% solution). These preliminary operations are to be performed by the laboratory technician or by a demonstrator, with the students assisting. In about one half hour the animal, now practically unconscious, is completely anaesthetised by administering ether. To do this pour about 20 c.c. on a towel and hold this firmly over the muzzle of the animal, being careful however to avoid suffocation. The animal is then tied, back down, on the animal board, meanwhile deep anaesthesia is maintained by giving ether. Count the pulse at the femoral artery; count the respirations and record the rectal temperature. Note any peculiarities about the animal (e.g., enlarged thyroid).

While the operator and anaesthetist are preparing the animal and performing the necessary operations, the technician and other members of the group proceed to get the manometer tubing and cannula ready, as directed in par. 3 below.

2. INSERTION OF TRACHEAL CANNULA. Moisten the hair in front of the neck and make a median incision down to the trachea (cut with the edge, not with the point of the scalpel). Pass a stout ligature under the trachea. Make a transverse incision half way across the trachea, insert the tracheal cannula and tie it by means of the ligature. The tracheal cannula is inserted to facilitate artificial respiration should this be necessary. If further anaesthetic is required, connect the tracheal cannula with the wide-mouthed anaesthetic bottle containing some ether.

3. INSERTION OF CAROTID CANNULA AND RECORDING OF MEAN ARTERIAL BLOOD PRESSURE.— Pull apart the skin flaps and separate the sterno-mastoid and sterno-thyroid muscles so as to expose the

carotid artery. With an aneurysm needle or a blunt-pointed curved seeker, free the carotid from its sheath for about an inch and place two ligatures under it. Tie the peripheral ligature (the one farthest

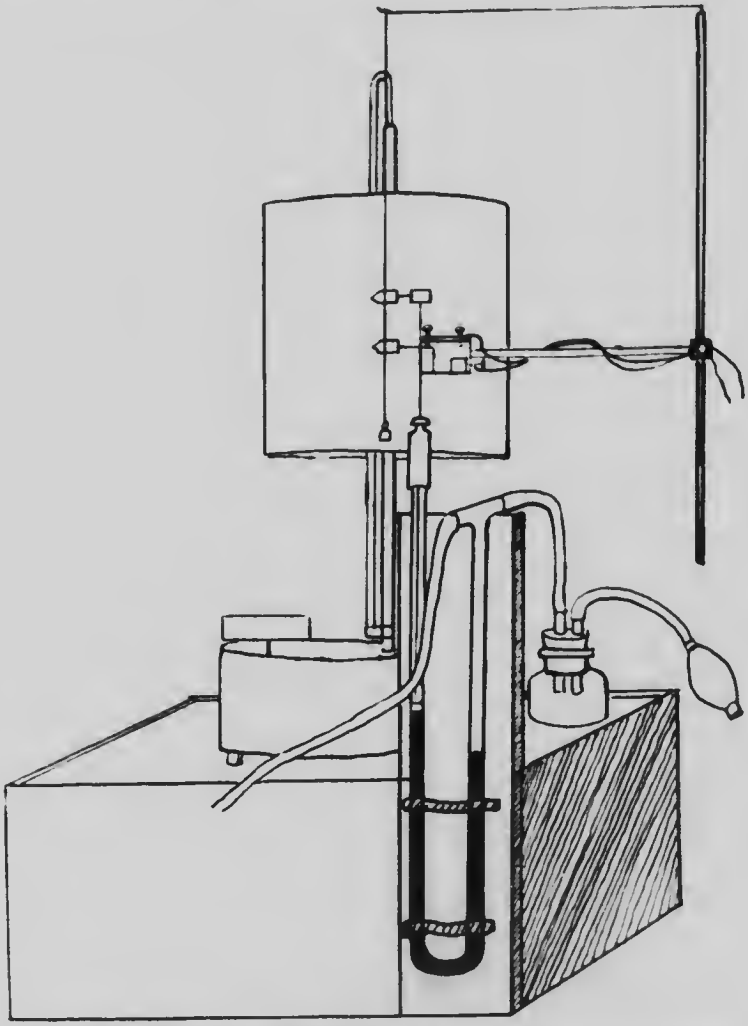


Fig. 23. Stand, manometer, pressure-bottle, etc., for recording blood pressure.

from the heart). Place a bull-dog forceps on the artery central to the central ligature.

Separate the vagus nerve from the carotid sheath on both sides of the neck, and place ligatures, without tying them, around both

nerves. Put bull-dog forceps on the ligatures to prevent these from slipping off.

Meanwhile a cannula of suitable size is connected to the manometer (Fig. 23) by rubber tubing which has been scrupulously cleaned of any old blood. This tubing is also connected by a T-piece with a bottle provided with a rubber bulb and containing a 2% solution of sodium citrate to prevent clotting of the blood. The cannula must be scrupulously clean and should be slightly oiled before inserting. Observance of these precautions greatly diminishes blood clotting. A pinchcock on the side tube which connects with the bottle is cautiously opened and by gentle compression of the rubber bulb, which is connected with the bottle, the tubing is filled with the citrate solution to the exclusion of all air bubbles. The pinchcock on the side tube is closed and a screw clip is placed on the tubing which connects manometer and cannula, and closed. The writing style of the chronograph is now adjusted so that it corresponds to that of the manometer. The line of the time tracing therefore serves as the line of zero pressure.

The cannula, filled to the tip with the citrate solution, is now inserted by the operator into the artery in the following way: the wall of the artery near the peripheral ligature is picked up by a forceps and, with a sharp scissors, a V-shaped cut is made into the artery without cutting it clear across. The tip of the V points peripherally. The V-shaped flap is lifted up with a fine pair of forceps and the cannula inserted into the artery and tied by means of the central ligature. The clip on the tubing connected with the bottle is opened and the bulb compressed until a pressure of about 120 mm. Hg. is recorded in the manometer. The pressure in the tubing prevents blood from entering it when the "bull-dog" forceps on the artery is opened, which is now done. The writing styles of the manometer and chronograph are adjusted to the drum, and a tracing is taken with a slow speed drum.

1. DEMONSTRATE THE EFFECT ON THE BLOOD PRESSURE OF VARIATIONS IN THE PUMPING ACTION OF THE HEART. STIMULATION OF PERIPHERAL END OF VAGUS NERVE.—Expose the vagus nerve in the neck on both sides and loosely tie the ligatures that were previously placed around each nerve. Start the drum and record a short piece of normal tracing, and then, without stopping the

drum, cut first one and then the other vagus between the ligatures. Note the effect produced on the blood pressure (Fig. 27). To signal the moment of cutting, short-circuit the time tracing for a second or two.*

Allow several inches of tracing to be recorded after both nerves have been cut, so as to be able to determine the after effects; then stop the drum and place the peripheral end of one vagus on electrodes coming from a short circuiting key which is mean[illegible]while

a *b*

FIG. 21. Arterial blood pressure tracing: (*a*) during moderate stimulation of the peripheral end of vagus; (*b*) during stimulation of splanchnic nerve.

[illegible]ed. Record a short piece of normal tracing and then by opening [illegible] short-circuiting key, stimulate the vagus with an induced current is just bearable on the tip of the tongue and note the effect produced on the blood pressure. Signal the time of stimulation as above directed. Demonstrate escapement.

*By short-circuiting the time marker in this way the seconds cease to be recorded and the break in the otherwise regular time tracing affords a useful signal indicating the moment of cutting or stimulation.

Repeat with the opposite vagus.

Repeat the experiment using varying strengths of stimuli.

Explain the results.

5. DEMONSTRATE THE EFFECT OF VARYING THE PERIPHERAL RESISTANCE ON THE BLOOD PRESSURE; STIMULATION OF SPLANCHNIC NERVE. Make an incision along the left costal margin starting about 1½ inches from the linea alba. After stopping all hemorrhage,* open the peritoneum and make out the suprarenal capsule lying over a transversely coursing vein. Just outside the suprarenal and above the vein expose the greater splanchnic nerve by blunt dissection. Tie a ligature loosely around the nerve and lay this on electrodes. While recording a normal tracing of the blood pressure stimulate the nerve (Fig. 21). In taking this tracing all details are to be followed as in par. 4.

Explain the result.

The splanchnic nerve is difficult to find unless the abdominal viscera, with the exception of the left kidney, are well retracted to the right. This is to be done by the assistant operator, who, standing on the right of the animal, covers the viscera with cloths wrung out with warm 0.9% NaCl solution, places the left hand with fingers extended over the cloth, and then with the finger tips just touching the posterior wall of the abdomen, pulls the viscera towards the right. With the right hand this operator also pulls down the kidney on the left side. By these procedures the suprarenal capsule is easily brought into view, and the operator isolates the splanchnic nerve by blunt dissection in the region just above the capsule.

It is often simpler to find the splanchnic nerve on the right side. In this case, after retraction of the viscera by the procedure described above, but towards the left side, the right kidney is exposed, and the vein, connecting the abdominal muscles with the adrenal vein, is ligated in two places and cut between the ligatures. The splanchnic nerve is then brought into view by little dissection.

*The hæmorrhage occurs mostly when the muscles are being cut. To arrest it firmly sponge the wound with surgical gauze, locate the bleeding points, catch them in the points of artery forceps (hæmostats) and while an assistant gently elevates the point of the forceps, tie a ligature around the tissue caught in them. For venous and capillary oozing, pressure with a piece of gauze wrung out in hot water is usually sufficient.

6. DEMONSTRATE THE EFFECTS OF HAEMORRHAGE AND OF TRANSFUSION ON THE MEAN ARTERIAL BLOOD PRESSURE.—Introduce a cannula into the femoral artery of one side and another into the femoral vein of the other side. The arterial cannula is introduced as directed above, and connected with a piece of rubber tubing through which to bleed the animal. The venous cannula is introduced as follows: The vein is exposed and two ligatures placed underneath it; a bull-dog forceps is applied central to the central ligature, and the peripheral ligature is tied. A cannula of suitable size, connected with about six inches of rubber tubing and filled

FIG. 25. Arterial blood pressure tracing showing the effect of a moderately slow hæmorrhage followed by a rapid hæmorrhage.

with 0.9% NaCl solution, held in by means of a clip on the rubber tubing, is inserted into the vein towards the heart in exactly the same way as in par. 3. When both cannulae are in position, the tubing on the venous cannula is connected with a burette.

HAEMORRHAGE.—After taking an inch or two of normal tracing of the pressure in the carotid artery, the clip on the femoral artery is partially or completely removed and the blood which escapes is collected in a basin and defibrinated. Note the effect on the blood pressure (a) of sudden and (b) of gradual bleeding (Fig. 25). It will be found when the artery is fully opened that there is an immediate fall in blood pressure due to release of peripheral resistance. If the

clip is only partially opened, considerable bleeding may occur before the blood pressure is materially affected. Continue the bleeding until a permanent (marked) fall of blood pressure is recorded. About 25 c.c. of blood per kg. body-weight must usually be removed to obtain this result.

SALINE TRANSFUSION. Now connect the venous cannula with a burette filled with a 0.9% NaCl solution, previously warmed to body temperature; remove the clip and allow the saline to pass into the animal, taking great care that no air bubbles are carried in with the solution. Meanwhile MAKE OBSERVATIONS ON THE VENOUS BLOOD PRESSURE (which is indicated by the height in the burette at which no more saline enters the vein); noting: (a) its height in mm. of water and (b) whether it shows any pulsations. Observe carefully the effect of the transfusion on the arterial blood pressure.

The blood removed in this and in all other experiments is to be defibrinated by beating in a clean dry basin with a bunch of wires. When fibrin formation has ceased the whipped blood is strained through muslin, and measured in a graduated cylinder.

BLOOD TRANSFUSION. Again bleed from the femoral artery, carefully noting the behaviour of blood pressure, respirations, etc. Note any differences in the character of the blood from that obtained previous to the saline transfusion. When the blood pressure is extremely low (40 mm.) transfuse with defibrinated blood and note the effect on the blood pressure as compared with that produced by saline solution. Note particularly with which solution the restored pressure is best maintained.

7. OBSERVE THE HEART BEAT IN THE OPEN THORAX UNDER ARTIFICIAL RESPIRATION. Since natural respiration is impossible after the thoracic cavity has been opened (explain why) it is necessary to start artificial respiration by means of a pump connected with the tracheal cannula. The air delivered from the pump is in a continuous stream. In order to interrupt it so as to simulate the respirations, a wide T-piece is placed on the rubber tube between the pump and tracheal cannula, and this side tube is opened and closed by a finger at a rate corresponding to the normal respirations. In order to open the thorax the skin is first of all incised down the mid line from the base of the neck to well on to

the abdomen and is quickly dissected to both sides sufficiently to expose the rib cartilages. The latter are then separately snipped through on both sides by a bone forceps and finally the soft tissues are cut through by a stout scalpel from below up. Arterial haemorrhage must be controlled by catching the bleeding points with a haemostat and tying, but venous oozing can be stopped by applying cloths wrung out with hot water.

Watch the heart while the vagus nerve is being stimulated. Place stout ligatures under the superior and inferior venae cavae and tighten them for a few moments; note the emptying of the heart. Place a ligature under the pulmonary artery and tighten for a moment. Note the effect on the heart. Repeat with the aorta.

Listen to the sounds of the heart with a stethoscope applied directly to various parts of it and study the effect produced on the sounds by tightening the ligatures referred to above. Stop the artificial respiration for a few moments and note the behaviour of the heart.

Afterwards excise the heart and open the right ventricle to observe the contractions of the papillary muscles. Note that the first sound is still heard in the excised heart. What conclusion do you draw from this observation?

CHAPTER VIII.

Experiment 32.

VASO MOTOR NERVES, DEPRESSOR NERVE, CIRCULATION TIME.

(The General Instructions for the Insertion of the Cannulae and the Conduct of the Experiment are the same as in Experiment 31).

1. PREPARATION OF ANIMAL.—Weigh the rabbit. Inject a 10% solution of chloral hydrate per rectum, 5 c.c. per kg. body weight. When the chloral effect has developed (about 30 minutes) cause the rabbit to inhale a little ether. To fix the head make an incision through the floor of the mouth and with an aneurysm needle pass a stout thread through the incision and out of the mouth. Tie the thread to the cross bar of the animal board. Tie out the limbs.

2. DEMONSTRATE VASOCONSTRICTOR FIBRES IN THE CERVICAL SYMPATHETIC.—Clip the hair from the front of the neck and make a median incision. Retract the skin. Separate the laryngeal muscles from the sterno-mastoid so as to expose the carotid sheath. Running with the carotid arteries will be seen one large and two small nerves. Of the latter, one is the cervical sympathetic, the other the depressor (see Fig. 26). Tie a pair of ligatures loosely around each nerve.* Now stretch the ear on the same side as the exposed nerve on a wire frame so as to render its vessels quite plain. Stitch the edges of the ear to the frame. With a good light behind it, examine the vessels of the ear, carefully noting their size. Note also the size of the pupils. Pick up the ligatures around one of the fine nerves, tie both ligatures and cut the nerve between them. Look for any effect on the vessels of the ear and on the pupils during both tying and cutting. Apply electrodes to the

*To obviate confusion the ligatures should be marked by placing bulldog forceps on the free ends. The nerves must be frequently moistened with 0.9 per cent. saline.

upper part of the cut nerve. Stimulate with an interrupted current of moderate strength and watch the effect on the vessels and pupils. If no change occurs, repeat with the other nerve. The nerve which

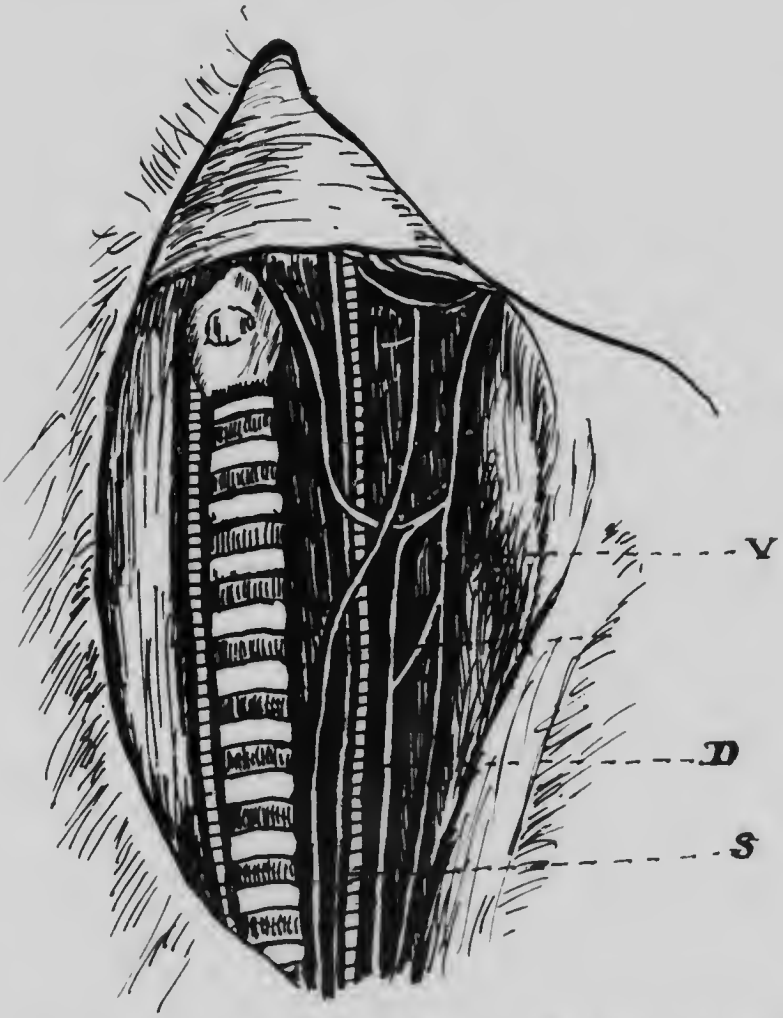


FIG. 26. Dissection of neck of rabbit to show relative position of nerves, etc.: (*c*) carotid artery; (*b*) vagus; (*s*) sympathetic; (*d*) cardiac depressor, (*m*) descending branch of hypoglossal nerve. (After Jackson).

causes definite effects is the sympathetic. Explain the causes of the results. It is often advisable to introduce the tracheal and carotid cannulae, as directed under par. 3, before attempting to stimulate the nerves.

3. DEMONSTRATE THE EFFECT OF STIMULATION OF THE VAGUS NERVE ON THE ARTERIAL BLOOD PRESSURE.—Introduce tracheal and carotid cannulae as described in pars. 2, and 3 (Exp. 31), and apply electrodes to the peripheral end of the vagus. Take about an inch of normal tracing and cut the vagus on one side while a tracing is being taken. Note any effect on the blood pressure. Apply electrodes to the peripheral end of the cut vagus and stimulate with an induced current of moderate strength (just bearable to the tip of the tongue). Repeat with stimuli of varying strengths. Remove the electrodes to the central end of the vagus and repeat the stimulation.

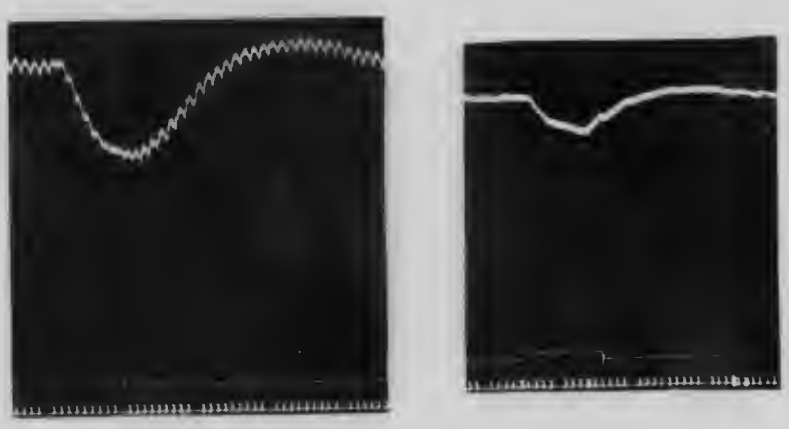


FIG. 27. Stimulation of the cardiac depressor nerve in the rabbit, showing effect on arterial blood pressure. In the tracing to the right the vagi were intact; in the tracing to the left they were cut. Note the slower respiratory oscillations after cutting the nerve.

In the above experiments, the drum should be revolving slowly while the stimuli are applied, and the moments at which this is done should be indicated by short-circuiting the time tracing. Explain the causes of the results.

4. DEMONSTRATE THE EFFECT OF STIMULATION OF THE DEPRESSOR NERVE ON THE BLOOD PRESSURE.—Pick up the ligature tied to the central end of the depressor nerve (the small nerve which is not the sympathetic), apply electrodes and stimulate. A fall in blood pressure should occur (Fig. 27). Look for slowing of the heart. Show that the fall in blood pressure, caused by stimulation of the depressor, is not entirely due to reflex vagus

inhibition, since it persists after both vagi are severed. What conclusions do you draw as to the cause of the fall? Finally stimulate the peripheral ends of the depressor and sympathetic nerves. The sympathetic stimulation sometimes quickens the heart.

5. ESTIMATE THE CIRCULATION TIME FOR THE LESSER CIRCULATION (Stewart's method).—Ligate the carotid and remove the cannula. On the same side of the neck insert a cannula (pointing towards the heart) in the jugular vein (for technique see par. 6, Exp. 31). Expose the carotid artery of the opposite side and place under it a strip of white glazed paper resting on a piece of thin rubber dam. Throw a strong light on the artery. Connect the venous cannula with a burette containing a 0.2% solution of methylene blue in physiological saline solution, at the temperature of the body. See that there is no air in the tubing. When all is ready, remove the bull-dog forceps and mark the exact moment that the methylene blue enters the vein (i.e., when the methylene blue is seen through the cannula to enter the vein). Carefully observe the carotid artery. The moment at which the blue appears in the artery is also noted. Repeat several times, using always the same amount of methylene blue. To obtain accurate results no methylene blue solution should be allowed to escape on to the wound. The results are to be given in relationship to (1) actual periods of time and (2) the heart-beats.

6. DETERMINE THE SEAT OF OXIDATION IN THE BODY.—Inject an excess of a stronger solution of methylene blue until the animal is killed by the injections, and make a careful autopsy; examining especially sections of the muscles, kidneys and liver, also the urine, the blood in the mesenteric vessels, etc. The methylene blue will be found to have stained the blood but not the tissues. The tissues have reduced it to methylene white. This is known as Ehrlich's experiment and it shows that the tissues are the seat of reduction in the body. Note the effect which exposure to air has on the colour of the cut organs. On standing exposed to the air the cut tissues will become blue because of oxidation of the methylene white to methylene blue. What conclusions do you draw from the experiment?

CHAPTER IX.*

POLYSPHYGMOGRAPH TRACINGS.

These experiments consist in obtaining graphic records of the pulses of the radial and carotid arteries, of the apex beat and of the venous pulse in the jugular vein.†

The experiments are important not only because the results if properly interpreted throw much light on the cardio-vascular mechanism, but because of the value of the technique in clinical diagnosis. The tracings are often difficult to obtain, and great care and patience must be exercised in adjusting the various receiving tambours. This is especially so for the radial pulse and apex beat. When these two cannot be satisfactorily obtained together, the carotid should be substituted for the radial. The observed person must remove his shirt and lie on a couch or table. Sometimes it is best to lie on the side, sometimes on the back. While cardiac tracings are being taken, the breath should be held for a few moments.

Experiment 33.

A. **The Velocity of Transmission of the Pulse Wave. (Propagation of Pulse Wave).**—With the arm easily extended and resting on the arm support, apply the button of the receiving tambour over the radial artery and adjust the pressure until a maximal movement of the recording lever is observed. Taking care that the radial tambour does not slip, now apply the carotid tambour by hand (apply opposite the angle of the jaw). When a maximal pulsation of both of the levers is obtained, adjust the writing points to a slowly moving clockwork drum. The writing points should be as nearly as possible in the same perpendicular, and should be

*For the experiments of this chapter two sessions of three or four hours each are necessary.

†Notes and tracings of these experiments are to be taken by every member of the class.

applied to the drum with a minimal amount of friction. This is done by manipulating the adjusting screws on the tambour stands, while the drum is moving slowly.*

When the adjustment is completed the drum is stopped for a moment so that the two levers may draw vertical lines showing their relative positions (alignment marks). The drum is then allowed to revolve at a medium speed and the tracings taken. The speed should be as great as is consistent with a definite upstroke of the pulse. Alignment marks should be inscribed at frequent intervals (by stopping the drum for a moment). A time record (0.2 sec.) is added to the tracing with the drum revolving at

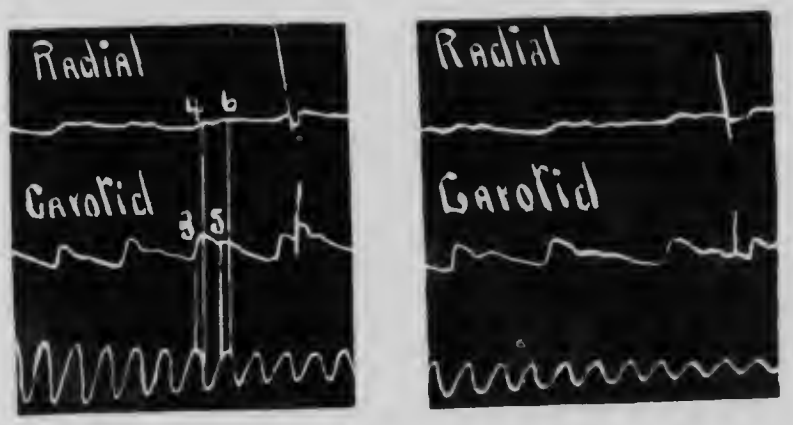


FIG. 28. Simultaneous tracings of carotid and radial pulses. The rate of transmission is determined by finding the difference between the beginning of each pulse, after correcting for alignment of levers, and measuring off this distance on the time tracing (1/50ths second.).

the same speed as when the pulse tracings were being taken. A vibrating spring provided with a writing point is used for taking the time tracing.

After the removal of the tracing from the drum, the distance of the beginning of the upstroke is measured from its alignment mark on each pulse curve (Fig. 28). The difference, interpreted in terms of the time tracing, gives the rate at which the pulse wave travels from the carotid to the radial artery. Note also the dicrotic notch

* This is more satisfactory than attempting to adjust to a stationary drum because friction is diminished. Before starting the drum, however, make certain that the tambours are so adjusted as to give the greatest possible movements.

and measure its distance from the beginning of the upstroke. Several measurements should be taken from each member of the group. How can the length of the pulse wave be calculated?

In the more or less arbitrary lettering of the waves observed in pulse tracings, the main upstroke is marked 3, in the case of the carotid and 4 in that of the radial; the dicrotic notch of the carotid is 5 and of the radial 6. The distances between 3 and 5 and between 4 and 6 represent respectively the time during which the heart is pumping blood into the arteries, i.e., the semilunar valves are open. It is called the *sphygmic period* or *period E*.

B. The Venous or Jugular Pulse Curve. Have the observed person lie down with his head slightly raised by a cushion and bent to the right side. Place the receiver (whistle funnel) over the jugular bulb on the right side of the neck. This lies immediately above the inner end of the clavicle. Bring the point of the lever of the recording tambour to write with a minimal amount of friction on a drum. Since a venous pulse tracing cannot be interpreted without a simultaneous tracing from an artery, adjust the button of a receiving tambour over the radial artery and arrange the writing style of its recording tambour so as to write on the drum in the same perpendicular as the style of the venous tambour. If no satisfactory tracing can be secured from the radial, try the carotid. Remember to inscribe alignment marks at short intervals. While the tracing is being taken it is usually advisable that the respiratory movements be suspended.

TO INTERPRET THE VENOUS CURVE.—Make a vertical mark on the arterial pulse tracing corresponding to the beginning of the pulse upstroke. If this is done on the radial pulse tracing, measure 1/10 sec. in front of it and make another vertical mark. (This mark is to allow for the time lost in propagation of pulse from heart to radial. It is determined according to Exp. A).

This line 3 corresponds to the beginning of the sphygmic period of ventricular systole, i.e., to the opening of the semi-lunar valves. Measure the distance from line 3 to the nearest alignment mark. By measuring off the same distance from the corresponding alignment mark of the venous tracing line 3 will be found to fall at the beginning of a small wave which is marked *c*. The small wave in front of *c* is marked *a* and is due to auricular systole. The large

wave of depression following *c* is marked *x* and is due to a fall of pressure in the auricle. What causes this fall in pressure?

The next point to determine is the end of the sphygmic period. This is found by measuring from the alignment mark to line 5 of the carotid or line 6 of the radial (less 1/10 sec.) and transferring to the jugular tracing. The line will be found to fall on a small wave on the upstroke of the depression. This wave is marked *V* and is due to the sudden opening of the tricuspid valves. Sometimes another small wave just precedes *V*. It corresponds to closure of the semilunar valves. Listen to the heart sounds while recording a tracing in order to determine this fact.

In preparing your report of the experiment, draw in the intra-auricular and intra-ventricular pressure curves in relationship to the venous curve.

C. Cardiograms.—Apply the button of the special receiving tambour to the apex beat and connect with a recording tambour. Adjust the position and pressure of the button until a maximal movement of the writing style of the recording tambour is obtained. Apply the writing point to a carefully levelled drum and with this running at moderately high speed, take a tracing. There is often difficulty in securing a satisfactory tracing, and it may be necessary to try another subject. Breathing should be suspended for a few moments while the tracing is being taken.

TO INTERPRET THE CURVE.—Adjust another receiving tambour to the radial or carotid pulse with both writing styles in the same perpendicular, and following the other directions described under "Venous Pulse", mark on the cardiogram:

1. The beginning of the sphygmic period, E (line 3).
2. The end of the sphygmic period, E (line 5).
3. The auricular wave (beginning of this wave is line 1; the end of it, line 2).
4. The beginning of ventricular systole (difference between 2 and 3 equals the presphygmic interval).
5. The opening of auriculo-ventricular valves (lowest point in tracing; somewhat difficult to determine).

When satisfactory tracings have been secured apply a stethoscope to the apex beat and after accustoming your ear to the sounds, mark as accurately as possible, by free hand, the position on the cardio-

gram at which they are heard. The second (sharp) sound can be heard best over the sternal end of the second rib on the right side.

Influence of Respiratory Movements on the Various Pulse Tracings.—Apply the respiratory tube to the thorax and connect with a recording tambour. Also arrange to take a tracing of one or the other of the pulses, or of the heart beat. Record simultaneous tracings of the respiratory movements and of the pulse. Note the effect of inspiration and expiration respectively on the pulse curves.

How do you explain the effects?

SECTION III.

THE CENTRAL NERVOUS SYSTEM.

CHAPTER X.

REFLEX ACTION IN THE FROG.

In a nerve fibre impulses are transmitted with equal facility in either direction (Exp. 18, p. 45), but in a reflex arc they go in one direction only. The nerve centre is responsible for this directive influence, and the centre may be situated either peripherally, as in ganglia, or centrally, as in the brain and spinal cord. The exact significance of the centres in the brain and spinal cord varies considerably in different animals according to their degree of development. In a general way the centres in the spinal cord are primarily the local centres for each segment of the body, but the reflex activities of other segments may be caused to cooperate so as to bring about complex movements. The centres in the brain, on the other hand, are to be regarded as affording higher nervous pathways which the reflex impulse does not necessarily traverse, but in which, when it does so, considerable modification may occur. The impulse may, for example, become suppressed (inhibited) or exaggerated (augmented) by its passage through nerve centres in which memory impressions have been stored away. If these memory impressions indicate that reflex movements would be harmful to the animal then the movements may be inhibited: under the converse conditions thay may be augmented. On account of this dominating influence of the higher over the lower centres it is clear that a precise analysis of the physiology of reflex action demands a simplification of the experimental conditions, by isolating the spinal cord from the brain. In the lower vertebrates this can readily be done by actually removing or destroying the latter. In the higher vertebrates, this operation is incompatible with life, so that the brain is merely isolated from the spinal cord by cutting the latter below the level of exit of the nerves to the

chief respiratory muscles (diaphragm). This so-called SPINAL ANIMAL offers a most suitable preparation for the study of reflex activities. Immediately following its isolation from the brain the cord enters into a depressed condition called SPINAL SHOCK. This is quickly recovered from in the lower animals, but may take months to disappear in the higher.

Experiment 34. Pith a frog, destroying the brain only and suspend the animal by fixing the lower jaw to a clamp (Fig. 29). Apply mechanical stimuli (by pinching) to the skin of various regions and note the location and character of the response. Commence the application of the stimuli immediately after decerebration and observe by repeating at intervals whether they improve with time. If they do so, what is your conclusion? Proceed now to demonstrate the duration of the *latent period* or *reflex time* using as the stimulus a 0.1 per cent. solution of H_2SO_4. Measure accurately the time which elapses between placing the foot in the acid and the movement of the leg. Remove all traces of acid from the skin by means of water. Make three observations with the same strength of acid using the two feet alternately and allowing some time (several minutes) to elapse between the stimulations. Repeat the observations using 0.3 per cent. acid. Are any differences in latent period and intensity of response observed, depending on the strength of stimulus?

It is likely, with the strong stimuli employed in the above experiments, that some spread (or irradiation) will have occurred in the cord, so that the opposite hind limb or the fore limbs show movements. How is this explained?

Experiment 35. To investigate the "march" of the irradiation more precisely and to demonstrate that the isolated cord is capable of synthesising a complicated and apparently purposeful group of movements saturate a piece of filter paper in 30 per cent. acetic acid, remove the excess of acid and place a small square of this paper on one of the flanks of the frog. After noting the character of the movements, wash away the acid and repeat, holding the leg on the irritated side.

The Spreading of Reflexes in the cord is greatly facilitated by strychnine, but the movements are not of the same "purposeful" character as those just studied.

FIG. 29. Decerebrate frog suspended for studying reflex action.

Experiment 36.— Inject a few drops of a 0.5 per cent. solution of strychnine sulphate into the dorsal lymph sac of the frog. Apply weak stimuli at intervals to the skin of different parts of the body and note the exact nature of the responses. Explain the action of the strychnine.

Inhibition of Reflexes.— Reflex activities may be inhibited either through afferent nerves or from the higher centres.

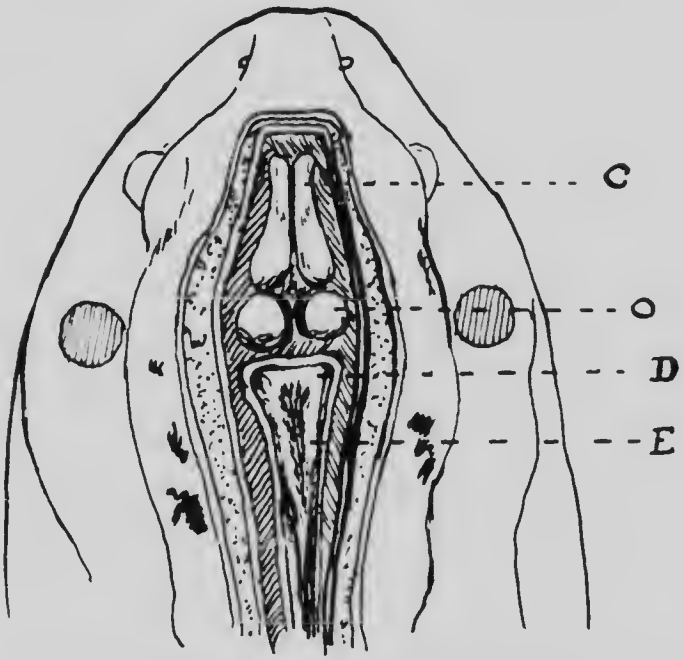


FIG. 30. Exposed brain of frog: (b) cerebrum, (o) optic lobes; (cb) cerebellum, (m) medulla.

Experiment 37.— Expose the sciatic nerve in the left thigh. Tie a ligature about the nerve near the knee and cut the nerve distal to the ligature. Immerse the right leg of the frog in 0.5% sulphuric acid and note the latent period for reflex action. Immediately wash off the acid. After three minutes place electrodes on the exposed sciatic nerve and as the right foot is again immersed in the acid, stimulate the nerve with a weak tetanizing current. Prove that the sensory endings in the skin are still irritable by the acid. Explain the inhibition.

To show that reflexes can be inhibited by influences from higher centres, place a crystal of salt on the optic lobes which have been exposed in a live frog by quickly cutting off the anterior portion of the head just behind the tympani with heavy scissors (Fig. 30). Now attempt to elicit reflexes by the methods described above.

THE FUNCTION OF THE SPINAL NERVE ROOTS.

Experiment 38.—Pith the brain of a large frog and fasten it ventral side down. Slit and evert the skin over the last four vertebrae; remove the muscles from the vertebral arches and then open the spinal canal by means of a strong scissors, keeping them close to the bone. Uncover three or four sets of roots. Pass a silk ligature under a dorsal root as far out as possible. Tie and cut distal to the ligature. Stimulate the central end of the root. Tie a ligature under another dorsal root close to the cord and cut the root central to it. Now stimulate distal to the ligature. In like manner test the function of the ventral roots. In this experiment the unipolar method of stimulation will be found best because of the shortness of the spinal roots. The plate electrode, attached by a wire to the secondary coil is placed in contact with the belly of the frog. The other wire is connected with a dissecting needle which then serves as the more active electrode to stimulate the nerve roots. Note the nature of the movements produced in the above experiments and draw a diagram showing the functions of the roots.

THE FUNCTIONS OF THE CEREBRUM IN THE FROG.

Interesting observations can be made on a decerebrate frog, but it must be remembered that they do not shed much light on the functions of the cerebrum in the higher mammals because in these the cerebral centres are relatively far more important and participate in many reflex activities in which the spinal cord alone is involved in lower animals.

Experiment 39.—Activities of a Decerebrate Frog. Anaesthetize a frog by placing it under a beaker together with a piece

of absorbent cotton containing ether. Slit and retract the skin over the skull and remove a triangular piece of the latter so as to expose the cerebrum. The base of the triangle should be on a line connecting the posterior border of the tympanic membranes and should be about a centimeter wide. Avoid injury to the brain beneath until the cerebrum is completely exposed, then carefully remove the latter and plug the cavity loosely with cotton. Sew the skin in place and keep the animal where the skin will remain moist until the next day. This can be done by placing the frog with a little water in an evaporating dish and covering all with a large funnel or something which will prevent escape, but at the same time allow access of plenty of air.

After 24 hours study the behaviour of the decerebrate animal compared with a normal frog, noticing especially: posture, ability to swim or hop, power of escaping from a vessel of water gradually heated, ability to turn over when placed on the back.

Again open the cranial cavity, without anaesthesia as there can be no sense of pain, and destroy the corpora striata and optic thalami. After recovery from shock observe the activities as before. Finally destroy the rest of the brain and notice any change in behaviour.

CHAPTER XI.

REFLEX ACTION IN MAN.

There are in general three types of reflexes elicitable in man and the higher mammals. These are: 1. The reflex movements produced by the application of hurtful or noxious stimuli to the skin (*nociceptive reflexes*). 2. The reflexes required to maintain the joints in such a position that the animal may stand erect and move about (*postural reflexes*). 3. So-called *myotatic reflexes* which are contractions produced by direct mechanical stimulation of muscles that have been brought into a hyperexcitable condition (hypertonus) through reflex action.

An example of nociceptive reflexes is the flexion reflex already studied in the spinal frog. In man this type of reflex, variously modified according to the part of the body from which it is elicited, is extensively employed in the diagnosis of nervous diseases. The particular value of the reflex is that its presence or absence indicates the condition of the reflex pathway at the various levels. For the study of reflex time it is useful to employ the palpebral reflex, but in doing so it must be remembered that there are several fundamental differences between this and the flexion reflex, such as the relationship between strength of stimulus and latent time as well as intensity of response.

Experiment 40.—By means of a strip of adhesive tape attach a thread to the upper eyelid. Pass the thread through the handle of scissors which have been clamped to a stand to serve as a pulley. Attach the thread to a heart lever. The head should be held firmly in such a position that when the upper eyelid is closed the writing point of the lever moves upward. To elicit the reflex the subject presses a pair of electrodes against the lower lid. The operator stimulates the subject with break shocks only, using a current which is just strong enough to cause the upper lid to move. The subject must not see the operator at work. With the aid of a magnetic signal determine the reflex time on a rapid clock-work drum. Calculate the average reflex

time from a number of observations on the same subject. Each individual of the pair should serve as subject.

There is no example of a postural reflex which can conveniently be studied in the frog or man. (The extensor thrust and the mark-time reflexes (see p. 218) of the spinal mammal will serve this purpose).

The myotatic reflexes are best illustrated in the KNEE JERK.

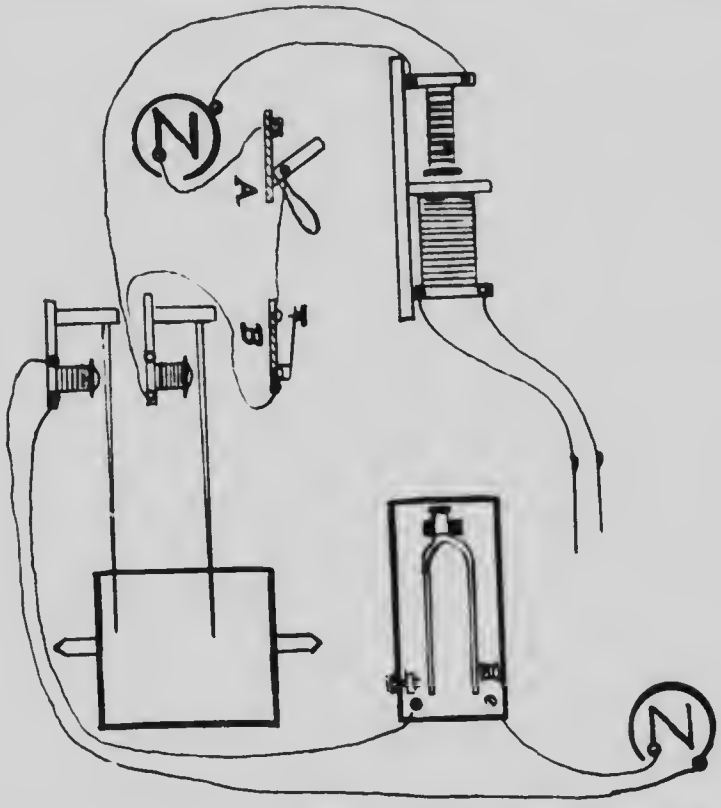


FIG. 31. Arrangement of apparatus for measurement of reaction time in man.

Experiment 41.—Let the subject sit in a comfortable position with one leg crossed over the other so that the patellar ligament is under an increased tension. The operator strikes the patellar ligament a sharp blow with the edge of his hand or a book. With a little practice it is easy to elicit a sudden contraction of the quadriceps muscle giving a sudden extension of the leg.

This should be tried on a number of subjects. After observing the extension in each subject, try the effect of REINFORCEMENT by the following methods:

1. Clenching the hands together vigorously.
2. Strong sensory stimulation such as pulling the hair.
3. Mental effort as solving a problem.

The magnitude of the response seems to depend on the tone of the muscle.

Tone depends on the condition of the nervous system, therefore the knee jerk is an important means of ascertaining certain pathological conditions of the central nervous system.

REACTION TIME IN MAN.

Akin to reflex latent time is the so-called REACTION TIME in man, that is the time which elapses between the application of a stimulus and a prearranged voluntary reaction.

Experiment 42.—In this experiment the apparatus is set up as in Fig. 31. With the operator's key A open, and the subject's key B closed, the operator closes, and the subject opens B whenever he feels the stimulus. The signal connected with the keys writes on a quickly revolving drum on which a time tracing in 100ths of a second is also inscribed. Determine the average reaction time. Now increase the stimulus to find whether the reaction time changes. What are the additional factors involved in reaction time as compared with reflex time? How do different subjects vary?

SECTION IV.

RESPIRATION.

CHAPTER XII.

ANALYSIS OF AIR AND COLLECTION OF ALVEOLAR AIR.

Experiment 43. In conducting experiments involving the use of gas analysis apparatus the greatest care must be exercised to avoid spilling of mercury, or fouling of the burettes or tubing with the absorbing solutions.

The analysis must be repeated until constant results are obtained.

The apparatus can be used for the analysis of air from whatever source it may be derived, but for practice it is best to use alveolar air obtained by the method described on p. 101.

Analysis for oxygen should be attempted only after several samples have been analysed for CO_2 alone with satisfactory results.

The gas burette (A) (see Fig. 32) is 10 c.c. capacity from the end of the oblique side tube (F) to the lowest graduation on the narrow portion. The uppermost graduation corresponds to 2.2. c.c. from the lowest, the distance between the two being divided into c.c. and 1/50ths c.c. The bulb part of the stem to just above the 2.2. c.c. mark is surrounded by a water jacket. Above the side tube (F) the burette is continued into a narrow-bored tube with two arms at right angles to each other. These are connected by thick-walled pure-gum tubing, with the absorption bulbs (C and D) containing respectively a 20 per cent. solution of NaOH and a 50 per cent. solution of KOH with 10 per cent. pyrogallic acid dissolved in it.*

Each bulb is connected below by rubber tubing with the overflow bulbs (G and H). A small bulb is blown on the stem of each absorption bulb to serve as a trap preventing the solutions from passing over into the burette. The vertical tube above the cross tubes is closed by the pressure adjuster,

*NaOH may be substituted for KOH, but it is not so satisfactory because of its viscosity. The best solution is made by dissolving pure NaOH (electrolytic, if possible) in an equal bulk of water, diluting 10 c.c. of this with 4 c.c. water, and then dissolving 10 gm. pyrogallic acid in it.

which consists, as shown in the small side sketch in the diagram, of a glass rod bevelled at one end and bored down the centre to within about 3 mm. from the bevelled end, where a lateral hole is bored. The adjuster is connected with the central tube of the burette by pure-gum tubing and, when this is pinched up opposite the side tube, the burette is brought into communication with the outside and the pressure in it remains undisturbed on allowing the tubing to fall back into place over the side tube.

The lower end of the burette is connected by thick-walled tubing with the reservoir (B), and on this tubing are a pinchcock (5) and screw clip (4). About 15 c.c. of mercury suffices to fill the apparatus. The reservoir is hung by a loop of wire around the neck on hooks placed on a wooden upright stand, the higher one being in such a position (marked I) that the mercury stands at the end of the side tube F, and the lower one, so that it stands exactly at the mark O on the burette.

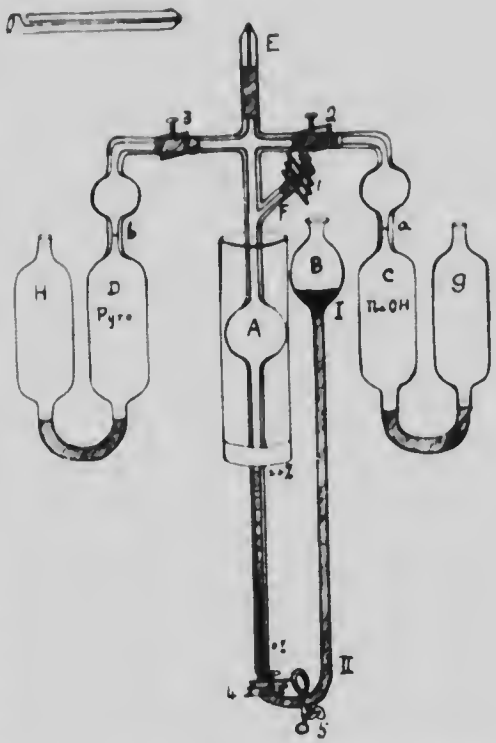


FIG. 32. Gas analysis apparatus. For description see context.

The first step is to fill the tubing between the side tube, F, and the absorption bulbs with nitrogen. This is accomplished by taking a sample of air in the burette by opening screw clip 1, placing the mercury reservoir in position II, and with the screw clip (4) open, cautiously opening the pinchcock (5) so that air enters the burette. Screw clip 2 is then opened and the reservoir (B) raised to position I. By cautiously opening pinchcock, 5, the air passes into C. With 5 held open the reservoir is raised and lowered several times so that all traces of CO_2 are removed from the air. With the pinchcock (5) closed, the reservoir is then put in position II and the pinchcock cautiously opened

until the NaOH stands at the mark (*a*) on the stem. Screw clip 2 is now closed and 3 opened, and the oxygen removed from the air by repetition of the same procedure. Screw clip 3 is finally closed. This preliminary filling of the burette with nitrogen is unnecessary if a previous analysis has been made.

The sample of air for analysis is now collected in an all-glass (Luer) syringe with the piston well lubricated with vaseline. With the screw clip 1 open, the burette is filled with mercury up to the end of the side tube F, and the nozzle of the syringe is inserted in the rubber tubing of the side tube. While making this connection gentle pressure should be maintained on the piston of the syringe so that no outside air becomes entrapped between the nozzle of the syringe and the mercury. To transfer the sample into the burette the reservoir is placed in position II and the pinchcock 5 carefully opened, gentle pressure being maintained on the piston of the syringe. After the air has been transferred to the burette the mercury meniscus should stand about 1 mm. below the O graduation, this being ensured by gentle pressure on the piston of the syringe. This leaves the gas in the burette under a slight positive pressure.

The pressure in the burette must now be made equal with that of the atmosphere. For this purpose all screw clips except 1 and the pinchcock (5) are opened and the adjuster is momentarily opened to the outside, as directed above. Being under a slight positive pressure, the current of air is outward.* The meniscus of mercury should now stand exactly at the O mark.

To absorb the CO_2 screw clip 3 is closed, and the reservoir raised (with 4 and 5 open) and lowered several times so that the gas passes in and out of C, in which the CO_2 is absorbed. In doing this, care must be taken that the mercury does not rise above the level of F, or that the NaOH solution is aspirated higher than *a*. Four movements usually suffice to absorb all of the CO_2. To determine its volume pinchcock 5 is closed and screw clip 4 halfway closed. With the reservoir placed in position II, the pinchcock is cautiously opened until the NaOH

*During this procedure a slight error is incurred because some of the sample of gas to be analysed presses up and mixes with the nitrogen above the side tube. The error thus incurred is, however, negligible for most purposes.

solution is as nearly as possible brought back to its original position at *a*. The fine adjustment of this level is effected by using the screw clip 4. The reading on the burette opposite which the mercury now stands gives the amount of CO_2 in 10 c.c. of gas. To make certain that all the CO_2 has been absorbed, the above procedure should be repeated.

To determine the oxygen the screw clip 2 is closed and 3 opened. The pyrogallic acid solution should now stand at its original position at *b*. The gas is then moved in and out of D several times, until all the oxygen is absorbed, as determined by no further shrinkage. This takes considerably longer than for CO_2. Since some O_2 still remains in the tube above the CO_2-absorption bulb, the air in the burette must be passed into this bulb, returned to the burette and again passed into the pyrogallate. This must be done at least three times to make certain that all O_2 is absorbed. The final volume of gas as read on the burette, less the volume of CO_2, gives the oxygen.

After completion of the analysis the apparatus should be left filled with nitrogen and with the screw clips all closed. A short piece of rubber tubing should also be connected with the upper end of reservoir *H* and closed by pinchcocks. This is to prevent undue oxidation of the pyrogallic solution.

As already stated, a slight error is incurred in using the above apparatus during the adjustment of the pressures. With a little practice, however, this becomes very small. It is very important to make certain that all the rubber unions are perfectly tight, which is best ensured by wiring the tubing.

Collection of Alveolar Air. Place the mouth piece of the expiration tube in the mouth and close the nostrils by a clip. Breathe quietly through the tube till accustomed to it. After a normal inspiration expire as deeply as possible through the tube and then close the mouth piece by the tongue. The operator at this moment aspirates a sample of air into the syringe attached to the side tube on the expiration tube. This is then analysed. The observation is repeated after a normal expiration.

After using, the glass mouth piece must be removed and placed in a vessel containing weak carbolic acid solution. Before using, it is rinsed in water.

CHAPTER XIII.

RESPIRATORY EXCHANGE IN MAN. (TISSOT-CARPENTER METHOD).*

The principle of the method consists in the collection of a definite volume of expired air in an accurate spirometer and the subsequent analysis of a mixed sample of it. It is then possible to compute the energy metabolism (Indirect Calorimetry). After correcting for temperature and pressure, the total CO_2 output and O_2 intake, and from them R.Q., are then computed.

Experiment 44 The subject sits on a chair and takes the mouth-piece A (Fig. 33) of the respiration tube in his mouth. The mouth-piece consists of an elliptical rubber flange with a hole in the centre connected with the respiration tube. Two rubber lugs are provided and these are gripped between the teeth, the flange being placed between the lips and the gums. The nose is tightly closed by a suitable clamp.

The Respiration Tube leads to *the valves*, *B*, (Douglas' or Pearce's), the end of the expiration tube of which leads to a large stopcock, C, connected with the interior of the spirometer. The latter consists of a 100-litre inverted aluminium cylinder, D, suspended from a pulley, E, in a water bath between the double walls of an upright stationary cylinder, F. As the aluminium cylinder rises out of the water on account of air entering it, its weight becomes greater. To allow for this, the wheel is made excentric, (G), so that as E revolves, with elevation of the spirometer, a weight connected by a thread to the circumference of the excentric exercises a progressively greater pull on the spirometer, and so exactly counterpoises it. The top of the cylinder is provided with holes for the insertion of a thermometer and of a tube and stop cock for drawing off the sample of air for analysis.

*Since the main thing to be learned in this observation, apart from the methods of analysis of the air, is the method of calculation of the respiratory quotient and of the total respiratory exchange, the experiment can be profitably assigned to a larger group of students than the usual. After the sample of air has been collected, certain members of the group will proceed to analyse it and make the necessary calculations. Since the greatest cause for delay in this experiment is in the analysis of the oxygen in the air sample, this procedure should be practised before starting the experiment, and a student who has already had experience with the gas burettes should demonstrate the technique to others.

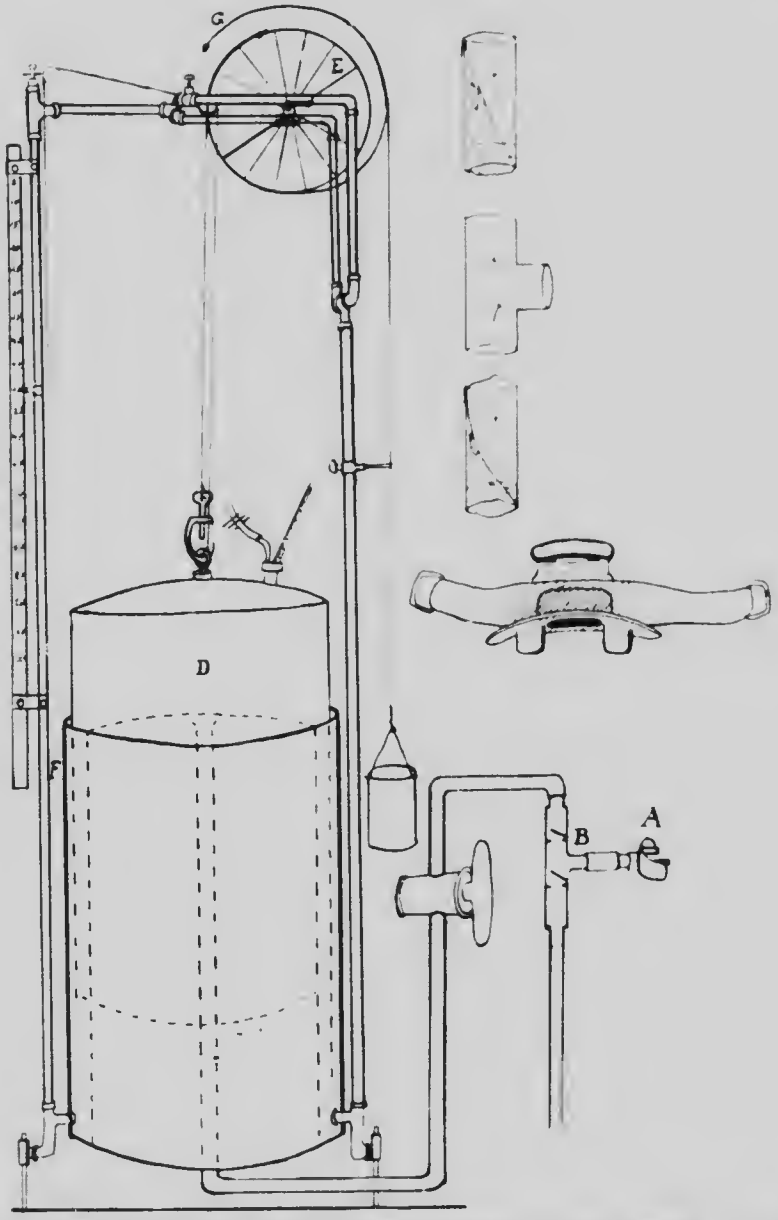


FIG. 33. Tissot-Carpenter Spirometer, Douglas mouthpiece and Pearce's valves. The dotted lines in the diagram indicate the outline of the aluminium cylinder while in the water seal between the outer and inner steel cylinders, the position of the latter of which is also indicated by dotted lines. For further references see context. The inserts show the Douglas mouthpiece and Pearce valve, the latter of which is made as follows. Prepared casings used in the manufacture of bologna sausage are obtained preserved in salt, and they will keep indefinitely on ice. When needed a short piece is taken, washed free from salt by allowing water from the tap to run through it, and softened in a weak glycerine solution. The gut becomes very soft and pliable, and does not dry quickly. A piece of the casing about 10 cm. long is threaded through a glass tube of about 15 mm. bore and 4 to 6 cm. long. One end of the casing is brought around the outside of the tubing and secured by means of a thread. The lower end of the membrane is pinched off and the casing is then cut a little more than half way across its middle, so that the opening will lie just within the free end of the tube when the casing is drawn back through it. The loose end of the casing is slightly twisted—an essential procedure—and is then secured by a thread on the outer side of the tube.

In conducting the observation, the spirometer is first set at zero on the scale at the side. The subject then breathes for some time through the valves and a side tube with the stopcock turned off. When the breathing is comfortable, the stopcock is turned so that the air enters the spirometer, and the side tube is closed by placing a clamp on the rubber tubing on its free end, the exact moment being noted. The air is allowed to collect for a definite period of time (1 to 5 minutes for a subject at rest), at the end of which the stopcock is again turned and the side tube opened so as to connect with the outside. The temperature in the spirometer and the barometric pressure are then carefully noted, as well as the volume of air in the spirometer. The number of respirations during the observation is also counted.

The sample of air for analysis is collected in an all-glass syringe, the nozzle of which fits into the rubber tubing of the stopcock on the top of the cylinder. To allow for the dead space of the tubing, the syringe, after filling, is removed from the tubing (after turning off the tap again), emptied and reconnected. The piston is then moved out and in several times, and the syringe, filled with air, is removed to the gas analysis burette and the analysis made according to the directions in Exp. 43.

It is particularly important to make certain that all the oxygen is absorbed by the pyrogallate and that the air entrapped in the tubing leading to the CO_2-absorption pipette is included in the analysis.

THE CALCULATIONS.—The following have to be determined:

1. Per cent CO_2 and O_2.
2. Respiratory Quotient.
3. Total vol. CO_2 and O_2 respired per kgm. body weight and per square metre of body surface.

An example will serve best to show the method of calculation: (The temperature and barometric pressure, as taken at the time of the experiment, were 20°C. and 747 mm. Hg. respectively)

CO_2 analysis:

1st reading of burette	10·60
2nd reading of burette after absorption of CO_2	9·60
CO_2 absorbed	0·40

= 4·0 per cent CO_2 in expired air.

O_2 analysis.

2nd reading of burette.	9.60
3rd reading of burette after absorption of O_2.	7.95
O_2 absorbed.....	1.65

= 16.50 per cent of O_2 in expired air.

Determination of R.Q.:

O_2 in atmospheric air	20.94 per cent.*
$O_2 - CO_2$ in expired air (16.50 + 4) =	20.50 per cent.

100 − 20.94 = 79.06 per cent, N in atmospheric air.

100 − 20.50 = 79.50 per cent, N in expired air.

Since the nitrogen is not changed in volume, the last figure shows that more oxygen must have been taken in during inspiration than $O_2 + CO_2$ has been given back in expiration. This obviously must be taken into account in the calculations. The amount of O_2 actually inspired for each 100 c.c. of air expired is found as follows:

$$\frac{\text{20.94 per cent. } O_2 \text{ in atmospheric air}}{\text{79.06 per cent. } N_2 \text{ in atmospheric air}} \times \text{79.50 per cent. } N_2 \text{ in expired air;}$$

or 0.265 (constant factor) × 79.5 per cent. N. (found for this observation) 21.06†.

21.06 − 16.50 = 1.56 per cent. O_2 actually absorbed.

100 − 0.03 (CO_2 in inspired air) = 3.97 per cent. CO_2 excreted.

$\frac{3.97}{1.56} = 0.87$, the respiratory quotient, or ratio of CO_2 excreted to O_2 absorbed.

Total Gas Exchange.—The volume of air expired in 15 minutes into the Tissot spirometer was found to be 100 litres measured at 20° C. and 747 mm. Hg. (brass-scale barometer). This volume of gas must be corrected so as to give the volume of dry air at 0° and 760 mm. Hg. To do this two things must be taken into account. (1) Since the expired air is saturated with water, the pressure due to water vapour must be subtracted from the observed barometric pressure to obtain the true pressure. The vapour tension of water for various temperatures is given in the Appendix, Table II. (2) The barometer tube lengthens or contracts with heat or cold, and therefore the barometric reading must be corrected. The figure corresponding to the temperatures is subtracted from the barometric reading in order to obtain correct barometric pressure.

In the above experiment, the correction for the barometer is 2.11 mm., and that for vapour tension at 20° C. is 17.4.

The formula applied to the present problem reads:

$$Vo = \frac{100 \times 727.2}{760(1 + 0.003665 \times 20)} = 89.2 \text{ liters.}$$

The latter calculation can be considerably simplified by using standard tables which give constants for corrections of gas volumes‡ (see Appendix).

* This is the constant O_2 percentage in air.

† This calculation can be simplified by using an abbreviated table giving the O_2 figure corresponding to the various percentages of N. in the expired air.

‡ For further details see Macleod, Physiology and Biochemistry in Modern Medicine (2nd ed.) C. V. Mosby Co., p. 554.

CHAPTER XIV.

DETERMINATION OF THE GASES OF BLOOD BY THE PUMP METHOD.

When blood is exposed to a perfect vacuum all of its gases are evolved. The evolved gases can then be transferred to a suitable burette and their nature and relative amounts determined. The gas cannot be completely removed by one exposure of the blood in a vacuum, because in such a case the gas would be evolved only until equilibrium had become established between the partial pressure produced by the evolved gas, and that still present in the blood. It is necessary to repeat the evacuation several times. For this purpose a mercury pump is usually employed, but satisfactory evacuation can also be brought about by using the simpler apparatus described in the following experiment.

Experiment 45. Place about 15 c.c. defibrinated ox blood in a 500 c.c. flask, and fill the latter with alveolar air by expiring deeply into it through a piece of wide-bore rubber tubing, or better, through a glass tube inserted through the stopper of a bottle filled with glass beads. This condenses and removes the water from the air. Rotate the flask, so that the blood forms a thin film on the walls, but do not shake in such a manner as to cause the blood to froth. While rotating, occasionally expire through the tube into the flask so as to maintain the percentage of carbon dioxide constant. Continue this procedure for about three minutes, and then close the flask. By this means the blood absorbs oxygen to full saturation, and carbon dioxide to the same extent as the blood in the pulmonary capillaries.

Meanwhile the bulb of the blood receiver (Fig. 34A) is partially evacuated by connecting it, by means of the attached piece of rubber tubing, to a water vacuum pump. The screw clip (D) is then tightened (leaving as long a piece of tubing beyond the clip

as possible), the side tube of the pump opened* and the blood bulb removed. A few drops of the anti-foaming solution (caprylic

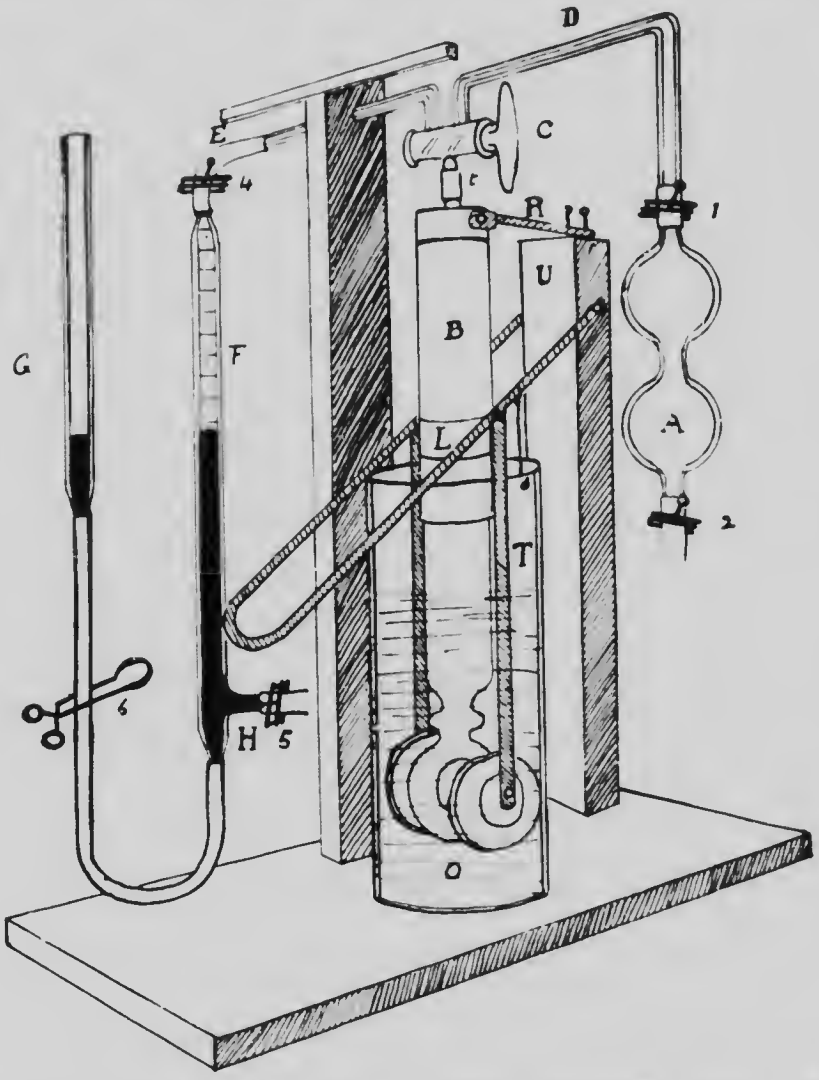


FIG. 34. Apparatus for measurement of gases of blood by pump method. For description, see Context.

alcohol or isoamylisovalerate), is placed in the receiver. This is

*The water must never be turned off from the pump while this is connected with a partially or completely evacuated vessel. Always allow air into the pump before turning off the water.

accomplished by taking about 0.5 c.c. of the fluid in a glass tube of sufficient external diameter to fit tightly the rubber tubing of the receiver (a 1 c.c. pipette with the delivery end partially cut off). Before inserting the pipette into the rubber tubing, the lumen of the latter beyond the clip is filled with the anti-foaming solution, so that no air may enter the receiver, and after inserting, the screw clip (I) is very cautiously opened and about 0.1-0.2 c.c. of the solution allowed to run in, after which the clip is again screwed tight, the pipette removed, and the solution still in it replaced in the stock bottle. The receiver is reattached to the water pump and evacuated as far as possible.

It is then attached to tube D of the blood pump and evacuation completed by manipulating the pump as described below for the evacuation of blood. When completely evacuated, as judged by the inability to suck over more air, screw clip I is closed and the blood receiver removed from the pump.

Ten c.c. of blood is now placed in the receiver. To accomplish this, blood is removed from the flask by means of the special 10 c.c. pipette, using only gentle suction and filling to the upper mark. All air is squeezed out of the tubing on the blood bulb, and the end of the pipette inserted in the tubing, being careful to see that no air bubbles are present at the union. Holding the pipette and blood bulb vertically, the clip is very cautiously unscrewed, and the blood allowed to flow from the pipette into the bulb until the lower mark on the former is reached. The capacity between the two marks is 10 c.c. After tightening the screw clip, the pipette is removed and the blood left in the tubing is squeezed out.

The blood pump must now be prepared.

This consists of a 50 c.c. all-glass (Luer) syringe (*B*), with vaseline between the walls and piston, the nozzle being connected by thick-walled rubber tubing, *t*, with the single tube of a three-way stopcock (*C*). Of the other tubes of the stopcock, one (*D*) runs to connect, by narrow-bore glass tubing, with the blood bulb and the other (*E*) to the gas burette (*F*)— a 10 c.c. graduated pipette is satisfactory. To avoid all risk of air leakage into the syringe, this is manipulated in an oil bath (*O*) as shown in the figure. The barrel of the syringe is clasped at its upper end by a brass collar (*L*) which is held by the iron rod (*R*) to the upright (*U*). To the latter is also hinged the free ends of another iron rod bent on itself (*S*), the bend being about 30 cm. from the free ends. Two strips of brass are loosely attached to the two arms of the bent rod on either side of the syringe where they cross it, and these run down to connect with the head of the piston of the

syringe. This connection will vary with the exact shape of the head of the piston, but in any case, free play must be possible at the joint. In the syringe used in this laboratory the head of the piston is ring shaped and is satisfactorily connected by fitting a wooden bobbin in the ring and attaching the brass strips to the end of the bobbin. The syringe is surrounded by a glass cylinder containing a fairly heavy mineral oil (Mobile oil) and this cylinder is attached to the iron lever so that it moves up and down with the piston.

The first step in preparing the blood pump is to get rid of all the dead space in the tubing and connections. This is readily accomplished, for *E*, by turning stopcock *C* so that *E* communicates with *B*, raising the levelling burette *G*, and simultaneously withdrawing the piston of the syringe by depressing the lever until about 20 mm. of mercury has collected on the top of the piston. The stopcock is then turned so that *B* and *D* are connected and the piston raised until all the air is expelled and mercury completely fills tube *D*. Any drops of mercury falling from the open end of *D* must be caught in a small beaker. The mercury left on the top of the piston seals this completely during the subsequent manipulations.

After squeezing all air out of the tubing on the blood receiver, this is connected with *D*, and immersed in a jug containing water at 45° C. Having turned *C* so that *B* communicates with *D*, the piston is then depressed to about the 20 c.c. mark, and while still depressed the screw clip (1) is opened. About this time the blood will begin to "boil" and the gases given off from it will pass into the vacuum above the mercury in the syringe. *C* is turned so that *B* is closed off and the piston allowed slowly to ascend. (It must not be allowed to ascend too rapidly, since this might break the syringe). The gas which has collected in the syringe is then expelled into the burette (*F*) by turning *C* so that *B* and *E* communicate and pressing up the piston. After all the gas is out of the syringe, the mercury is allowed to run into *E* a short distance, being careful not to allow any to get into *F*. This first process obviously removes only a small fraction of the total gas in the blood, and it must be repeated several times exactly as described above, until no more gas can be secured. The dislodgement of the gas from the blood is greatly accelerated by warmth and by occasionally removing the bulb from the water-bath and shaking briskly.

It is now necessary to measure and analyse the evolved gas. For this purpose the piston is cautiously pushed up, with *E* and *B* in communication, until mercury stands at the zero mark on the neck of the gas burette. The clip (4) is then screwed down and the levelling burette (G) lowered until the menisci stand at the same level in it and the burette. This brings the gas to atmospheric pressure and the volume is read and noted. The reading gives the C.C. of gas in 10 c.c. blood. The volume should be reduced to standard temperature and pressure (for calculation see Exp. 44). To analyze the gas a 40 per cent. solution of sodium hydroxide is sucked from a watch glass into a 2 c.c. all-glass syringe, and the tube of the syringe inserted in the side tube (H). All air must be expelled from this tube. With the pinchcock (6) closed, the clip (5) is opened, while gentle pressure is being maintained on the piston of the small syringe so that the mercury may not run into it. The NaOH runs up to the top of the mercury column (F), and when it is all in, clip 5 is again screwed down. The syringe (I) is removed and *F* inverted several times so that the carbon dioxide in the gas contained in it may be thoroughly absorbed. On now opening clip 6, the mercury will rise in *F*, and by adjusting the levelling tube the shrinkage in volume due to the absorption of CO_2 can be ascertained and the percentage of this gas determined. The reading is taken which corresponds to the top of the NaOH solution, a similar amount of NaOH solution being placed on the top of the mercury in the levelling tube. Care must be taken to see that all the CO_2 is absorbed.

To absorb the oxygen, about a gram of pyrogallic acid is dissolved in 2 c.c. of water in the watch glass, the solution is introduced into *F*, and the further manipulations conducted in the same manner as for the NaOH solution. The gas which remains when both CO_2 and O_2 are absorbed is nitrogen. There should not be more than 0.1-0.2 c.c., any larger amount being due to air leakage into the apparatus during the manipulations.* By taking proper precautions, however, the residual nitrogen should never be more

*If any considerable amount of nitrogen is left, its volume should be measured, and after subtracting O_2, the volume of O_2 that must have been introduced, as air, calculated and subtracted from the actually observed O_2.

than 0.3-0.5 c.c. The results are to be calculated to give percentages of each gas in 100 c.c. of blood.

When the analysis is completed, the mercury is run out from the burette by the side tube (*H*), after removing the stopcock (*c*), and the burette thoroughly washed with water. The mercury and alkali pyrogallate solution (which is now brown in colour) are then washed in running water until the washings react neutral to litmus paper. The mercury should then be transferred to a separating funnel containing a dilute solution of sulphuric acid. The blood bulb should also be cleaned immediately, since otherwise a sticky precipitate which is difficult to remove adheres to the walls.

CHAPTER XV.

DETERMINATION OF BLOOD GASES BY THE CHEMICAL METHOD.

Instead of pumping the gases out of blood, they may be displaced by chemical means. Each method has its own advantages. By the pump the gases are obtained unmixed with air so that their analysis tells us directly how much of each is contained in the blood. By the chemical method the oxygen that is loosely combined with haemoglobin is expelled by shaking the laked blood with potassium ferricyanide (the oxygen liberated during the reduction of this salt into ferrocyanide displaces the oxygen which is loosely combined with the haemoglobin, and takes its place to form methaemoglobin). The carbon dioxide is expelled by adding a non-volatile acid (saturated solution of tartaric acid). The volume of the expelled gases may be measured in a suitable burette, if proper care is exercised to avoid change in volume due to variation in temperature. The chemical method is the more practical for most work, but since the gases become mixed with the air originally present in the apparatus, it is not suitable for proving what these gases may be. Logically, therefore, in studying the blood gases the pump method should precede the chemical.

Experiment 46. – The technique is as follows: The water jacket of the burette (Fig. 35) (G) and the water bath of the blood bottle (D) are filled with water that has been standing for some time in the same room and the temperature is noted. With the stopper removed from the bottle the fluid (solution of calcium chloride) in the burette is adjusted to the zero mark by raising or lowering the levelling tube (H). 20 c.c. of CO_2-free weak ammonia water* is then placed in the bottle (C), and 2.5 c.c. (indicated by file mark) of a freshly prepared saturated solution of potassium ferri-

*0.5 c.c. Aq. Ammonia per 1000 c.c. of distilled water to which some barium hydroxide has been added and then some ammonium sulphate, the resulting precipitates of $BaCO_3$ and $BaSO_4$ being allowed to settle.

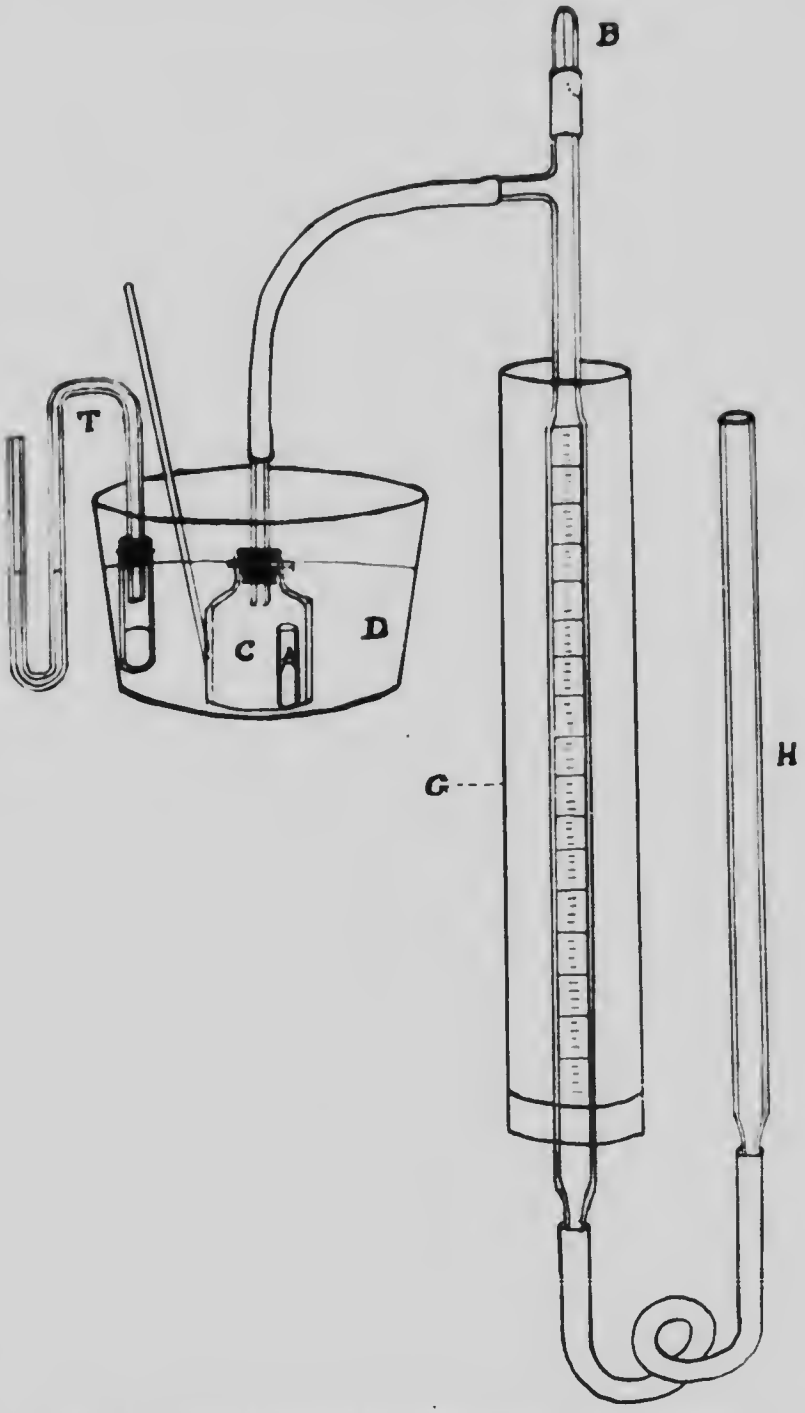


FIG. 35. Haldane's apparatus for analysis of blood by chemical method.

8—

cyanide in the small flat-bottomed tube (A). About 15 c.c. of defibrinated blood is exposed in a 250 c.c. flask, to alveolar air obtained by expiring deeply into the flask and rotating so that the blood forms a film on the walls. The blood will become saturated with oxygen and it will take up CO_2 until there is equilibrium between the tensions of this gas in the air and blood. (Is this all the CO_2 with which the blood could combine?) The rotation should be kept up for about two minutes, the air in the flask being meanwhile repeatedly replaced by alveolar air.

Immediately it has settled to the bottom of the flask, 10 c.c. of the blood is removed by the pipette and, after wiping the tip with a cloth, slowly delivered under the ammonia solution in the bottle. The bottle is then gently shaken until the blood is completely laked and a transparent red solution is obtained. (In cases where the blood is not saturated with oxygen, as, for example, in venous blood, it is necessary to postpone the laking process until after the bottle has been closed and connected with the burette, since otherwise O_2 would be absorbed from the air.) Having placed the flat-bottomed tube (A) upright in the bottle and with the air outlet of the burette (B) open, the stopper is firmly inserted into the bottle, which is then immersed in the water bath, and the water stirred. Whenever the fluid in the burette ceases to move further—indicating that the temperature of the air in the bottle has become the same as that of the water bath—the air outlet is again opened to allow the meniscus of fluid in the burette to return to the zero mark.

To displace the oxygen, the bottle is removed from the water bath, and while holding it in a towel, to prevent its becoming warmed by the hand, it is tilted so that the ferricyanide spills into the laked blood. The bottle is shaken for about one minute without allowing the contents to come in contact with the stopper or tubing. The expelled oxygen depresses the fluid in the burette, and as it does so, the levelling tube should be lowered so that there may not be increased pressure in the apparatus, which would encourage leaks. The bottle is returned to the water bath, and the water stirred until the level of fluid in the burette remains constant. The reading on the burette, taken when the levels of fluid in it and the levelling tube are exactly the same, gives the c.c. of oxygen expelled from

10 c.c. of blood. To make certain that all the oxygen has been expelled, the above procedure must be repeated. The two readings should agree. Great care must be taken to keep the apparatus away from drafts or other influences that might cause the temperature of the water to change. If this occurs, the temperature in the bath and jacket must be brought back to the original temperature before the final reading is taken. A small air thermometer T, in the shape of a U-tube (containing coloured water) connected with a test tube, (weighted with sand) is also placed in the bath. If the menisens of fluid in the thermometer changes during the observation hot or cold water must be added to the bath and this stirred until the original temperature is regained.

The carbon dioxide is measured by a repetition of the same technique, using tartaric acid in place of ferricyanide solution. The steps are as follows: the air outlet is opened, and the menisens of fluid in the burette brought back to zero by raising the levelling tube, and removing the stopper of the bottle. The reagent tube is withdrawn and washed into the bottle with as small a quantity of CO_2-free water as possible (water that has been boiled and cooled). 2.5 c.c. of the tartaric acid solution is placed in the tube, the stopper reinserted with the air vent opened, temperature adjustment made, and the CO_2 displaced and measured by the same manipulations as for oxygen. The bottle must be very thoroughly shaken since the CO_2 is difficult to dislodge from the viscid mixture of precipitated blood proteins now present in the bottle.

When the estimations are completed, the flask, pipette, bottle, reagent tube, etc., are washed thoroughly clean, and any fluid that may have passed into the connecting tube, cleaned out thoroughly with water and a pipe-cleaner.

CHAPTER XVI.

THE DISSOCIATION CURVE FOR OXYGEN AND THE CO_2-COMBINING POWER OF BLOOD.

For accurate determination of the relative amounts of reduced and oxyhemoglobin in blood exposed to atmospheres containing varying partial pressures of oxygen no method surpasses that of Barcroft and his coworkers. The principle of this method is to expose a small quantity of blood in a thin film on the walls of a relatively large cylindrical vessel (tonometer) containing a mixture of nitrogen and oxygen gases until equilibrium has become established between the partial pressure of the oxygen in the atmosphere and the absorption of oxygen by the blood. Some of the blood is transferred to a small bottle connected with a differential manometer and shaken with dilute ammonia water, whereby it becomes laked, and by taking up oxygen, causes shrinkage in the volume of air in the bottle, the degree of which is indicated by the manometer. The oxygen-saturated blood is then shaken with ferricyanide of potassium which dislodges the oxygen and causes the pressure in the bottle to rise. From the relative displacement of the fluid in the manometer in the two observations, the percentage saturation of the blood with oxygen is readily calculated.

For use by a class of students the method is not practical because of the difficulty of providing suitable mixtures of oxygen and nitrogen with which to fill the tonometer, and because of the expense of the differential manometer. The first of these difficulties is overcome by exposing the blood to a partial vacuum instead of a mixture of gases, the partial pressure of oxygen being readily calculated from the degree to which the tonometer is evacuated as measured by a barometer. After exposure to the partial vacuum, the blood is transferred to a simple form of differential blood gas manometer.

Experiment 47. The following apparatus is required:

The Tonometer consists of a wide glass tube (the tonometer T, Fig. 36) of fairly stout glass, tapering down to narrow tubes at both ends. The capacity

should be at least 200 c.c.* The narrow tubes are connected with thick-walled (pressure) rubber tubing which should be wired on to the glass tubes. The rubber tubes are closed by screw clips (1 and 2). File marks are made at one of the tapering ends of the tonometer, the distances between them corresponding approximately to one cubic centimeter.

THE BAROMETER consists of a vertical thick-walled glass tube about 1.25 metres long and of about 3 mm. bore bent on itself near one end, and with the

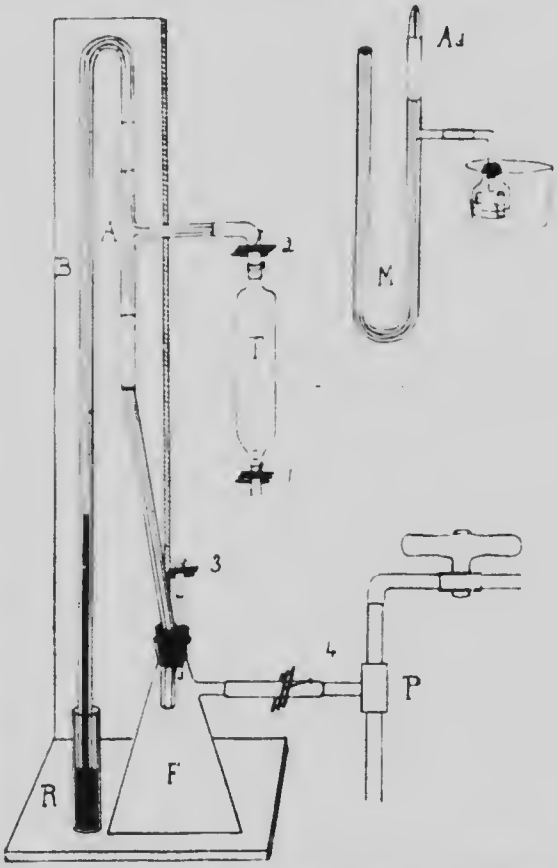


FIG. 36. Apparatus for the determination of the Dissociation Curve.

other end dipping into mercury contained in a wider flat-bottomed (specimen) tube (the mercury reservoir) closed by a perforated cork. The barometer tube and reservoir are firmly mounted on a stand furnished with a millimeter scale, which is attached to the stand in such a way that it can be adjusted to bring it-

*It would be preferable to use a tonometer twice as large since this would diminish errors due to the addition of the gas given off from the blood.

zero to the surface of mercury in the reservoir, as this varies at different pressures. The free end of the barometer tube is connected by rubber pressure tubing to a glass T piece *A*, one limb of which is similarly connected to a stout walled filtration flask *F* joined to a good water pump *P*. A capillary tube closed by a piece of rubber tubing and a screw clip (3) also passes through the stopper of the flask.

THE DIFFERENTIAL MANOMETER AND GAS BOTTLE. This apparatus is made from a piece of narrow bored glass tubing (bore 1 mm. bent into a U shape with one limb about 200 mm. long and the other about 150 mm. (*M* in illustration). To the shorter limb is connected a T-piece (of narrow bore tubing) which should be fused to it, although rubber pressure tubing is quite satisfactory. One limb of the T-piece is connected with red rubber tubing of moderately thick wall, closed by a pressure adjuster (*Ad*) as described elsewhere*. By pinching the rubber tubing lying over the lateral hole of the adjuster, the manometer is brought into communication with the outside so that the air in the bottle and manometer are brought to atmospheric pressure. The other tube of the T-piece is connected by pressure rubber tubing with a glass tube which passes through a rubber stopper that accurately fits a small wide-mouthed bottle *b* of about 15 c.c. capacity. Finally a small flat-bottomed test tube 15 mm. high and 6.7 mm. diameter is required. Instead of attaching the T-piece to the short limb of the manometer, it may be inserted in the rubber stopper, one limb being connected with the manometer (which in this case simply consists of a U-tube—and the other with the pressure adjuster. The fluid used in the manometer is clove oil.

The first step is to rinse out the tonometer with 0.9 per cent. saline and connect it with the side tube of the barometer T-piece *A*. The pump *P* is turned on with screw clips 1 and 3 closed, but screw clip 2 open and the pressure lowered until the mercury stands at a constant level in the barometer. Screw clip 4 is closed and the mercury observed, to see whether there is any leak. Provided there is none, clip 3 is cautiously opened and the mercury allowed to fall almost to the level in the reservoir (*R*); clip 2 is tightened, the tonometer, *T*, removed and the pump turned off. Defibrinated or oxalated blood (whipped ox blood is most suitable for large classes, but in any case blood from an etherized animal must not be used) is now sucked into the tonometer, by placing some of the blood in a small evaporating dish, and, with the rubber tube dipping into it, cautiously loosening clip 1; 3 to 4 c.c. of blood should be allowed to enter the tonometer. This is then reattached to the T-piece *A* of the barometer and with clips 2 and 4 open (but 1 and 3 closed) the pump is turned on and the mercury allowed to rise as far as it will go when clip 4 is closed and the pump turned off. Clip 3 is

* See page 98.

now cautiously opened until there is a partial pressure of about 20 mm. Hg. oxygen in the tonometer.*

When the mercury has reached this level, or one near it, clip 3 is closed and the height at which the mercury stands very accurately noted. Clip 2 is closed, after which the mercury is allowed to fall to zero by opening 3. The tonometer is removed and rotated so that the blood becomes spread out as a thin film on the walls, after which it is placed in a water-bath kept about 40° C. in which it is constantly rotated for about 15 minutes.

On removal from the bath the pressure in the tonometer must again be measured. For this purpose the tonometer is reattached to *A* and the pump is turned on (with 3 closed) until the mercury has risen to the level at which it previously stood. Clip 4 is closed and 2 opened. If there has been no leak, and time has been allowed for the tonometer to cool down, there will be practically no difference between the two readings. If a difference of more than 5 mm. is observed it must be noted and the pressure prevailing in the tonometer taken as the average between the two readings.

Meanwhile 3 c.c. of freshly prepared weak ammonia water containing a trace of saponin (0.5 c.c. aq. ammonia in 500 c.c. water) has been placed in the blood gas bottle *B*. A pointed glass tube about 30 mm. long is now attached to the rubber tubing of the tonometer and this is removed from the barometer and held in a vertical position above the bottle. The screw clip 2 is opened so that the

*This is computed as follows: After suitable adjustment the standard barometer in the room is read and from the reading is deducted the tension of aqueous vapour at the temperature of the room (for Table, see page 236 of Appendix). The difference gives the pressure in mm. Hg. of an atmosphere of dry air. Since air contains 20.96 oxygen, the partial pressure of this gas in the tonometer must be equal to $\frac{20.96}{100}$ ths. of the difference between the height to which the mercury is raised in *B* and the corrected barometer reading. Thus, suppose the room barometer to be 753.4 mm., the corrected barometer reading is 753.4-17.4 = 736 mm. and the temp. 20° C. Then $\frac{20.96}{100} \times 736 = 154.2$ mm. H_2O

and 20 mm. $O_2 = \frac{20 \times 736}{154.2} = 95.45$ mm.

That is the mercury in the barometer must be raised to 736 − 95.45 or 640.55 mm above the level in the reservoir (*R*).

air enters the tonometer, the clip 1 is then cautiously loosened to let a drop or two of blood flow out from the tip of the glass tube,* and after closing it again the end of the tube is wiped free of blood and placed in the bottle so that it dips under the ammonia solution. Clip 1 is now cautiously opened and about 1 c.c. of blood allowed to flow under the ammonia. If this is done carefully the blood does not mix with the ammonia water which floats on the top of it as a layer and so prevents any diffusion of oxygen between the blood and the air. The bottle is firmly closed by its stopper, the pressure adjuster being meanwhile held open so that the level of the clove oil in the manometer is not disturbed. The bottle must then be submerged in a water-bath containing water at about room temperature, in which it is left until, with the adjuster closed, no further contraction of volume, due to cooling, is observed to occur.

The bottle is now removed from the bath and vigorously shaken so that the blood becomes laked and absorbs O_2 from the atmosphere of the bottle. On replacing the bottle in the bath and allowing time for cooling the difference between the levels of clove oil in the two limbs of the manometer is noted. With the adjuster open to the outside, the stopper is removed from the bottle and about 0.25 c.c. of a freshly prepared saturated solution of potassium ferricyanide is placed in the small flat-bottomed test tube, which is then lowered by means of a forceps into the fluid in the bottle, without allowing any of the ferricyanide to mix with the laked blood. After reinserting the stopper and cooling, the bottle is again removed from the bath and shaken so that the ferricyanide, by mixing with the laked blood, drives off the loosely combined oxygen and raises the pressure, which is measured by the manometer.

The relative amounts of reduced haemoglobin and oxyhaemoglobin present in the blood are proportional to the first and second readings of the manometer; when all is reduced haemoglobin the diminished pressure (shrinkage) recorded in the first shaking of the bottle is practically the same as the increased pressure recorded in the second. They will not be exactly the same, since the volumes

*Enough blood should be run out to bring the meniscus of blood in the tonometer to the upper file mark.

of bottle and tubing in the two cases are not the same, but the error thus incurred is inconsequential for most purposes (cf. Boyle's law).

The calculation of the percentage saturation of haemoglobin with oxygen is made by subtracting the first reading from the second, dividing by the second reading and multiplying by 100. Suppose in the observation made at 20 mm. partial pressure of O_2 the first reading is 24 mm. and the second, 108, then

$$\frac{108-24}{108}\times 100 = 77.0\%\ HbO \text{ and } 22.3\%\ H_b$$

The result must now be plotted on coordinate paper with the percentages of H_bO along the ordinates and the partial pressure of oxygen on the abscissae. The experiment should be repeated, using 10 mm. and 40 mm. pressures of oxygen, and the results similarly plotted. By joining the points, the dissociation curve for blood is obtained. Care must be taken to see that the bottle is sufficiently shaken so that the partly reduced blood absorbs all the oxygen and gives it up again with ferricyanide. It is particularly in the latter operation that care must be taken.

The Influence of Carbon Dioxide in Lowering the Dissociation Curve can be readily shown by the method. The procedure is as follows: After the pressure has been reduced to the desired degree in the tonometer, the latter is placed in a horizontal position so that the blood lies along the walls, free of the ends. A CO_2 generating apparatus (Kipp's) or a bottle containing this gas is then connected by suitable tubing with the free end of the tonometer, care being taken before making the connection, to fill the tubing with CO_2. To accomplish this a slow stream of the gas is maintained and the air in the tubing beyond the screw clip (1) is squeezed out before connecting with the CO_2 generator. The most suitable partial pressure of CO_2 to work with is 40 mm. and to attain it the CO_2 apparatus is first of all opened and the screw clip 1 very cautiously loosened until, with clip 2 open but 3 and 4 closed, the mercury descends about 40 mm. in the barometer. Clips 1 and 2 are then tightly screwed down, and the tonometer removed, the further procedure being exactly as described above.

The effect of the 40 mm. of CO_2 will be found in the above example, where a partial pressure of 20 mm. O_2 was used, to reduce the percentage of HbO from 77 to about 35.

THE CO_2-COMBINING POWER OF THE ALKALINE RESERVE OF THE BLOOD.

After completing the estimations necessary for finding the percentage of oxyhaemoglobin, in the experiments in which CO_2 is present in the tonometer, it is of interest to determine the amount of this gas with which the blood has combined. This will represent its ability to act as a buffer towards foreign acids. To perform the estimation it is necessary, however, to measure accurately the amount of blood which is removed from the tonometer to the blood gas bottle. This can readily be done by attaching a 1 c.c. pipette to the tubing of the tonometer (beyond clip D), a few drops of blood being allowed to escape from the pipette before delivering under the ammonia solution in the bottle, and precautions being taken not to take any of the upper layers of blood that had been exposed to full atmospheric pressure when the tonometer was opened. This is ensured by removing the pipette from the tonometer before all the blood has run out.

To dislodge the CO_2 from the blood, the stopper is removed with the usual precautions and about 0.25 c.c. of a saturated solution of tartaric acid placed in the small test tube. After closing and allowing for temperature changes, the acid is shaken with the mixture of blood and ferricyanide, and the CO_2 thereby evolved, measured by multiplying the displacement of the fluid in the manometer by a figure (the constant of the apparatus) obtained by a preliminary experiment in which a known amount of a standard carbonate solution is similarly treated.

SECTION V

SPECIAL SENSES

CHAPTER XVII.

VISION.

In order to gain accurate information through the sense of sight about objects in the external world, their size and shape, and their positions relative to one another, two things are necessary. There must in the first place be a group of cells sensitive to light waves and so connected to the central nervous system that stimulation of any part of the sensitive surface gives rise to a sensation different from that caused by stimulation of any other part. Secondly some system of lenses is needed so that each point in the sensitive layer does not receive rays from all directions in the external field, but has focussed on it only those which arise from a single part of the field. In the eye the sensitive elements are contained in the retina, and the cornea and lens together make up the focussing apparatus. In the discussion and experiments which follow we shall first consider the way in which light waves are brought to a focus in the eye and later the response of the retina to them. Before taking up the complex arrangement of refracting surfaces which exist in the eye it is well to review some of the simple cases of the formation of images both by lenses and by mirrors. Although the focussing in the eye is entirely done by refracting surfaces and reflection plays no part in it, it is by reflected rays that one examines the condition of the eye and on this account it is necessary to have clearly in mind the laws which underlie the reflection, as well as those of refraction.

Physiological Optics.

Reflection.

When rays of light diverging from a point are reflected by a mirror their course is so changed that they appear to an observer

to come, not from the actual object itself, but from some other point known as the IMAGE of that object. Each point on a luminous object has a point image corresponding to it. The position of the image of the whole object may be found by defining the position of the image of each of its extreme or limiting points. To find these one applies the LAWS OF REFLECTION, which state (1) that the incident ray (that is, the ray before reflection), the reflected ray, and the perpendicular to the surface at the point of incidence, are all in one plane, and (2) that the angle between the incident ray and

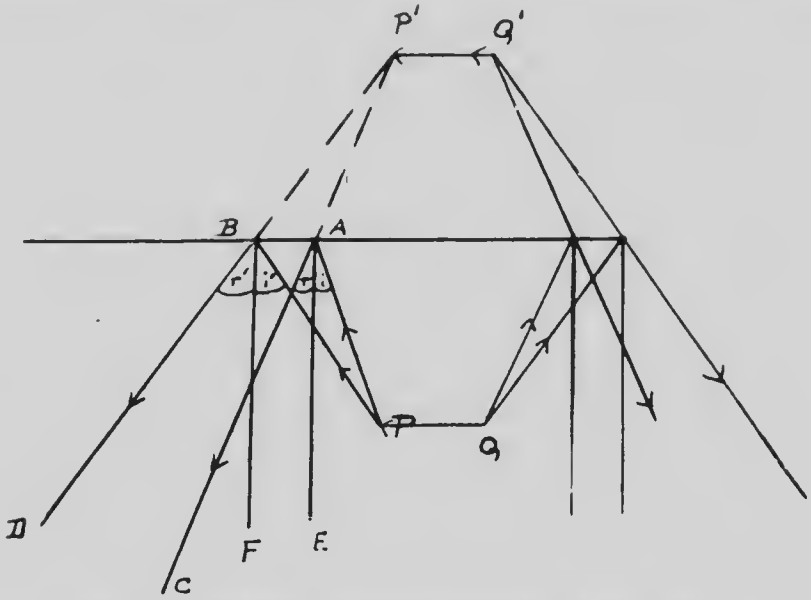


FIG. 37. To illustrate the formation of an image by a plane mirror.

the perpendicular (or the ANGLE OF INCIDENCE) is equal to that between the perpendicular and the reflected ray (the ANGLE OF REFLECTION). Fig. 37 shows the construction for a plane mirror. PQ is the object. PA and PB, incident rays, and AE and BF, the perpendiculars to the surface at the points of incidence. These, together with the reflected rays, AC and BD, are all in the plane of the paper. i and i′ are the angles of incidence. After reflection the rays AC and BD pass in such a direction as to make each angle of reflection equal to the corresponding angle of incidence ($r = i$, $r' = i'$.) These rays both appear to come from a single point

behind the mirror P′, the position of which is found by projecting the lines of the reflected rays AC and BD back until they meet. Since ALL rays from the point P on the object appear after reflection to come from one point on the image, P′, the place from which two of the rays seem to come, must be the apparent source of all the others, in other words, P′ must be the image of the point P. The construction for Q is similar, Q′ being its image. When the

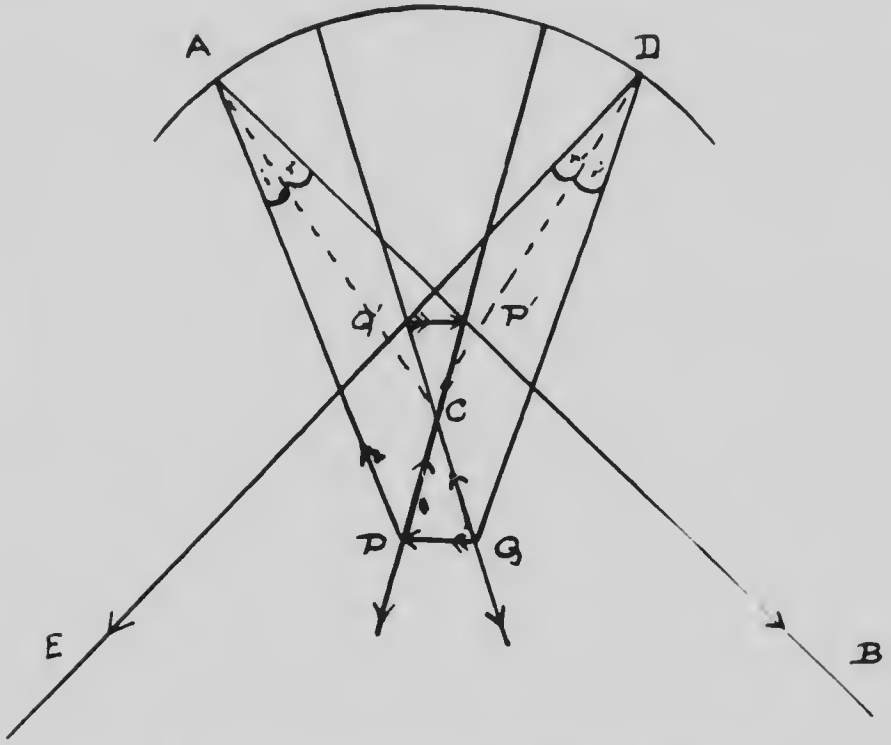


FIG. 38.—To illustrate the formation of an image by a concave mirror.

reflected rays only appear to come from the image, as in these cases, and do not actually pass through it, the image is called a VIRTUAL one. It will be noted that it is erect.

Fig. 38 shows the construction for reflection of a similar object by a concave mirror. C is the centre of curvature of the mirror; any line from C to the surface is perpendicular to it, since it lies on one of the radii. One ray from P, passing through C on its way to the

surface, lies along such a line. It is reflected back along the path on which it came (since $i = o$ in this case, r must also $= o$). PA, another incident ray from P, is so reflected (ray AB) that the angle of incidence (i) equals the angle of reflection (r). The place (P′) where this crosses the reflected ray PC is the image of the point P, from which these and all other reflected rays from P appear to

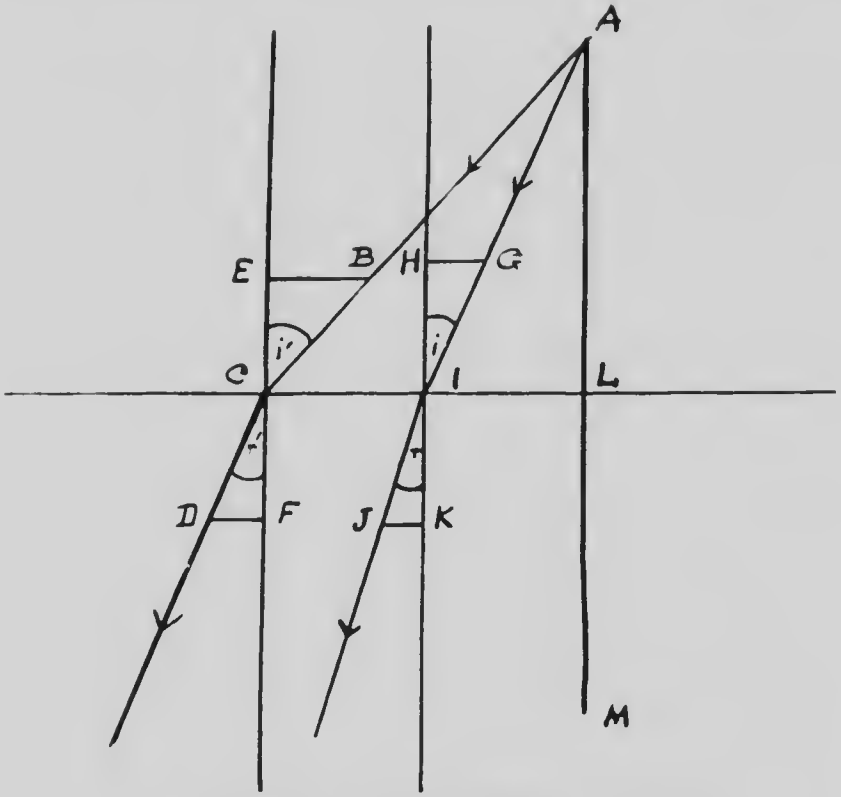


FIG. 39. To show refraction of rays passing from air into water.

come. The construction of the image, Q′, of the other limiting point, Q, is similar. The rays in this case actually pass through the image from which they seem to come, and the image is therefore called a REAL one. Real images are always inverted.

Refraction.

Refraction at a Plane Surface.—When a ray of light passes from air into a denser medium such as glass or water, the direction

in which it travels is altered and it is bent toward the perpendicular to the surface between the two media (Fig. 39). The angle between the incident ray and the perpendicular is the angle of incidence (i and i'), that between perpendicular and refracted ray, the angle of refraction (r and r'). The extent to which this bending or REFRACTION occurs depends on two things. One is the angle of incidence. The greater this angle is, the more the ray is refracted, provided of course that the medium is the same. The other factor is the nature of the medium; the denser the medium the more it refracts. It is found that for a given medium there is a constant relationship between the direction of the incident ray and that of the refracted one; the sine of the angle of incidence divided by the sine of the angle of refraction is always the same for the same medium, greater when the medium is dense and less when it is rare. It is usual to express the refracting power of a medium in this way and to call it the REFRACTIVE INDEX, designated by μ. The example in Fig. 39 will make this clear. AC and AI are incident rays, CD and IJ, the rays after refraction. The angles of incidence are i and i', the angles of refraction, r and r'.

$$\frac{\text{Sine of angle of incidence}}{\text{Sine of angle of refraction}} = \mu \text{ for water} = 1.3;$$

that is $\dfrac{\dfrac{BE}{BC}}{\dfrac{DF}{CD}}$ or (since BC = CD) $\dfrac{BE}{DF} = 1.3$. Similarly $\dfrac{GH}{JK} = 1.3$.

The ray ALM, being perpendicular to the surface, is not refracted but passes through unchanged in direction.

If the direction of the light is reversed and if it passes from the dense to the rare medium the direction of refraction is also reversed, the rays being bent away from the perpendicular instead of towards it.

Refraction at a Convex Surface. It is through a convex refracting surface that the light first passes on entering the eye, as it goes from the air into the layer of tears and the cornea.

The central point on a curved refracting surface is called its PRINCIPAL point (P.P. Fig. 40); a line joining this with the centre of curvature (N.P.) is the principal axis of the refracting surface

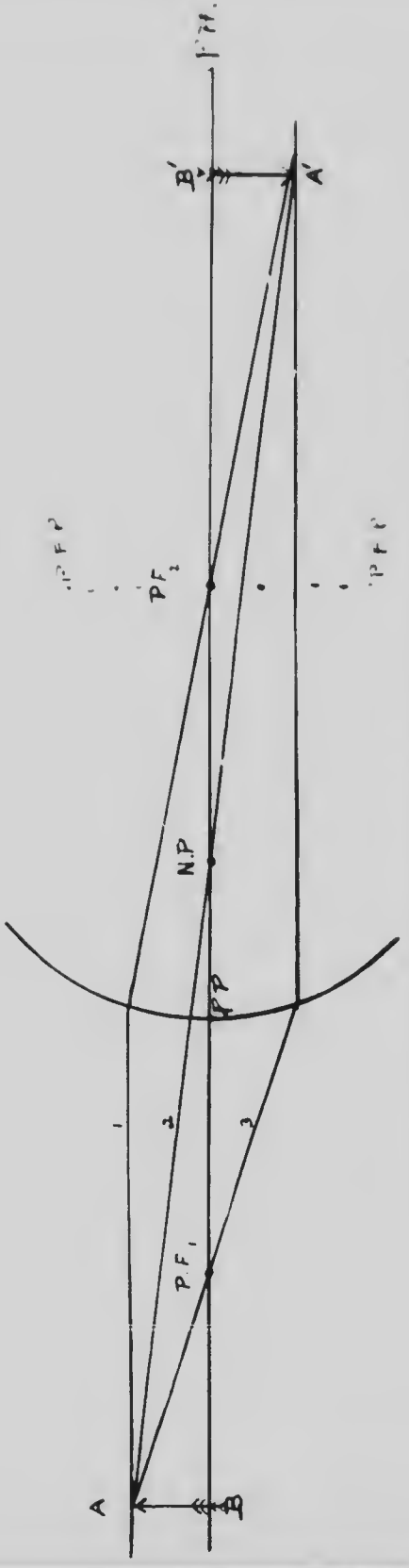


Fig. 40. To show refraction of rays passing through the axes of a convex surface

(P.A.). That ray from the object which is directed straight towards the centre of curvature or NODAL POINT (N.P. of the refracting surface lies of necessity on one of the radii to the surface; it is therefore perpendicular to it and is not refracted (ray 2). If a pencil of rays are parallel to one another and also parallel to the principal axis (see ray 1), they are all brought to a focus after refraction at some point on the axis, this point being known as the SECOND PRINCIPAL FOCUS (P.F$_2$, Fig. 40). This lies nearer the surface if the curvature on the surface is sharp and the refractive index high, farther back if the surface is part of a large circle, or if the refractive index of the medium is not very great. Light to be parallel must in theory come from objects at an infinite distance. In actual fact, however, rays from points more than about ten metres away diverge so little that, for the eye at least, there is no practical difference between their focus and that of rays which arise from further away. All parallel rays which reach the surface inclined at a small angle to the principal axis are brought

to a focus at some point in the plane of the principal focus or the PRINCIPAL FOCAL PLANE (P.F P.). Rays which are appreciably divergent when they reach the surface (that is which arise from objects less than about ten metres away) have their foci behind the principal focal plane. The focus moves farther back the nearer the object is brought until finally a position of the object is reached such that the rays from it after being refracted meet only at infinity, that is, they are parallel to one another. This position is known as the first principal focus (P.F. Fig. 40). If the object is brought nearer, the rays are merely rendered less divergent after refraction and are not brought to a focus at all.

Fig. 40 shows the way in which the position may be found of an image formed by refraction at such a surface. From each limiting point on the object, any two rays, the paths of which are known, are followed until they meet. The place where they cross is the image of the point from which they arise.

Refraction by a Convex Lens. Rays, after they have entered the eye, have to pass through a convex lens, the crystalline lens, before they reach the light-sensitive cells. The refraction in this case is similar to that which we have just considered: like a simple convex surface, a convex lens converges rays which pass through it. The extent of the refraction depends on how sharply the surfaces of the lens are curved and on how much denser its substance is than the surrounding medium. The lens has a principal axis, which joins the centres of curvature of the two surfaces, a first and second principal focus, and a principal focal plane. The point where the principal axis cuts the surfaces is the principal point; rays which pass through here are not appreciably bent if the lens is a thin one.

Experiment 48.—Set in front of the opening of the lantern the ground glass screen, and the diaphragm with a vertical slit. Through a convex lens throw an image of the slit on the black wooden block. The slit is to serve as the "object". Now place against the lens a sheet of paper perforated with two holes, about 3 mm. in diameter, horizontally placed and less than the diameter of the lens apart. This stops all but two pencils of rays from the object. Note that the image does not disappear, but becomes less bright. Cover one hole and see the further dimming of the image.

Move the object further from the lens. There are now two blurred images of the object. Cover the left hole and note that one image disappears.

Place the object at a distance from the lens less than the first. Note that in this case also a double image is formed. Again cover the left hole and see whether the image which disappears is the same one as before.

Draw diagrams of the formation of images by the lens when the object is (a) at a distance from which the rays are focussed on the screen, (b) at a greater distance, (c) at a less distance. Show whether the image in each case is real or virtual.

Since the focal length of a lens varies inversely as its strength, being shorter the stronger the lens, it is usual to express the strength of lens in terms of the reciprocal of its focal length. The standard is a lens which has a focal length of one metre and this is said to have a strength of one dioptre. $D = \frac{1}{F}$, where F = focal length, D = strength in dioptres.

Refraction in the Eye.

If the rays from an object pass in their course through a succession of focussing surfaces, a calculation of the position, size, etc., of the image which they finally form can be made along the lines which we have indicated as long as the surfaces are only two, or at most three, in number. When they are more than that the problem becomes unwieldly. In such a case, however, it is often possible by mathematical calculation to arrive at an ideal, or imaginary, single convex refracting surface which will have approximately the same power as that of all the surfaces together. For this the radii of curvature and the refractive indices of all must be known and the surfaces must be "centred". Surfaces are said to be centred when they all lie along the same principal axis. Light entering the eye has to pass through a large number of refracting surfaces, through cornea, aqueous humour, lens, and vitreous humour. Of these, the cornea, the aqueous humour and the vitreous humour have refractive indices which are much alike, all about equal to that of water ($\mu = 1.3$). The lens is denser and all parts of it have not the same composition. It is made up of more or less concentric layers about a central core and the density increases from the outer layers ($\mu = 1.40$) to the core ($\mu = 1.41$). When they enter the cornea from the air, light rays undergo the main part of the refraction which occurs in the eye, because the densities of the two media are so different. The other considerable refraction occurs on the passage of the rays through the denser substance of the crystalline lens. In the complex system of the eye the refractive

and the radius of curvature of each part is known and the surfaces, although not accurately centred, are sufficiently nearly so to make it possible to find mathematically a single surface which represents the whole. This is used in the construction of the SCHEMATIC EYE (Fig. 41). The single refracting surface is made to lie a few millimetres behind the real cornea. In the unaccommodated eye distant objects are clearly seen, which must mean that the rays from them, which are parallel or nearly so, form sharp images on the light-sensitive surface. The refracting surface of the schematic eye, to represent this, has its principal focal plane on the retina. From a distant point object situated in any part of the visual field, we know the course of one ray, that which is directed straight for the nodal point of the simplified eye and which is not changed in its direction before reaching the retina. By the previous argument we have shown that *all* rays from such a point meet at the retina. Therefore to find the image on the retina of any object, all we need to do is to draw straight lines from its limiting points through the nodal point of the schematic eye.

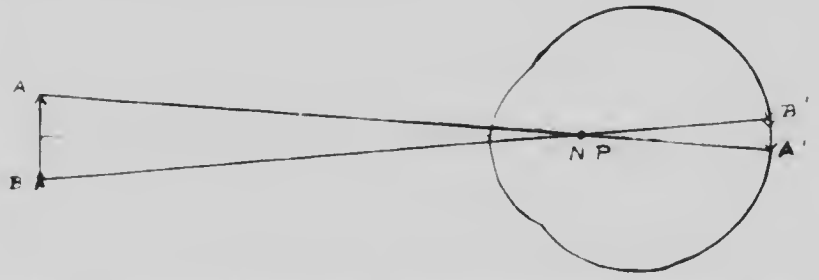


FIG. 41. The formation of an image by the eye as represented by the schematic eye.

It will be seen that according to the foregoing construction the retinal image is an inverted one. That we interpret this to ourselves as an erect picture of the object may at first sight appear confusing. It should be remembered, however, that we are not born with the faculty of associating stimulation of any particular part of the retina with the presence of some object in a particular part of the external world. We learn this in infancy through our other senses, mainly the sense of touch. Because a point on one side of the retina is as a matter of fact always affected by light from some object which we know by our other senses to be on the opposite side of the field, we form the habit of interpreting the stimulation in this way. We associate stimulation of the right side of the retina with the presence of something in the left side of the visual field and so on. To put the matter more generally, we refer stimulation of any part of the retina to the presence of some object in the out-

side world situated along the line which joins that part with the nodal point of the eye. That this is actually the case may be seen from the following experiment.

Experiment 49.—Close the right eye and direct the eyes as far as possible to the left. With the tip of the finger press lightly on the right eye at different points near the margin of the orbit and note the positions in the visual field of the resulting "phosphenes".

In this experiment although we press the right side of the retina all the light spots which we see appear to be on our left, in the upper field if we press below and in the lower if we press on the upper part of the eyeball.

CHAPTER XVIII.

ERRORS IN REFRACTION.

PHYSIOLOGICAL ERRORS. ACCOMMODATION. SPHERICAL ABERRATION.

In the description of refraction by lenses it has been stated that a spherical lens brings all the rays from one point on the object to a focus at the same point. As a matter of fact this is not strictly speaking the case. The rays which pass through the outer parts of the lenses are more refracted than those nearer the centre; they are therefore brought to a focus a little in front of the central ones, and, passing on, blur the image which the latter form. The difference between the foci becomes greater the nearer the object and the more divergent the rays from it.

Experiment 49.—Fill the bottle with water and use it as a lens.* Cover the opening in the lantern by the diaphragm with a 2 mm. opening, and set it about a meter away from the bottle. Move the black screen in the pencil of light coming through the lens until you have the light as sharply focussed on it as possible. Note that the region on either side of the focus is dimly lighted. Interrupt the light coming through the outer parts of the lens and note that the focus becomes sharper. Bring the lantern nearer the lens, set the screen at the new focus and interrupt the outer refracted rays as before. The improvement in the sharpness of focus is more marked.

Cover the round opening of the optical box with the clear glass slide. Set the bottle immediately inside the opening, light the incense in the cork and cover the box. When it has filled with smoke place the lantern about a meter away, so that the

*The lens in this case is of course a cylindrical one. Since, however, we have to do only with those rays which diverge horizontally, we may use the refraction which the curved surface effects in these to represent refraction by any one plane of a spherical lens.

rays pass through the bottle, and look directly down on the refracted rays. The boundaries of the light pencil are curved, and not plane surfaces because the outer rays, being more refracted than the inner, intersect the latter. Move the lantern nearer and note that the curvature of the surfaces increases.

The refraction in the eye is corrected for spherical aberration to some extent by the difference in the refractive indices of the different parts of the lens. The central rays pass through the part of the lens which is the most dense and so they are refracted more, in comparison with the rays through the periphery, than they would be if all the layers were of the same composition.

Chromatic Aberration. Light which arises from objects seen under ordinary circumstances is made up of waves of different lengths. In passing through the refracting media each wave length is bent to a slightly different extent and this causes another error in the refraction of the eye. The shorter waves of the violet end of the spectrum are brought to a focus nearest the lens, the long ones of the red end are least refracted, while the foci of those of intermediate length lie between these two extremes.*

Experiment 50. Chromatic Aberration in Refraction by a Convex Lens. Set in front of the opening of the lantern the ground glass screen and the diaphragm with a 2 mm. opening. Place the block holding the convex lens about 15 cm. from the opening. Using a sheet of paper as a screen move it back and forth in the path of the refracting light until you find the focus. Note that it is not pure white, but made up of coloured bands. Cover the right half of the lens with a card. The light has violet fringe. Uncover the lens and move the screen a little nearer to it. The disc has a violent centre and red border. Move the screen beyond the focus. The colours of the disc are reversed.

Experiment 51. Chromatic Aberration in the Eye. Cover with a card the outer half of the right pupil and, closing the left eye, look at an electric light filament. It appears to have a red border at the right and a violet one at the left. Draw a

*For the way in which chromatic aberration is done away with in lenses of fine optical instruments by using layers of different dispersive power, the student is referred to his text-books on Light. There is no such adjustment in the eye.

diagram of the course of the red rays and of the violet ones, using the schematic eye, and explain the apparent contradiction between the results of this and of the preceding experiment.

When we turn our eyes to look at a certain object we habitually place them so that the image falls on the fovea centralis. This is the part of the retina which is capable of seeing most acutely, of making out differences smaller than can be perceived by any other part, and the line which connects it with the nodal point is called the visual axis. If this corresponded with the optical axis the refraction would be the best that the refracting media of the eye are capable of. In point of fact, however, there is an angle of about 5° between the two and this is a slight additional source of error in refraction in the eye.

Errors Tending to be Pathological. — The defects in refraction which have been described so far are found in every eye. As well as these there are several types of faulty formation of images which are often found, any one of which if it is at all pronounced makes the vision of the eye abnormal.

Near-Sightedness or Myopia. — In near-sighted eyes light from distant objects, that is for practical purposes parallel light, is brought to a focus in front of the retina instead of exactly on it. The rays therefore when they reach the retina are already diverging from their focus and they form a blur on the light-sensitive layer instead of a sharp point of light (Fig. 42 B). This may be because the curvature of one or all of the refracting surfaces is unusually sharp, the length of the eyeball being normal, or it may, as is more frequently the case, be due to an unusual length of the eyeball. There is no physiological correction for this condition. In practice it is rectified by placing in front of the eye a concave lens. Light from distant objects is thus made divergent before it reaches the eye and its focus therefore lies on the retina, further back than the principal plane of the short-sighted eye.

In Long-sightedness or Hypermetropia the condition is reversed. The principal focus of the refracting media lies behind the retina and the parallel rays from distant objects have not yet arrived at their focus when they are interrupted by it (Fig. 42, C). An increase in the refractive power of the eye is needed to bring the focus forward and give a clear image. An effort of continued accommodation (see below) will accomplish this, but such an effort gives rise to various nervous symptoms, headache, irritability and the like. If convergent glasses of suitable strength are used, the focus may be advanced the necessary amount without any effort on the part of the patient.

Astigmatism. So far as we have considered the eye as refracting all rays to the same extent no matter in what plane they diverge from their object nor whether they pass through the upper and lower parts, or through the right and left sides, of the refracting surfaces. Cases are fairly common, however, in which this does not hold good. In these eyes, known as astigmatic, all the meridians

are not curved alike. In the most common form the vertical meridian is an arc of a smaller circle than is the horizontal and, being more sharply curved, it refracts more than does the latter. Rays which diverge in a vertical plane from a point

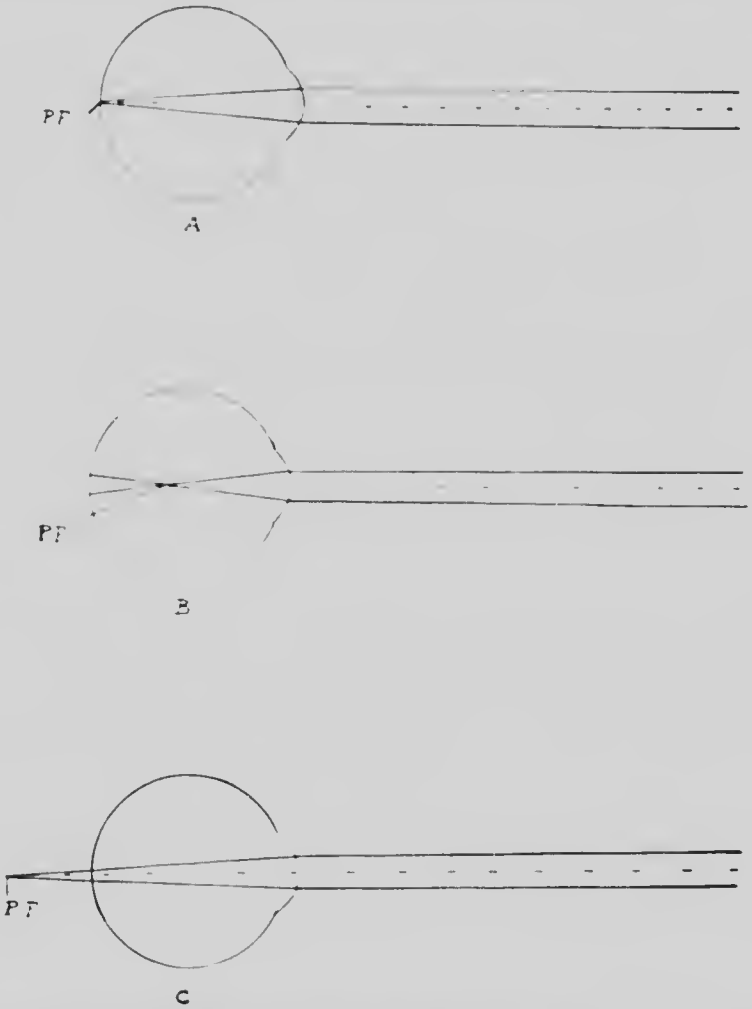


FIG. 42. The focussing of parallel rays from a distant object by (A) a normal, (B) a short-sighted and (C) a long-sighted eye. PF in each case shows the position of the principal focus of the combined refracting surfaces of the eye, represented by the single surface of the schematic eye.

object are brought to a focus by such eyes in front of the focus for the horizontal rays. The result is that the eye can never see a point image of a point object. If the rays through one meridian are focussed on the retina then those which pass through the meridian at right angles to the first must reach the retina as a pencil

of rays, either because they have already passed, or because they have not yet reached their focus. The unlike meridians are not necessarily arranged in this way, the vertical is not always that of greatest curvature, although this is the most common form, nor are the meridians which differ most always at right angles to one another. When they are the astigmatism is a REGULAR one; when the angle between is not a right angle it is an IRREGULAR astigmatism. Regular astigmatism is easy to correct by glasses ground so as either to converge the rays which are to pass through the meridian of least refractive power or to render more divergent those directed towards the meridian of greater refractive power. Irregular astigmatism is more difficult to correct. In some eyes the astigmatism is of neither of these types but consists in irregularities in the curvature of the same meridian, a condition which cannot be made right with glasses. Even in a normal eye there is something of this irregularity. The image of a star is not a single point, as it would be if the refracting surfaces were of even curvature throughout, but it has an irregular shape which is different for each individual, because of the slight defects in the curves of the various surfaces.

Accommodation. We have seen how parallel rays from a distant object are brought to a focus on the retina of a normal eye. When however the gaze is directed to an object close at hand the eye receives rays which diverge from one another by a considerable angle. If no change takes place in the refractive power of either cornea or lens these rays tend after refraction towards a focus lying behind the retina. In consequence when they are intercepted by the retina they form a blur and not a sharp point. To make the image of the near object a clear one either the distance between retina and lens must be made greater than it is for distant vision, just as one increases the distance between lens and plate in a camera when focussing for objects close at hand, or else the refraction by the eye media must be increased. The latter is the change which is brought about in the mammalian eye by the act of accommodation. The curvature of the anterior surface of the lens is made sharper and its refraction therefore greater.

To understand the most generally accepted explanation of the way in which this is done, one must have clearly in mind the anatomical relationship which the lens bears to neighbouring structures in the eye (Fig. 43). It is held in its place by the numerous fibres which together make up the suspensory ligament of the lens. These are more or less radially arranged about the lens, being continuous at their inner ends with the capsule near the margin, while the outer ends of the threads are connected to the surface of the ciliary body near its free inner edge throughout its entire circle. The arrangement is such that those fibres which arise from the posterior surface of the lens capsule go to the anterior surface of the ciliary body and those which arise from the anterior surface of the lens are at-

tached to the processes of the posterior surface of the ciliary body as far back as the ora serrata of the retina. The contents of the eyeball are under a pressure which is greater than atmospheric by about 25 cms. Hg. They therefore press outwards on the coats of the eyeball and as a result of this the choroid coat and the ciliary body, its forward continuation, pressed out by the vitreous humour, pull backward as well as outward on the lens ligament. This in its turn exerts more pull on the anterior than it does on the posterior surface of the lens capsule. The lens itself is fluid in nature and its capsule is elastic. Its natural shape is a

FIG. 43. Diagram of the attachments of the lens, and of the neighbouring structures.

more or less rounded one but, being flexible, it yields to the pull of the ligament and both its surfaces are flattened, the anterior however much the more so. The act of accommodation is a contraction of the circular and radial muscle fibres which are contained in the ciliary body. This makes the circle of the free margin of the body smaller, brings it nearer to the lens, and causes the processes of the posterior part to move forward, dragging with them the anterior part of the choroid, with which they are continuous. The fibres of the ligament

which pass between the posterior processes of the body and the anterior surface of the lens are slackened by these changes and the lens is allowed to bulge forward by its own elasticity.

The extent to which the curvature can be increased by accommodation is limited only by the elasticity of the lens.

Experiment 52. The Near Point. The least distance at which an object may be held away from the eye and still be clearly seen is the NEAR POINT. Find how far away your own near point lies by looking fixedly at a pin or pencil-point with one eye closed and gradually bringing the object closer to your face. A distance is found nearer than which if the object is held it is seen blurred. The blurring means that your accommodation is no longer sufficient to bring all the rays from a single point to a focus on the retina, the focus lies behind the retina, and the rays from each point reach the sensitive cells as a pencil, and not as a point of light.

The near point in a young child's eye is generally about 10 or 12 cms. away; as time goes on this distance increases and as a rule at 40 or 50 years of age the near point has receded past the length at which it is convenient to hold a book. Convex glasses are then used for reading and hand-work and by this means the divergence of the rays is reduced so that objects may be comfortably held and still throw sharp images on the retina. The change in elasticity has no effect on distant vision, the shape of the lens in the eye at rest is the same as before.

When the gaze is directed to an object near at hand two other changes occur, associated with the act of accommodation. The visual axes, which during rest or distant vision are parallel, are converged so that they meet on the object at which one looks, and make the image of it fall on the fovea centralis of each eye. This is done by the contraction of the internal recti, which rotate the eyeballs inward. At the same time the ACCOMMODATION REFLEX of the pupil occurs; the circular muscle fibres which are contained within the free margin of the iris contract and reduce the size of the opening. The iris acts as a curtain or adjustable diaphragm for the eye, limiting the size of the pencil of rays which enter it. By this reflex contraction of the opening in near vision all rays are shut out except those which pass through or near the centre of the

refracting surfaces. By this means spherical aberration is cut down in near vision, in which great accuracy is usually required and in which the aberration would otherwise be marked, because the incoming rays are very divergent. The image is made somewhat less bright by this device, since there are fewer rays to contribute to it than there would be with a wider pupil, but, the object being near, the number of rays which the eye receives from it is in any case large and the loss is not of much importance. All three groups of muscles which contract in near vision, the ciliary, the circular muscle fibres of the iris, and the internal recti, are supplied by the oculomotor nerve. The nerve supply of the radial fibres of the iris, the contraction of which dilates the pupil, comes from the cervical sympathetic.*

Some indication of the nature of the change in the eye media during accommodation may be got from the following experiments.

Experiment 53. In a dark room arrange the lens and watch glass so that they are centred, with the lens behind the watch glass, which is placed with its convex side forward. Hold a candle beside your head and a little behind your eyes and look at the images of the flame formed by the three refracting surfaces. These let most of the light through, but reflect some small portion of it. The reflected rays form the images which you see. There are three of them, one inverted and two erect.

Experiment 54.– Purkinje Images. Look at your partner's eye in the way in which you looked at the lens and watch glass in the last experiment and ask the subject to gaze past you into the distance, along a line half way between your eye and the candle. As before, three images may be seen but in this case one of the erect images is larger and dimmer than the other. Now ask the subject to focus on your finger held in his line of vision, at about the distance of your head away. The small, bright, erect image and the inverted one are unchanged in position, but the larger erect one moves nearer the first and

*A stopping-down of the pupil, similar to that which occurs for near vision, is brought about reflexly when the eye moves from a darker to a lighter place. If the change in the intensity of the light is great the contraction is more marked at first, becoming gradually less for some minutes afterwards as the eye becomes "accustomed to the light". This is known as the "LIGHT REFLEX" of the pupil.

becomes smaller than before. This is the image from the anterior surface of the lens. It is large and dim in the unaccommodated eye because the surface which forms it is fairly flat. During accommodation when the surface becomes more curved the image becomes smaller and appears to move forward because of the movement of the reflecting surface.

Experiment 55. Scheiner's Experiment. The course of rays which enter the eye, from objects at distances for which the eye is not accommodated, can be traced by blocking all but two pencils of them and finding out which regions of the retina are affected by the two pencils of light. In a piece of heavy paper prick two holes less than the diameter of the pupil apart. Stick two needles upright in a strip of wood, one 30 cms. and one 60 cms. from the end. Hold the screen with the holes horizontal in front of one pupil and, closing the other eye, look along the stick and accommodate for the far pin. Note that the image of the near pin is double. Cover one hole in the screen and see which image disappears. Repeat, with the eye accommodated for the near pin. From your results draw diagrams of the course of rays entering the eye (a) from an object at the distance for which the eye is accommodated, (b) from one at a greater distance, (c) from one at a less. Compare with the diagrams from the results of Exp. 18 and explain the differences.

CHAPTER XIX.

EXAMINATION OF THE REFRACTION OF THE EYE AND OF THE INTERIOR OF THE EYEBALL.

THE OPHTHALMOSCOPE.

Most of the light which enters the eye passes through the retina and is absorbed by the black pigment layer behind it but a small proportion of the rays are reflected from the retina itself. The rays reflected from each point are refracted again by lens and cornea being bent away from the perpendicular in this case and they pass out in the same general path as was taken by the incoming rays which illuminated the point. That is, they travel back towards the source of light. Since the eye of the observer is not under ordinary circumstances placed at such a source it does not receive any of this light and therefore any pupil at which one looks appears to be black. If however one arranges to have the observer's eye at the source of light, or a very little distance from it, some of the rays reflected from the observed retina can be seen.

This is the principle on which the ophthalmoscope is constructed. It not only gives a view of the interior of the eye but also, as we shall see, affords a method of examining its refraction. The instrument is a slightly concave mirror with a tiny hole in the centre of it. One holds it in front of the eye which one wishes to examine and a light is placed beside the eye and a little behind it (L, Fig. 11 A). The mirror sends rays from this into the eye. LAA′ and LBB′ in Fig. 11 are two such rays. There will of course be many others, lighting up the whole area between A′ and B′ and some of them falling on either side. Each point of this illuminated area, reflecting part of the light which it receives, acts as a source of rays, as is shown for the point X. The observer looks from behind through the little hole and some of the reflected rays (as XY and XZ) returning towards the mirror pass through the hole and afford him a view of the interior of the subject's eye.

The subject is asked to stare ahead of him, in order to relax his accommodation, or, what is more effective, an application of atropine is made to the eye. This paralyses the endings of the third nerve in the iris and the ciliary body and prevents accommodation, and reflex contraction of the pupil under the stimulus of the strong light. The rays from the retina, arising at the principal focus if the eye is a normal one, pass out after refraction parallel to one another. If these are to be clearly focussed on the observer's retina his eye must be unaccommodated. He must not look there-

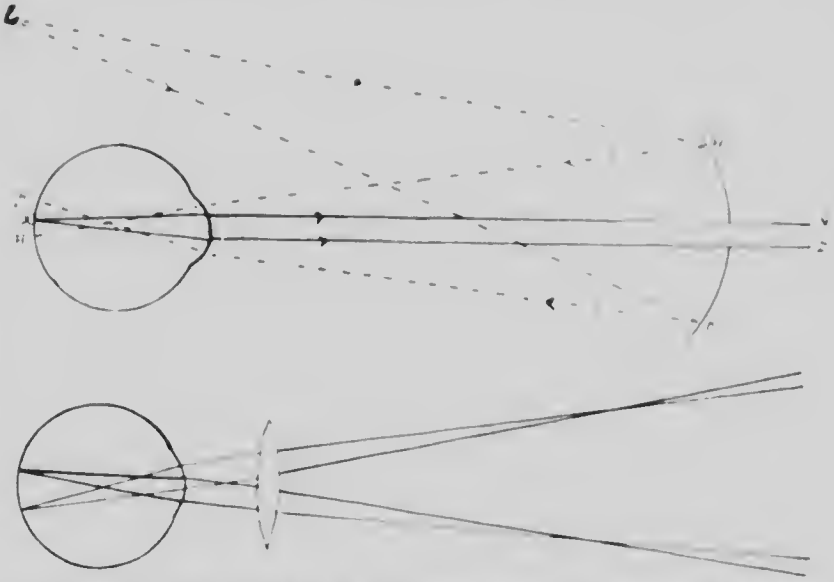


FIG. 14.—To illustrate the use of the ophthalmoscope. Upper diagram shows the course of the illuminating rays and of the outgoing rays, by the direct method. Lower diagram shows the course of outgoing rays when the indirect method is used; the illuminating rays have been omitted.

to at the retina which he wishes to see but must gaze through it to the distance. If the observed eye is shortsighted, the principal plane, the plane from which rays would have to come to be rendered parallel after passing out through lens and cornea, is somewhat in front of the plane of the retina. Light rays from the retina, therefore, since they diverge less than would rays from the principal plane, are too much refracted by the eye media and converge after refraction. If they are to make a sharp focus on the unaccommodated eye of the observer they must be rendered

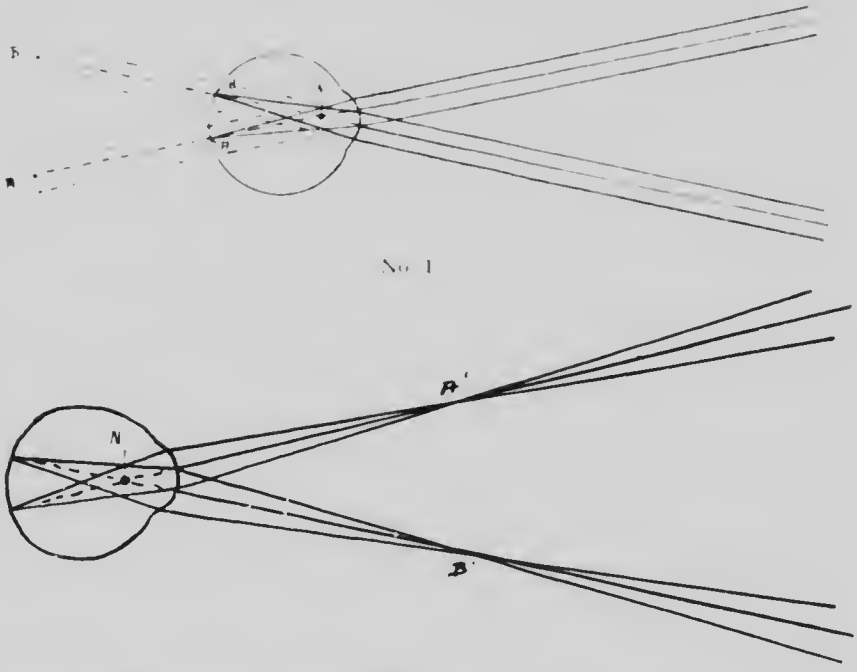
parallel by a divergent lens, and the strength of the lens which one must use for this gives a measure of how much the rays differed in direction from the parallel paths which they should have taken. If the eye is LONG-SIGHTED the condition is reversed. The retina is farther forward than the principal focus of the eye, the rays diverge too sharply and after refraction they still diverge to some extent. The observer to see clearly must in this case use a convergent lens and, as before, the strength of the lens gives a measure of how different the eye is from the normal. This is known as the DIRECT METHOD of using the ophthalmoscope. The part of the retina which one can see at one time in this way is a small one and for that reason, and sometimes too because it is difficult for the observer, if he is inexperienced, to relax his accommodation completely, the INDIRECT METHOD is often used (Fig. 44, B.). The arrangement of light and mirror is on the same plan as before, but in this case a convex lens is held immediately in front of the subject's eye. This focusses the rays emerging parallel from the eye and forms an inverted real image of the bright spot of the fundus, at the principal focal plane of the lens. It is this image at which the observer looks.

Experiment 56. The use of the ophthalmoscope for the examination of the human eye is not suitable as a laboratory experiment since a beginner to have success needs both time and quiet. Each student is expected, however, at some time during this part of the course, to procure an instrument, of which there are several available in the supply room, and to practise with it until he can see for himself the interior of a normal eye. Using both the direct and indirect methods, work at the experiment until you can see many or all of the details of the interior surface of the eyeball described below (p. 147). Before doing this it is wiser to practise a little in the laboratory, using the instrument according to both methods to examine the eye of a rabbit, the pupil of which has been dilated with atropine.

Direct Method. Place the light and ophthalmoscope, as shown in Fig. 44, and look from behind the mirror through the hole. Start with it about eighteen inches away from the observed eye and move it slowly about until the pupil is seen as a bright red spot. Now move the mirror gradually nearer,

keeping it always in the same line, until when you are about two or three inches away from the subject's eye the bright spot of the retina comes clearly into view. Both observer's and subject's eyes must be unaccommodated.

Indirect Method. The arrangement is as before except that a converging lens is held two or three inches away from the observed eye and steadied by resting a finger of that hand



No. 1.

No. 2.

FIG. 45. Diagrams to illustrate the use of the retinoscope. No. 1 shows the path of rays from a normal eye, No. 2 that for rays from a normal eye in front of which is held a converging lens, and of rays from a short-sighted eye.

against the subject's forehead. Hold the ophthalmoscope about twenty inches away and move it about and move the lens back and forth until the image of the bright spot is clearly seen. The observer must accommodate his eye for a point near at hand.

THE RETINOSCOPE. Another instrument which is used to examine the refraction in the eye is the retinoscope, a flat mirror with a little peep-hole in the middle, which can be made to rotate through a small angle on its handle. The principle of its use depends on the following considerations. If a spot of light is

reflected on a wall and the mirror reflecting it is rotated upward, the movement of the spot is in the same direction as that of the mirror. If a spot of light be reflected into a normal eye and the mirror moved, the movement of the light on the retina appears to the observer's eye, placed at the hole in the instrument, also to be in the same direction. This is shown in Fig. 45, No. 1. A is the first position of the spot of light and B the second, moved by an upward movement of the mirror. The rays from A, when after refraction they come out of the eye, appear to come from A' at infinity. Those arising from B seem to come from B', also at infinite distance. The mirror was moved up and the image also moves up. If a strong converging lens is held in front of the observed eye so that the rays coming out are made to form inverted images, at some short distance from the lens, of the retinal points from which they arise, and if the rays are diverging from the images again when they reach the observer's eye, the condition is reversed. The spot of light appears to move in a direction opposite to that of the movement of the mirror. This is shown in Fig. 45, No. 2. The rays from A, converged after coming out of the eye, make the image at A', those from B at B'. If the observer's eye be farther away than A' and B', the rays from the first (lower) position of the spot of light seem to come from the upper image, those from the second place of the spot, which was really above the first, from an image lower than the first image. The direction of movement is thus reversed. If the observer's eye be nearer than the focus of the lens (that is, nearer than A' or B'), the movement appears to be in the direction of the movement of the mirror, as it was without the lens. Suppose the observer's eye is always at a distance of one metre from the subject. Then if the lens is stronger than one dioptre (i.e., has a focal length of less than one metre) the spot will move in the opposite direction. If the lens is weaker than this there will be no reversal, and the spot will move in the same direction as the mirror. Now, if the subject's eye is not normal but long-sighted, the rays when they come out diverge from each other. To bring these to a focus in front of his eye, still at one metre distance, the observer will have to use a stronger lens than before and the more long sighted the eye is, the stronger the lens will have to be. Rays coming from a short-sighted eye are already converging when they come out. If the defect is small, a converging lens will still be needed to make the movement of the spot appear reversed to an observer at one metre, though not so strong a one as is needed for the normal eye. If the defect is great, the focus of the rays without any lens at all may lie at some point between observer and subject, and in this case the spot of light moves in the opposite direction to the mirror from the first. The condition will be thus like that already described for Fig. 45, No. 2, only that no lens need be imagined. A divergent lens must be used to cause it to go to the similar direction and, as before, the strength of the lens which must be used gives a measure of the abnormality. An examination of this kind can be done in any plane of the eye and the method can therefore be used to measure astigmatism. In this case the strength of lens necessary to give reversal when one plane of movement is being tested is different from that for another plane.

CHAPTER XX.

THE RETINA.

The view of the interior of the eyeball which one gets with an ophthalmoscope reveals a whitish lining, the retina, over the surface of which are fine branches of arteries which supply it with blood. There are two spots on it which differ in their appearance from the rest of the surface. One of these, situated directly opposite the centre of the pupil, is the MACULA LUTEA. This is slightly raised, yellowish in colour, and has a tiny depression in its centre, the FOVEA CENTRALIS, the part of the retina capable of the most accurate vision. The blood vessels skirt around the yellow spot and do not pass across it. Towards the nasal side of this and some little distance from it there is a shining white spot, the OPTIC DISC, which marks the passage through the retina of the fibres of the optic nerve.

In order that the student may have the structure of the retina fresh in his mind as a basis for his experiments, he is reminded of the details of its anatomy and histology which follow.

The fibres of the optic nerve enter the eyeball from outside arranged in a cylindrical fashion, enclosing in the middle an artery, branches of which we have seen ramifying over the surface of the retina. When they have pierced the outer layers of the retina, the nerve fibres of the cylinder divide and spread out over its whole surface, thus forming the innermost retinal layer of all. The whole retina extends forward in the eyeball only as far as the beginning of the ciliary processes. From here on it is represented simply by the layer of black pigment cells which clothes the posterior surface of the ciliary body. The cells which are sensitive to light waves, the fundamental part of the whole organ of vision, are contained in one of the layers of the retina. The retina itself is to be regarded not simply as comparable to a group of sensory nerve endings connected each by a single fibre directly to the central nervous system, but as a part of the brain itself, from an outgrowth of which it is formed during the development of the fœtus. Accordingly it contains, as well as the light-sensitive cells themselves, two layers of relay cells through which the impulses arising in the sensitive elements must pass on their way to the brain (Fig. 46). The elements which respond to light waves, the rods and cones, are placed farthest away from all the incoming light. They are the free ends of the outer cell layer, sticking out towards the choroid coat from which they are separated only by a layer of black pigment cells. The nuclei

corresponding to them are nearer the centre of the eyeball and each cell sends a connecting fibre inward into the outer nuclear layer of the retina, where it is connected with the dendrites of one of the first relay cells, the bipolar cells. The rods have slightly thickened inner, and straight slender outer, limbs. They are connected with their nuclei by fine thread like processes. The cones, which are shorter than the rods, are also divided into inner and outer limbs. Their outer limbs are shorter and sharper than those of the rods; the inner ones are much thicker and connected each by a substantial process with its nucleus, which lies nearer to it than do those of the rods. The dendrites of the bipolar cells, which are the next units on the path of the impulse, are connected by short thick processes with their cell bodies, the nuclei of which make up the inner nuclear layer. The axones of these cells are short. They end in the inner molecular layer, where they establish connection with the dendrites of the second relay cells, the giant cells. It is the axones of the giant cells which, passing over the surface, make the inner layer of the retina and join together at the optic disc to go out as the optic nerve. In all parts of the retina except the fovea centralis the bipolar cells connect each with several fibres from rod cells or cone-cells, and are in turn

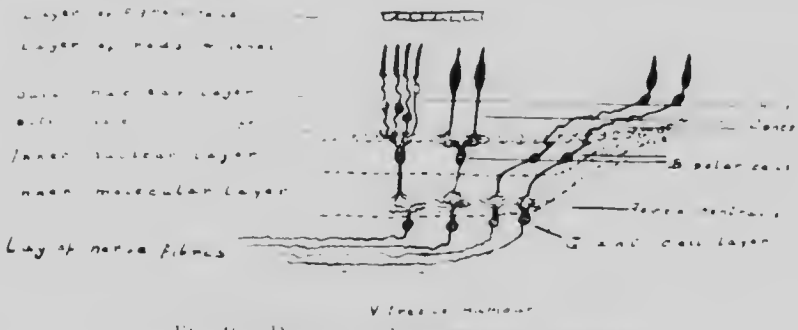


FIG. 46. Diagram of the structures in the retina.

several of them connected with one giant cell, so that the number of nerve elements represented in the optic nerve is a good deal less than the number of cells in the retina, and one fibre in the nerve may carry impulses which arise from any one or from all of a number of rods or of cones. The histology of the fovea is different in this as in some other respects from that which we have described. It consists only of the light-sensitive cells and the fibres which connect them with the first relay cells. These, together with the giant cells to which they lead, are swept aside from the fovea itself and, piled up around its margin, cause the slight thickening of the retina which surrounds it. The sensitive cells in the fovea are all cones, no rods are to be found, and they are each of them connected through a different bipolar cell to a single giant cell and so to a separate fibre of the optic nerve. The arrangement affords some histological basis for the acuteness of vision which we have in the fovea, since each cone has a separate path to the brain. It is found that two points in the external world may be distinguished as separate, provided that the angle by which they are separated at the eye is 5 minutes or

more, and provided that they are looked at directly, that is, that their image falls on the fovea. Construction by the schematic eye shows that an angle of this size represents an image of about .0035 mm. on the retina, approximately the distance from the centre of one cone to that of another. If the points are looked at, not directly, but with 'the tail of one's eye', they must be much more widely separated than this to appear distinct from each other; the distance necessary increases, broadly speaking, the farther they are removed towards the periphery of the visual field.

That the cells which are most deeply placed of all should be those which respond to the vibrations of the light waves would at first sight appear improbable. There is abundant evidence, however, that this is the case, as the two following experiments show.

Purkinje Figures. The retinal blood vessels, being situated between the source of light and the receptive surface cast shadows on the retina. These are very small and by habit are disregarded. If, however, they can be made to fall on some part of the retina which ordinarily does not receive them they may be seen.

Experiment 57. Make the subject turn one eye inward and look towards a dark wall. With a lens concentrate a good light upon the exposed sclerotic, focussing so as to make a small, strongly illuminated area. Now give the lens a circular or gently rocking motion. The field appears to the subject to be reddish yellow, with dark branching figures on it. These are the shadows of blood vessels. In the direct line of vision a small spot free from shadows may be seen, the macula lutea or yellow spot.

The path of the light rays through sclerotic and choroid and the vitreous humour is shown in the diagram (Fig. 47). When the source of light is moved there is a movement of the shadow of the blood vessel on the retina, which is projected outward by the subject along the line which connects the point stimulated with the nodal point, and which is interpreted as a movement of a shadow in the field of vision at the distance for which the eye is accommodated. A is the first position of the light, A″ the place on the retina where it throws the shadow, A‴ the place on the screen at which the eye appears to see the shadow. When the light is moved to B the shadow moves to B′ and its apparent place in the field to B″. n.p. is the nodal point of the eye, b.v. the position of the blood vessel. Now triangles A″B″ n.p. and n.p.A′B′ are similar and we know the lengths A″B″, n.p.A′ and n.p.A′. We can therefore find

A'B', the distance the shadow actually moved on the retina. But triangles A'B' b.v. and bv..AB are also similar. Here we know A'B', A.B. and (approximately) bv.A., so that we can find bv..A', the distance between light-sensitive layer and the blood vessel. This is found to be about 0.2-0.3 mm., which is sufficiently like the distance actually existing between the retinal blood vessels and the layer of rods and cones to support the conclusion that these are the elements sensitive to light.

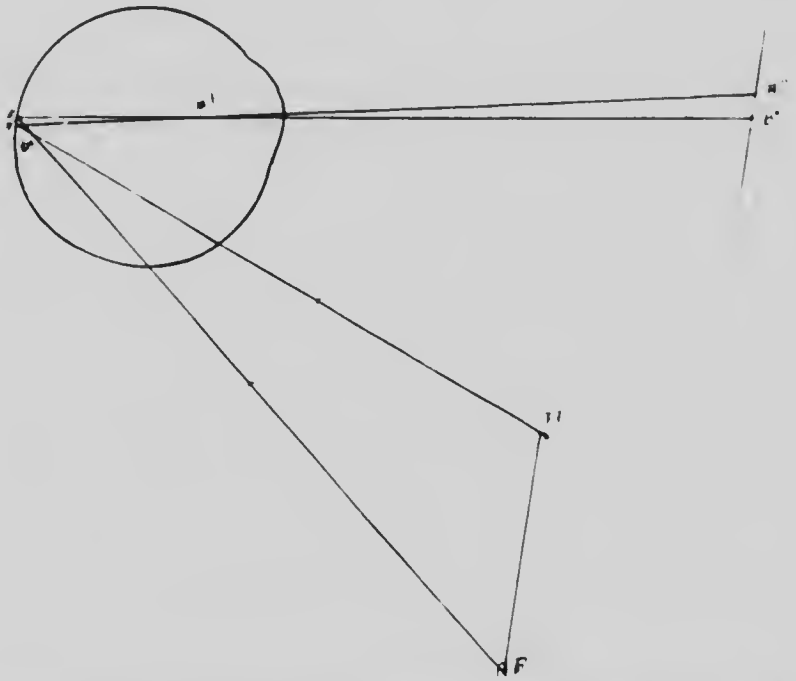


FIG. 47. To illustrate the course of the rays which cast the shadows in Purkinje's figures, and the direction along which these shadows are projected outward into the visual field.

Blind Spot. It may easily be shown that, although we are unconscious of it, there is an area in the field of vision of each eye which is blank; objects placed there are not seen. Construction with the schematic eye shows that light rays from this area fall on the optic disc. Here, as we have seen, there are no rods or cones, the layers which contain them being interrupted to allow of the passage through it of the fibres of the optic nerve.

Experiment 58. Take a thin strip of paper and blacken about 1 mm. of its end. Hold a large sheet of paper with a dot in the centre vertically in front of you. Keeping your head quite still, close one eye and look fixedly at the dot with the other. Move the blackened end of the paper strip slowly over the field of vision and mark the points when it seems to disappear. From these points map out the area of the blind spot in the visual field. Using the schematic eye, show where it must be in the retina.

The Response of the Retina to Light. In addition to the information from subjective experiments such as those which we are about to discuss, which of course involve activity of the brain as well as the eye, some interesting knowledge as to the nature of the retina's reaction to light stimuli has been got from direct experiments on the eye itself. The technique which shows the movement of the cones, the negative variation in the retina, and the formation and destruction of the visual purple, is unfortunately too difficult for class experiments. The student must turn to his text-books for information as to the nature and significance of this work.

DARK ADAPTATION.—It is familiar that eyes at night or in a dark room can see lights so faint that they would not be made out at all by day. This is in part due to an actual lowering of the threshold of the light-sensitive elements, and one may satisfy oneself by simple experiments that the main part at least of this change in threshold occurs, not in the line of direct vision, but in the peripheral field. The observations are entirely subjective ones and the student must make them for himself under suitable conditions of lighting. If one looks at the sky at twilight searching for the first star it may appear on one side or another of the direct line of vision, only to disappear when looked at directly. Small pieces of white paper on a black background in a darkened room are visible as long as they are not in the direct line of vision, but disappear, each as its image is made to fall on the fovea. Indeed, workers in this field have not been able to find that any lowering of the threshold at all occurs in the fovea during adaptation to the dark and this makes it probable that the change in the threshold of the peripheral field is a change in the rods and is not shared by the cones. The adaptation which the rods undergo is thought to be associated with the presence in them of visual purple, the coloured substance which is formed in them in the dark. One of the chief reasons for this is the striking resemblance between the sensitiveness of the pigment on one hand, and of the dark-adapted eye on the other, to waves of different lengths. When several different colours are seen under similar lighting conditions it is only a matter of judgment which of them is

the brightest, but most normal individuals if shown a spectrum in a brightly lighted room, pick out the yellow as the brightest part of it, brighter than either red, orange, green, blue or violet. If however all light is shut out of the room except that coming through the prism which makes the spectrum, and if the eyes are allowed to become adapted to the dark, the place of greatest brightness in the spectrum appears to shift from yellow to green. This colour, it is to be noted, is also the most effective in bleaching the visual purple. The spectrum under these conditions also appears shortened at the red end, that is to say the long waves are ineffective for the eyes in this state, as they are also found to be on the purple itself.

Some further evidence which bears on the same question is got from observations on the ACHROMATIC (or colourless) THRESHOLD. If, after the eyes have become adapted to a dark room, light is gradually introduced, all objects in the room of any colour except red look colourless when first they become perceptible, as if they were made up of varying shades of white and grey, and it is only on farther illumination that they appear in their own tints. The interval during which they seem uncoloured is the achromatic interval. Red objects have no such interval; they appear at once in their own colour, but they require a stronger light to make them visible than is needed to make the other objects show as colourless grey ones. The whole phenomenon can be seen out of doors at dawn, or, in the reverse order, at dusk. At the first daylight everything comes into view as if in a print, all black, white or grey, and it is only as the light increases that the leaves change from grey to green, blues, yellows and violets appear, and red flowers turn from black objects to brightly coloured ones. These results are interpreted as follows: in the light the cones have a threshold lower than that of the rods and, since they are capable of distinguishing colours as well as merely brightness, we see all light in which there is more of some wave lengths than of others as coloured light, no matter how faint it may be. In the dark adapted eye the threshold of the cones is not changed, but that of the rods is lowered, probably because of the presence in them of the visual purple, so that they become more sensitive than the cones. The rods are not organs of colour vision, however, when stimulated they give rise only to sensations of brightness. Therefore faint lights of wave lengths other than red are first seen by the rods of the dark-adapted eye as colourless rays and only affect the cones and appear coloured when they have become a good deal stronger. Red lights do not affect the rods at all, and their effect does not appear until they are strong enough to stimulate the cones.

There is an abnormal type of vision known as TOTAL COLOUR BLINDNESS, which shows a good many points of resemblance to the vision of a dark-adapted eye. People who have this defect see, as far as one can determine, all lights as colourless, rays of different wave-lengths differ to them only in brightness and any two colours can be made to look exactly alike to the colour blind by varying the brightness of one or the other. Two colours which match in their judgment look equally bright also to the normal eye in its condition of dark adaptation, but are different not only in colour but in brightness under ordinary conditions of lighting. Like the dark adapted eye the colour-blind is more sensitive to the

shorter wave lengths, a green which appears to the colour-blind eye to match a certain yellow seems to the normal in daylight to be not only of a different colour from the yellow chosen, but also less bright than it. The sight of people who have this defect is easily fatigued in bright light and is much less acute than the normal. In the dark it far surpasses the normal in accuracy. Many colour-blind people have a blind spot in the fovea, so that they see nothing in the direct line of vision but only in the peripheral field. It is thought that the condition is one in which the rods mainly function. The parallel between these eyes and the normal dark-adapted eyes is not complete. The vision of the colour-blind is much the more acute and is often enough so to enable the subjects to read, which cannot be done with normal dark-adapted eyes.

CHAPTER XXI.

COLOUR VISION.

It is a familiar fact that when ordinary white light is passed through a prism the waves of which it is composed are retracted to a greater or less extent according to their length. As a result the single beam which was made up of waves of all lengths is spread out into a band of light, one end of which is formed by the shortest of all the visible rays, the part immediately next to it by those a little longer, and so on throughout the whole band until the other end is reached, which contains the longest waves that the eye can perceive as light. The physiological effect of this re-arrangement of the waves is to make the light, which when mixed was uncoloured or white, appear to be made up of a whole series of colours, known as the spectrum. The colours are arranged in a definite order according to the length of the waves of each part of the band of light. The shortest of all look violet, those next them blue, the waves next these again green, then yellow, then orange, and then red, first scarlet and then, at the end of the band, crimson. Each colour shades imperceptibly into the one next it, so that as well as those which we have mentioned we can find in the spectrum numbers of others, such as greenish-blue, violet-blue, greenish-yellow, and so on.*

There are two other ways in which lights may differ from one another. One is in BRIGHTNESS. Of two rays of the same colour if one is more intense than the other it looks brighter.

Experiment 59. Take two sheets of paper of the same colour. Place one near an electric light of low, and the other near a

*Each of the spectral colours may also be produced by the use of pigments. The colours of these depend on the fact that, from the waves of different lengths in a beam of white light falling on them, they absorb all except those which belong to a very limited area of the spectrum. Blue paint, for instance, reflects only the blue waves and a few of the green and absorbs all the others. Yellow glass absorbs most of the wave lengths falling on it except the yellow ones, and light which has passed through it consequently has that colour.

light of high candle-power. The second looks brighter than the first.

The other way in which two samples of the same colour may differ is in the proportion of white light which they contain. A colour which contains no white light at all is, physically speaking, completely SATURATED or full, the more white one mixes with it the less saturated, or the paler, it becomes.

Experiment 60. Take two sheets of yellow tissue paper and put one over a piece of heavy paper of the same shade and lay the other over a sheet of white paper. They both have the same colour but the first is fuller or more saturated than the second, paler one.

The evidence which we have examined so far would lead one to think that the sensation of a given colour was entirely dependent on the action on the retina of waves of a certain length. It would seem that the sensation of yellow, for instance, was simply the physiological result of the action of waves from that particular part of the spectrum. The student's attention however is directed in this regard to the results of the two following experiments.

Experiment 61. The Effect of Stimulating the Retina with Two Different Colours at the Same Time. From the paper discs provided choose an orange and a green, colours situated immediately on either side of yellow in the spectrum, the waves of one being longer and those of the other shorter than the yellow waves. Arrange them in the rotator so that about half of each shows and spin them rapidly until they seem to fuse.* The colour which together they produce is a yellow

*The ideal way to mix colours on the retina is to cause the rays from those parts of the spectrum which contain the desired colours to converge again, so that they actually fall on the retina at the same time. A simpler means is that adopted here, in stimulating the retina with alternate flashes of the colours following each other in very rapid succession. Advantage is taken of the fact that the reaction of the retina does not cease immediately the stimulus is withdrawn but lasts for a fraction of a second after it. The result cannot be got by mixing together pigments of the colours which it is desired to combine. For instance, although blue and yellow light thrown together on the retina give white or grey, blue and yellow pigments mixed give a green colour. The reason for this is that both blue pigment and yellow pigment reflect, along with their particular colours, some green rays. When they are mixed the blue absorbs yellow light which the yellow pigment would have reflected, the yellow pigment does as much for the blue, and the only colour left over is the green, which was completely absorbed by neither.

much like the pure spectral colour, only a little less saturated. Change the disks, taking this time reddish-orange and yellowish-green. These colours are also one of longer and one of shorter wave length than yellow but they are more widely separated from it in the spectrum than the first two were. When you spin them you again get a yellow but this time there is a much greater proportion of white in it, it is less saturated than before. Replace these colours with two others still further removed from yellow, although still lying one on either side of it; use scarlet and blue-green. When spun together this combination looks whitish or pure grey, the yellow is no longer produced. Two colours such as these, which give a sensation of colourless or white light when combined on the retina, are COMPLEMENTARY COLOURS. There are innumerable such pairs, a few of them being the following; orange with blue, bright yellow with a more violet blue, green-yellow with violet. Try these for yourself. It may be necessary to show in each case a little more of one of the pair than of the other, depending on whether or not they are equally bright. In experimenting on these complementary colours notice that when they are arranged in any proportion other than that which gives white, they produce a sensation of some colour which lies between them in the spectrum, just as those pairs did which were less widely separated.

Now take pairs of colours too widely separated to be complementary, for instance red and blue, or orange and violet, and see that combining them gives a sensation of purple, a colour which does not exist in the spectrum at all. It is the complementary colour to green.

We have seen that a colour sensation, yellow for instance, can be produced (a) by waves, acting alone, of the length of those which make up the yellow part of the spectrum, or (b) by a great many combinations in pairs of waves of other lengths, waves which falling alone on the retina give colours which have no yellow in them at all. We have also seen that two beams of different wave lengths, as well as producing each a definite colour sensation when it acts alone, can if combined in varying proportions give rise to quite a large number of the other colours. All the colours, how-

ever, can not be got by using any one pair, to get the whole range of possible colour sensations you must take at least three spectral colours and vary these. There are many combinations of three which can be taken, we have chosen those most generally used.

Experiment 62. Effect of Simultaneous Action on the Retina of Three Primary Colours. Put into the rotator a red, a green, and a violet disk. Keep on altering the proportions of each until you have got (a) a colourless light, (b) each of the colours of the spectrum and (c) a purple.

As you have seen, all the various colour sensations which we are capable of receiving can be produced by combined action on the retina of three simple stimuli. To put the matter differently, the whole range of the response of the retina to light can be expressed as a function of three independent variables.

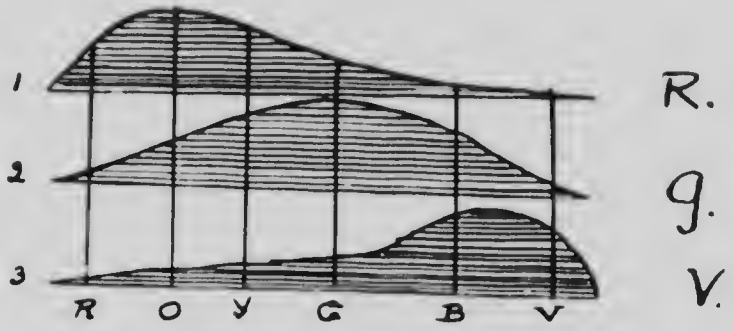


Fig. 18. To illustrate the Young theory of colour vision.

Young-Helmholtz Theory of Colour Vision. Any theory which is to be advanced in explanation of colour vision must be based on the fundamental facts which we have just described. The earliest of the modern theories, and one which has given rise to a great deal of experimental work, was first suggested by Young and later elaborated by Helmholtz. According to this there are three different components in the retina, whether anatomical units, or chemical substances, is not stated. Each of these is supposed to be most effectively acted on by light waves of a quite limited part of the spectrum, and those waves which influence one of the substances most have very little effect on either of the other two. For the sake of illustration, red, green and violet, were selected as the 'primary colours.' One component is supposed to be sensitive to red waves, less so to orange and responsive in a gradually decreasing degree to the rest of the spectrum. The green component is affected most by green, to a less extent by yellow or blue, and little by the colours at either end. The third component,

as well as responding to violet, is sensitive to blue, but little to the other wave lengths (Fig. 48). If any one of these components is stimulated much more than the others the result is a sensation of the primary colour corresponding to it. Sensations of colours which lie intermediate to the primary colours in the spectrum are simply the result of less unequal stimulation of two of the primary substances: that explains why you can get such a sensation either by using a wave length such as blue which affects two components violet and green, one more than the other, or by stimulating the retina with waves of two other colours violet and green but unequal intensities, one of which stimulates mainly one, and one mainly the other, of the same components. If any two components are stimulated equally a sensation of white light results; complementary colours are therefore merely those which have an equal effect on two of the components. Whether or not a colour is more or less saturated depends on whether the components other than the one mainly involved are responding much to the light or not.

Experiment 63. After Images. Look fixedly for a few seconds at a bright object (the filament of an electric light will do) and then transfer your gaze to a black background. The bright image does not disappear at once but only fades gradually away. This is a POSITIVE AFTER-IMAGE. Repeat, after the image has quite faded, but this time move your eyes to a brilliantly lighted white background. There is a persistent image in this case also, of the same shape as the object, but whereas the object was a bright one the image looks dark. This is a NEGATIVE AFTER-IMAGE.

Look steadily at a tiny spot in the centre of a square of bright red paper, well lighted. After a few minutes shift the gaze to a white background. You see the square still, but tinged with greenish-blue, the colour complementary to red. Repeat, after waiting until the after-image has faded, with a yellow square, then try with a green one. The negative after-images are coloured with the shades complementary to those of the objects.

The explanation of the positive after-image of a white object probably lies in the fact, which we have already noticed in another connection, that the reaction of the retina does not cease immediately the stimulus is removed. The positive image is merely the after-discharge of the light-sensitive cells in the retina. The phenomenon of the negative after-image is comparable to the "rebound" which occurs after a reflex, as Sherrington showed. When a reflex arc has been for some time under the influence of one reflex, its

responsiveness to a stimulus which tends to bring about the opposite reflex becomes increased. Fatigue following a reaction of one kind makes the way easier for the opposing reaction.

In more detailed form, according to the Young theory, the reason why the after-image of a coloured object is tinged with the complementary colour is as follows. During the time that the gaze was fixed on the object all three components in the cells of the retina on which the image fell were being stimulated but the component or components which are most sensitive to the colour of the object were being stimulated most. When the object is withdrawn and the place on which the image falls is uniformly stimulated by white light all three components respond. The one which was mainly affected by the former light is fatigued however and responds less than the others. This enables the colour complementary to it to come into prominence.

Partial Colour Blindness. This abnormal condition of vision is interesting in the discussion of the theories of colour vision as it affords a comparison with the normal. The defect, fairly common among men, less so in women, is physiologically quite different from total colour blindness, with which it must not be confused. People who are partially colour blind distinguish differences in light waves of different lengths, no matter whether their intensities are equal or not, but they see fewer differences than normal people. If they are shown light of different colours (the best to use light coming from lanterns with windows of coloured glass) they select as being of similar shades the yellows and the blues and the violets which look much alike to the normal eye, but in choosing shades similar to a green they also take, as well as other green lights, different shades of red, and in matching a red standard they pick out as having a colour similar to it not only other reds but also greens as well. The condition of their light-receptive mechanism seems to be not so much different from, as simpler than, the normal: roughly speaking, partially colour-blind vision is a reduction form of ordinary sight. Whereas all the various shades which one sees with normal colour vision cannot be produced with combinations of less than three given colours, two only, mixed in different proportions, will match all the colours seen by a subject who is partially colour-blind.

There are two types of the defect. In the first, most common, form the eye is relatively insensitive to red light. To match an olive green, people with this type of the defect choose a scarlet which to the ordinary eye appears much brighter and the visible spectrum is shorter for them at the red end than it is for other people. Subjects whose vision belongs to the second type match a green with a red which is about equally bright and the red end of the spectrum appears to have its usual length.

The explanation of these facts, according to the theory of the three visual components, is that in eyes of the first type the red component is left out and so at all wave lengths have only the other two components to act upon. In the second type it is the green which is missing. This would express the actual conditions well enough if only these abnormal types were accurately, and not merely approximately, reduction type of the normal. More recent work however has

emphasized the fact that this is not the case and so far it has not been possible to cover adequately the conditions of the defect with this, or indeed with any other, theory.

HERING'S THEORY OF COLOUR VISION.—The only other theory which has rivalled that of Young as a starting point for experimental work is the one brought forward by Hering. This is also based on the laws of colour mixing which we have already outlined, but it affords as well some physiological explanation for the important fact that in our sensations light may not only be divided into different colours, but these again appear to fall into two great classes, the 'warm' or 'bright', cheerful colours and the 'cold' or 'dull' ones. To the 'warm' class belong reds, oranges, and yellows; they are bright of their own nature as it were, quite apart from the brilliancy with which they are lighted. The dull ones are green, blue and violet. There are many examples which show that we recognize this inherent brightness or dullness of colours in everyday life. We "see things through rosy spectacles" or we "feel blue". Decorators use yellow in north rooms with no sunlight, but blue or green are said to be too 'cold'; children are dressed in red in winter because it is "such a warm colour". The suggestion which Hering made was this: there are three substances in the retina each of which may be changed by light stimuli in two ways, being either built up or broken down. One of them is the white-black substance; light waves of more than a certain intensity break this down, no matter what their length, and give as a result the sensation of white light. Darkness causes it to be built up and a sensation of blackness arises from this. Of the other two components, one is acted on only by red light, which breaks it down or katabolises it, or by green, the complementary to red, which builds it up. Katabolism of the third substance is caused by yellow light, anabolism by blue, its complementary. All other colour sensations are mixtures of the sensations arising from the anabolism or katabolism of the three substances. All colours which break down the components are similar to white, which also does this; that is why they are specifically bright. Colours which build up are like darkness and hence in themselves are dull. Complementary colours give white light because, since they act equally on a single substance, the katabolic effect of one cancels the anabolic effect of the other and the only reaction left over is the katabolism which they both bring about in the white-black substance.

According to this theory the reason why the after-image of, for instance, a green object is coloured red is that the action of the green light having built up a great deal of the red-green substance, the red waves contained in the white light from the bright field which follows have more substance to act on than the other waves. The after-image of a yellow object is blue because the yellow waves have broken down the yellow-blue substance to a great extent and the anabolic reaction to the blue waves in the succeeding light is more intense, tending to restore the equilibrium, than it would be if the equilibrium had been undisturbed.

This theory gains some support from other observations on vision. Blackness seems to us to be, not a negative, but a positive thing. In the ordinary resting state of the retina we do not see blackness; our visual field is covered with vague

light-waves and is roughly speaking grey, certainly not pure black, as it ought to be as far as the Young theory goes. According to the Hering theory the equilibrium of the white-black substance is the resting-state, associated with grey sensation, and the black sensation belongs to active katabolism.

When it comes to an explanation of partial colour-blindness this theory breaks down. Absence of the red-green component would be the most obvious explanation to advance but this does not hold in view of the two different types of the defect. There are also difficulties arising from more detailed experiments on after-images which stand in the way of accepting this theory as a complete expression of the facts of colour vision. Indeed no theory has as yet provided that, a fact not to be wondered at in view of the extreme complexity of the reactions involved.

CHAPTER XXII.

SIMULTANEOUS CONTRAST. VISUAL JUDGMENTS.

In the foregoing discussion it has been tacitly assumed that the only light which has anything to do with the visual sensations which arise from stimulation of any particular part of the retina is the light which actually falls on that part. As a matter of fact this is not so. Strong stimulation of one area, as well as causing the appropriate sensation there, also tends to produce sensations of the opposite nature in the parts nearby.

Experiment 64. Take two pieces of the same paper, neutral grey in colour, and lay one on a white, and one on a black back-

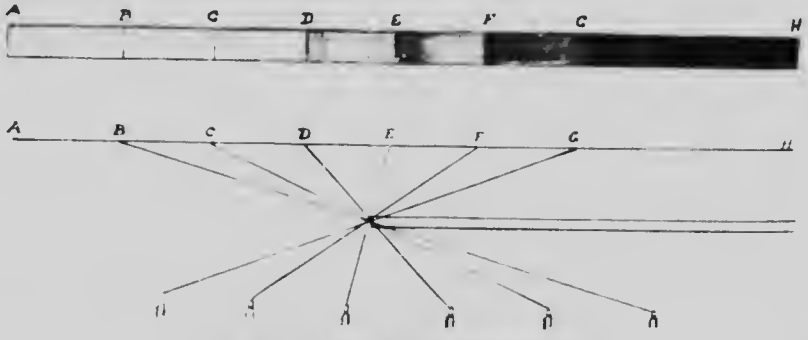


Fig. 49. To show simultaneous contrast.

ground. The first appears darker and the second lighter than before because of the contrast with the surrounding field. The effect may be increased by veiling the whole with a white tissue paper.

Experiment 65. —Arrange a white background with a horizontal row of candles in front of it and thrust a screen in from one side, part way between the two (Fig. 49). One large area of the background (GH) receives none of the candlelight and one (AB) receives light from all the candles. Between these there are a number of vertical strips, on the first of which (FG) the light from only one candle falls, on the second the light from

two DEFs, and so on. One would expect each of these strips to seem of even brightness throughout; actually however, as the figure shows, each one appears lighter at the margin which is next the darker area and darker at the other edge, which borders on the more brightly lighted strip.

Experiment 66. Lay the piece of grey paper on a background of bright red. Cover them both with white tissue paper. The grey area looks tinged with green-blue, the colour complementary to that of the background. Change the background several times, using various brilliant colours, and note what influence each has on the apparent colour of the neutral grey.

The phenomenon of simultaneous contrast is seen in countless ways in ordinary life. Shadows on sand in the sunlight look bright blue because of their yellow back-ground. So, too, places in a brightly lighted room which are shaded from the artificial light but faintly illumined by daylight appear to be blue, though as a matter of fact the light falling on them is colourless, only because of the yellow in the light on the surrounding areas of the field. The principal, consciously or unconsciously, is used in art, in posters and on the stage, to make the effect of vivid colour more vivid or to modify the shade of fainter ones. It is not clear how the phenomenon is brought about. It does not happen in the retina, for simultaneous contrast has been shown for the blind spot, where there is no retina at all. The modification must take place somewhere on that part of the brain which is concerned with sight. In this connection it is interesting to remember that there are other instances of the activity of one sensory area influencing the sensitivity of another. Sherrington showed the stimulation of one part of a receptive field lowered the threshold of the response to a succeeding stimulation of another part of the field, for the same or for an allied reflex (Immediate Induction).

Judgment of Space and Depth. External objects, no matter what their shape, can only form flat images, images of two dimensions, on the retina. In spite of this we see the objects in three dimensions, as having depth as well as width and height, and it is of interest to analyse some of the factors which contribute to this judgment as well as those which have to do with another closely allied to it, the judgment of distance. It is not practicable to do in the class room many of the experiments on the factors concerned

in visual judgments because they are so largely subjective in nature. The student is expected to make observations for himself along the lines indicated below. He should also use the various charts which have been prepared to assist in this part of the work.

Experiment 67. Some of our accuracy depends on the use of both eyes together. Try to touch with the tip of your pencil a black dot in the middle of a sheet of white paper which you hold obliquely in front of you. Now close one eye and see whether or not you can perform the movement as well as before.

MONOCULAR ELEMENTS IN JUDGMENT OF DISTANCE. One of these is the HAZINESS or clearness of the air between the object and the observer. Objects at a distance are hazier, all other things being equal, than those near by, so that we usually interpret hazy objects as being distant. That is why objects "loom large" in a fog. Since the things look hazy we conclude that they are farther away than they really are. The size of the images which they cast on the retina is not changed, however, and this together with our idea of their distance is the only clue which we have as to the size of the objects. If they were really as far away as we imagine them to be, to throw images of this size on the retina they would have to be larger than they really are, and we form our judgments accordingly.

A second and more important factor in monocular judgment is that known as MATHEMATICAL PERSPECTIVE. It is familiar that lines which are really parallel to one another appear as they recede into the distance to come closer and closer together. The rails of a level stretch of track, for instance, appear to one standing between them to form a V, with its point beyond the horizon. We are so familiar with this effect of distance that when we see lines which we believe to be parallel, or nearly so, appearing much closer together at the farther than at the nearer end of an object, we judge the object to have a good deal of depth.

THE MUSCLE SENSE of the ciliary muscles gives important information as to the depth of objects which are close at hand, because of the difference in the extent of accommodation necessary to bring into focus first their nearest and then their farthest point. The greater depth they have, the more difference there is in the effort of accommodation in the two cases.

THE SIZE OF FAMILIAR OBJECTS is used to judge distances. If the images on the retina of people or animals are small we assume that they must be far away and other things which are evidently near them, of which we do not so accurately know the size, are judged also to be distant.

BINOCULAR OR STEREOSCOPIC VISION. — When we look with both eyes at a flat object the image which is thrown on one retina is of exactly the same shape as that made on the other. If the object has depth, however, the two images are not exactly alike. The right eye sees a little more of the right side and a little less of the left than the left eye does, and vice versa. For instance, if one looks from above at a pyramid with its top cut off, the right eye receives an image of it of the shape of diagram A in Fig. 50, the left, one like B. Each of these diagrams alone looks perfectly

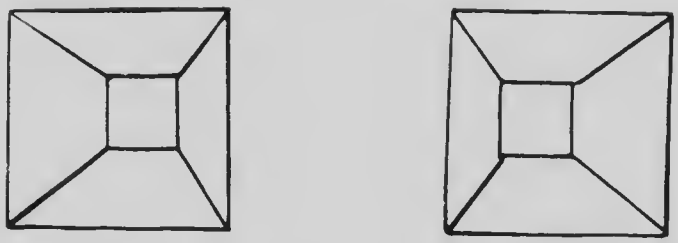


FIG. 50. — [illegible]

flat. But hold them in front of your eyes about six inches away and stare straight through them, relaxing your accommodation; the two appear to fuse and the picture that results is one of a pyramid which has depth as well as breadth and height. This is in all probability the main factor in the better judgment of depth which one gets from using both eyes. The principle is used in the stereoscope. Two photographs are taken of the same view, but the first from a position a little to the right of that from which the second is taken, the distance between the two positions being greater than the distance between the eyes. These pictures are mounted on a frame and, by an arrangement of prisms, the image of the one which was taken from the right side is thrown on the right eye, and the image of the left picture on the left. The result is a view in which the relief or depth appears even greater than it really is.

CHAPTER XXIII.

HEARING.

Almost all that is known about the physiology of hearing has been got by interpreting the structure of the ear. The subject lends itself hardly at all to experimental investigation, and on this account no attempt has been made to have this chapter similar in treatment to the others. It is inserted merely in order to round out for the student the outline of the physiology of the special senses.

THE PHYSICAL BASIS OF SOUND.

Preliminary to the consideration of the ear itself the student is reminded of the following facts in the physics of sound. Sound, like light, is carried by waves, but while light waves are strains in an intangible ether and have nothing to do with the molecules of the medium in which they travel, sound waves are vibrations of the medium which carries them, and they do not pass across a vacuum. The vibrations of the air molecules by means of which a sound wave is carried are in the direction in which the wave itself is travelling, each particle swinging to and fro alternately in front of and behind its mean position. The movement of the particles close to the source of the sound is started by the vibrations of the source itself. Those molecules in turn set those swinging which are a little farther away, these communicate the movement to the next, and so the wave is carried. It follows that in air in which a sound wave is travelling there must be some place where the number of air molecules is greater than the average and somewhere where it is less, alternate places of compression and of rarefaction (Fig. 51, No. 1). The condition in the column of air at any one time may be shown diagrammatically by a curve such as in Fig. 51, No. 2. Here, lengths along the abscissæ represent distances from the source along the line of the wave, lengths along the ordinate show the number of molecules per unit area. Zero represents the average number, lengths above, numbers per unit area greater than the average (compressions), and those below, numbers less than the average (rarefactions). If the sound is musical the wave will be a regular one and the distance from one crest to another, or from one trough to another, always the same. Unmusical sounds, or noises, are made by waves which have no regular shape.

Between musical sounds, which will chiefly occupy us, it will be seen that there are several possible differences. (1) They may differ in *rate*, that is, in their distance from one trough of the wave to the next, or from one crest to the succeeding one. The rate of the wave determines the *pitch* of the note; the faster the wave the higher the note. The note of the second wave in Figure 52, for instance, whose period is half that of the first, is an octave higher than the first one. (2)

Waves may differ in *intensity*, in the distance from zero line to crest or trough; the greater the intensity, the *louder* the note. (3) The waves may be musical, that is, each single wave may be a repetition of the last, and still not have the simple form shown for 1 and 2 in Fig. 52. These curves are such as would be made by a swinging pendulum, writing on an evenly moving surface, and they are known as *pendular* or *sine* curves. The sound waves which a tuning fork gives have this form. Waves 3 and 4 in Figure 52 on the other hand, are musical ones, but not of this simple shape. They are each made by adding, algebraically, waves 1 and 2 at different relative phases. Curve 3 is got by adding 2 at *a'* to 1 at A.

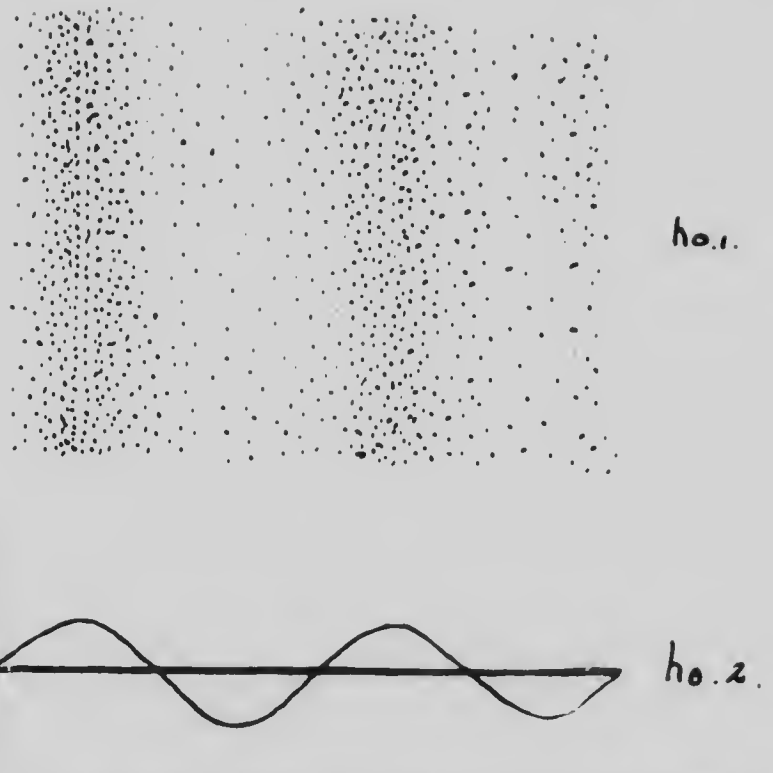
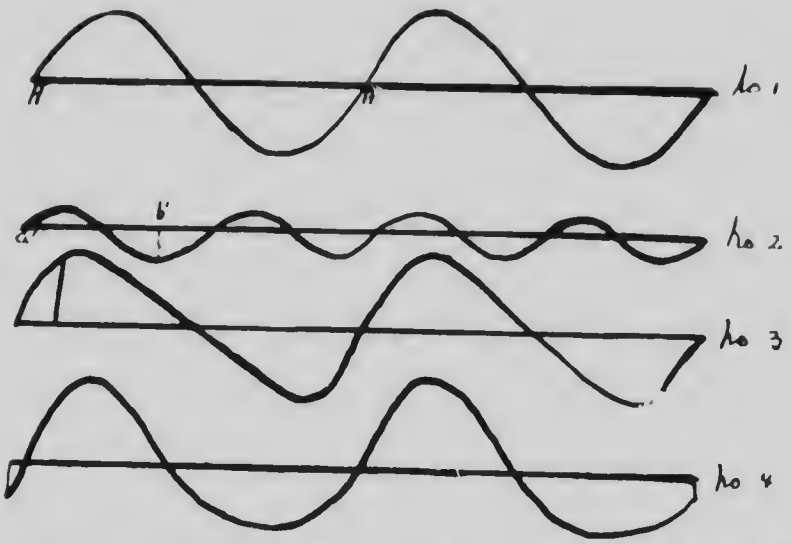


FIG. 51. — No. 1. To show the [illegible] of a vibrating body, the air of which a sound wave is supposed. No. 2. To show the expression of the condition of No. 1 in form of a curve.

curve 1, 2 at *b'* was added to 1 at A. Obviously, the number of such composite ves which one might make is unlimited, since one can also choose components different relative rates. For instance the period of one might be to the period the other, as one to three, or one to four, or two to five, and so on. Further, might add three or four waves together instead of only two. The period of composite is always that of the slowest component wave which it contains. low note is known as the *fundamental*. It is the *shape* of the wave which



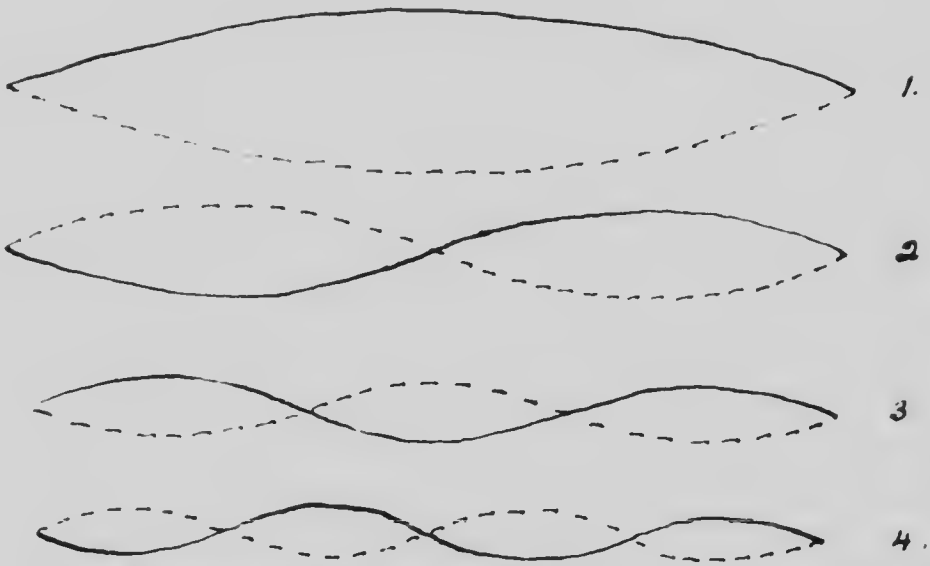


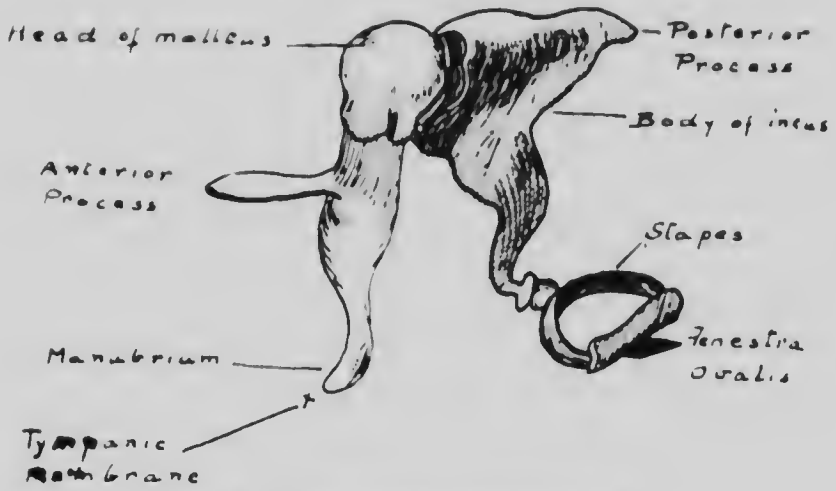
FIG. 53. Diagram of a vibrating violin-string to show how the various overtones arise.

gives it its *quality*. If, for instance, one hears middle C sounded on a violin it has a quality different to that of the same note on the piano, and this again is different from the sound of the tuning fork, which, as we saw, gives a simple pendular curve. The reason for this difference is that none of the notes in the common instruments swing with a single vibration. A violin string, for instance, swings as a whole, the rate of vibration of the whole length determining the pitch of the lowest or fundamental tone in the composite curve of its note. As well as this, however, the string tends to swing as if it were divided into short lengths, halves, quarters, thirds, etc. (Fig. 56). The point dividing these lengths swing with the vibration of the whole, the parts only swing with the rhythm of the whole and with that of the part as well. The rate of vibration of the divisions is to the rate of the whole inversely as the length of the division is to the length of the whole. The result of this is that the sound given out from the string is a composite one, like those which we have described, made up of a wave with a period like that of the vibration of the whole string, upon which a number of other waves have been superposed, waves with rates in simple arithmetical ratios (1, 3, 1/4, 1/5, etc.) to the first. These arise from the vibrations of the parts and are known as the OVERTONES. It is the number and intensity of the overtones which determine the quality of the note, and it is because these differ on different instruments that we are able to distinguish from one another the notes that they give.

THE TRANSMISSION OF SOUND WAVES IN THE EAR.

Of the three parts of the ear, the external and middle chambers are only concerned with the transmission of the sound waves, the essential organ of hearing being entirely contained in the internal ear. The waves travel in air as far as the inner end of the EXTERNAL AUDITORY MEATUS. Here the alternation of compression and rarefaction of which they are made up sets in motion the TYMPANIC MEMBRANE. This membrane is made up of two epithelial layers, enclosing between them a layer of connective tissue which contains both radial and circular fibres. In shape it is somewhat peculiar, being slightly vaulted, with its convex side inward. The apex of the vault, or UMBO, which forms a centre for the radiating and circular fibres, is not exactly at the middle of the membrane but a little above it. Because of its peculiar shape the membrane responds to waves of a very large range of rates, instead of having a strongly marked natural period of its own and being set in motion mainly by waves of this period, as would be the case if it were simply a flat drum head.

THE MIDDLE EAR. Sound waves travelling in the middle ear are no longer carried by air particles but by the swinging of a chain of three small bones, one end of which is fastened to the end of the tympanic membrane and the other to the membrane of the fenestra ovalis, one of the openings in the bony partition between the middle and the inner chambers. The connection with the tympanic membrane is through a long process, the manubrium of the MALLEUS, the first of the three bones. The body of a malleus is connected by a joint with the second



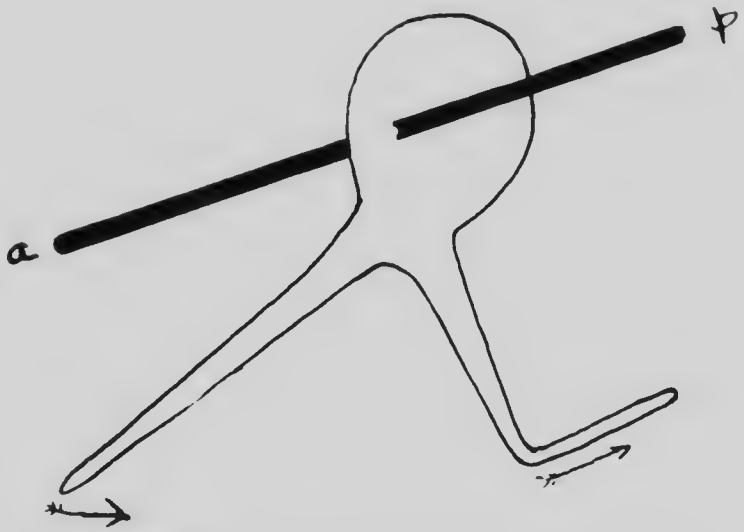


FIG. 55. Diagram of a lever drawn to show the mode of action of the bones of the middle ear.

case, the incus. This in its turn is in connection, by a process running down and with the third bone, the stapes, the inner end of which communicates with the membrane of the fenestra ovalis. Fig. 54 shows the shape and relationship of the three bones. The malleus is rather loosely attached by a ligament to the upper part of the outer wall of the chamber and besides this has a second process, a slender one, nearly at right angles to the manubrium, running back to the anterior wall and firmly bound there. The bone with which the body of the malleus is jointed, the incus, has two processes, one of which, already mentioned, extends down and in to make connection with the stapes, while the other, directed backwards, is firmly bound to the posterior wall of the chamber. The stapes lies almost horizontally between the top of the process of the incus and the fenestra ovalis. The whole chain of bones acts together as a lever, one arm of which is the manubrium of the malleus, the other the downward process of the incus, together with the stapes, the fulcrum being somewhere in the lower part of the malleus near its joint (Fig. 55). When the membrane of the tympanum swings in the top of the manubrium is pressed inwards. This would tend to move the malleus and incus bodily inwards except that they are firmly bound to an anterior and posterior wall by the attachment of the processes to the anterior and posterior ligaments which we have already mentioned. These act as an axis around which the three bones swing. When the top of the manubrium is moved in, the head of the malleus and the body of the incus swing out. Outward movement of the body of the incus means movement in of the process which is attached to the stapes, and this thrusts the stapes ahead of it. It will be seen in the diagram that the manubrium, the power arm, is about half as long again as the process in the incus, the load arm. The lever therefore reduces the excursion of the waves which enter it, but at the same time it increases their intensity. Movement communicated to the fluids of the internal ear need only be very tiny indeed to stimulate the sensitive cells, so that this cutting down of the magnitude of the sound waves is not a disadvantage. At the same time, even these tiny columns of fluid have an appreciable inertia and on this account the increase in power of the wave is important. A further increase in power is got from the concentration of the force of the wave received by the whole surface of the tympanic membrane on to the much smaller area of the fenestra ovalis.

As we have said, a movement inwards of the membrane causes the head of the malleus to move out, locking with the body of the incus and drawing it along. If the membrane is moved out to any unusual extent, however, as happens for instance when the surrounding pressure falls, the inward movement of the head of the malleus which results is not followed by the incus. As Helmholtz pointed out, the joint between them is a cog or ratchet joint and locks firmly for movements in one direction, but slides for large movements in the other. This is probably important as a protection to the other parts of the middle, and to the internal, ear.

There are two tiny muscles in the middle ear. One of these, the TENSOR TYMPANI, has its origin in a groove above the Eustachian tube, that is, from the inner wall, and is inserted into the neck of the malleus below the axis around which the bone rotates. When this contracts it must draw the manubrium of the

malleus inward and through it exert a pull on the membrane. What the usefulness is of this extra tension on the membrane, and the slight increase in pressure of the middle chamber which it causes, is not clearly understood. It has been suggested that the action is protective, damping the response of the membrane to excessive sounds. The suggestion has also been made that the muscle contracts reflexly when the attention is directed to sounds of high pitch, or rapid vibrations, to increase the tension of the membrane and thus to make its natural vibration period more rapid. The second muscle in the middle chamber, the STAPEDIUS, arises from a projection on the inner wall and is inserted into the neck of the stapes, on which, when it contracts, it exerts a lateral pull. The conjectures as to the use of the tensor tympani apply also to this muscle.

If the tympanic membrane is to swing freely in response to sound waves in the air the pressure on both its surfaces must be kept equal. This is ensured by the opening of the EUSTACHIAN TUBE which occurs during swallowing and gives a communication between the middle ear and the pharynx. When

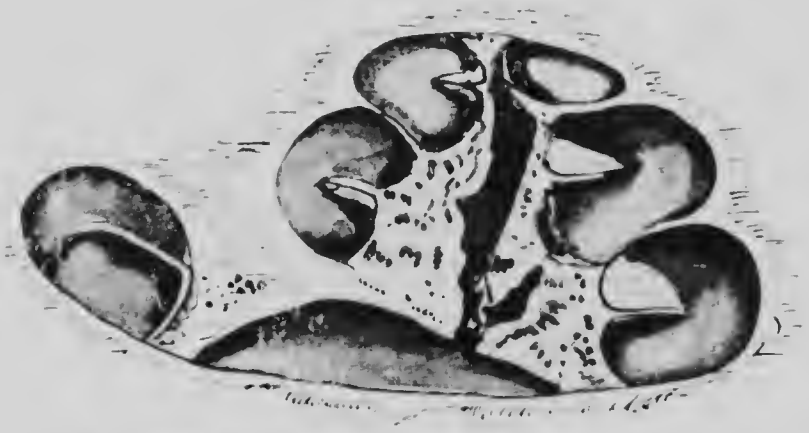


Fig. 56. Cross-section of the bony labyrinth showing the spiral of the cochlea.

the mucous membrane of the tube becomes swollen and the passage closed, as in severe colds, the gradual absorption which goes on of the air contained in the chamber of the middle ear lowers the pressure in it, the membrane no longer vibrates freely, and a partial deafness results.

PHYSIOLOGY OF THE ESSENTIAL ORGAN OF HEARING.

THE INTERNAL EAR. The cavity of the skull which contains this part of the labyrinth is spiral in shape, gradually tapering off to a point (Fig. 56). It is lined with membrane and is filled with fluid. From the part of the bone which forms the central pillar of the spiral a ledge projects into the cavity, reaching about half way through its width and running almost the entire length of the spiral. This partial partition of the chamber is completed by two membranes attached at one side to the ridge of bone and at the other to two lines a little distance apart on the membrane of the outer wall (Fig. 57). As a result the fluid of the spiral

divided into three columns, one on ie of the partition of bony ledge and the first membrane, one on the other s the bony ledge and the second membrane, and the third, enclosed between the membranes, roughly triangular in shape, its third wall being made by the membrane of the outer wall itself. Of the two outer columns one, the SCALA VESTIBULI, is closed, at the end which is directed towards the middle ear, by the membrane of the fenestra ovalis and the other, the SCALA TYMPANI, by the second membrane-covered opening in the partition, the fenestra rotunda. At the top of the spiral these two columns communicate over the tip of the middle triangular one, which ends blindly a little short of the upper end. Fig. 58 shows diagrammatically the relationship of the three movements of the membrane of the fenestra ovalis cause vibrations of the same

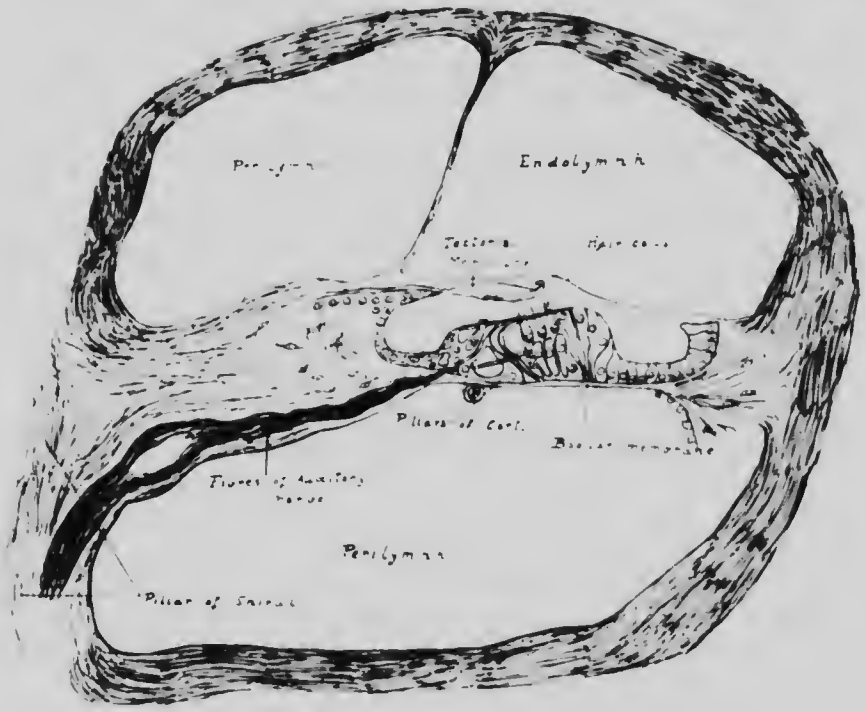


FIG. 57. One turn of the cochlear spiral, enlarged to show the organ of Corti.

and shape in the double column of fluid contained in the two outer chambers, being allowed by the membranous end of the second column at the fenestra rotunda, which moves out when the ovalis moves in and vice versa. Because the partitions between them are only membranes, it is to be expected that the oscillations of the two enclosing columns must affect the contents of the third. It is among these structures that the endings of the auditory nerve are found and we must look here for the essential organ of hearing.

Of the two membranes which form the walls of this triangular chamber (fig. 57) one is histologically quite simple. The other, the BASILAR MEMBRANE supports a double row of pillars, standing with their bases apart and their tips

leaning together and projecting into the fluid of the middle column, the endolymph. These are known as the PILLARS OF CORTI. Above these pillars on either side, supported by the tent-like roof which they form, are several rows of cells, among them a large number which have hair-like projections up into the endolymph. The whole is known as the ORGAN OF CORTI. The fibres of the auditory nerve, which runs within the inner pillar of the spiral, pass out by the bony projection along its entire length and end in the hair cells, which must therefore be the cells sensitive to the waves of sound.

As we have already seen, the ear is capable of distinguishing not only pitch and intensity but also quality, in any waves which it receives. Not only do notes of the same pitch, one without and one with many overtones, not sound alike but with training the hearer can distinguish what the various overtones in the notes are, so that our theory of hearing must afford a physiological basis for this analysis. We have no problem of simplification to deal with in the ear as we had in the eye; in hearing the sensations are as numerous as the stimuli which evoke them. The most generally accepted theory is one proposed by Helmholtz, the resonance theory. If one places a number of tuning forks of different periods near a vibrating fork or reed, giving out a simple note, among those forks which have not been struck the one with the same natural period will begin to vibrate and give out its note, while the others remain silent. If the vibrating source is a

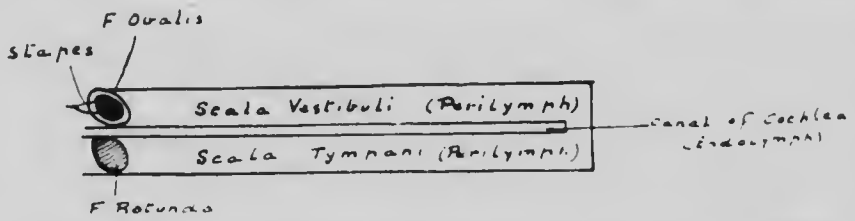


FIG. 58. Diagram of the relationship of the three fluid columns of the internal ear.

violin string, which gives a note with overtones, not only will the fork with a period like the fundamental respond but those with periods equal to each overtone as well. Helmholtz suggested that some such apparatus must be contained in the ear. He at first thought that it was the rods of Corti which, having each a different natural period, acted as resonators to the composite vibrations of the fluid of the outer columns. The ear however is capable of distinguishing more notes than there are rods; besides, rods are absent from the ears of singing birds, and this idea had to be abandoned. It was then suggested that the resonators might be found in the basilar membrane itself. It contains a series of radial fibres running from the bony ledge to the outer wall and the width of the membrane, and therefore the length of these fibres, increases progressively from the base of the spiral to its apex. If these fibres are the resonators the arrangement is like that of the strings in a tiny piano, with the short treble ones at the bottom of the cochlea and the bass ones at its top. The difficulty is to see how even the longest of these very small fibres can have a natural period as slow as that of the lowest note which we can hear. Of course the period of them all is increased because of the fluid about them but, even so, the difficulty has not been completely met.

CHAPTER XXIV.

SKIN SENSATIONS.

There are three types of skin sensations, the appreciation of differences of temperature (temperature sense), the sense of touch, and the sense of pain. Since our knowledge of the normal behaviour of each of these senses depends on subjective phenomena, each student must perform the fundamental experiments as given below, both on himself and on others, and must accurately describe the results in his notes.

TEMPERATURE SENSE.

It is the rate at which heat is being gained or lost by the skin, and not the actual degree of temperature of the applied object, that is determined by our sensations.

Experiment 68. Place a finger of one hand in water at 2° C. and the corresponding finger of the other hand in water at 40° C. After no temperature sensations are felt by either finger, transfer them simultaneously at 30° C. Note the nature of resulting temperature sensations in the two fingers.

The temperature sensations are received by "hot" and "cold" pots scattered over the skin, the cold spots being the more numerous (Fig. 59).

Experiment 69. Mark out an area of skin on the back of the hand, say 20×20 mm. square. The hand should be resting comfortably on a table and the subject blindfolded. With a thermo-aesthesiometer (a test tube drawn out to a point will serve the purpose), containing water at 40° C., proceed to explore the selected area, systematically, in parallel lines, marking with ink the spots at which a distinct sensation of warmth is experienced. When all the area has been explored accurately, transfer the spots to ruled paper, and with the aesthesiometer containing water at a temperature of 15° C., proceed in the same way to determine the cold spots. Note that the cold spots, besides

being more numerous, are more sharply defined than those of warmth; the latter are also much more readily fatigued. This fact must be remembered in repeating the observations.

More intense heat stimuli, besides stimulating the warm spots, also stimulate those of cold, causing a cold sensation.

Experiment 70. Test this using a temperature of about 50° C. Mechanical and electrical stimuli, etc., applied to hot and cold spots, may call forth thermic sensations.

Experiment 71. Select a few pronounced cold and hot spots, and stimulate them by tapping lightly with a small round pointed object, or by applying the tetanizing electric current.

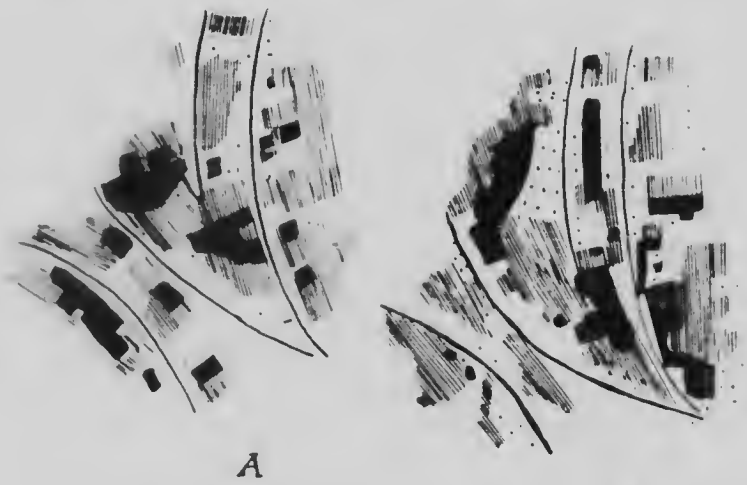


FIG. 59. Heat and cold spots on the skin of the palm of the left hand: *A* heat-spots, *B* cold-spots. The depth of the shading in each case represents the intensity of the sensation.

The sensation of temperature remains for some time after the condition causing it has been removed ("temperature after-sensations").

Experiment 72.—Place on the forehead a coin which has been either cooled or warmed, and note the nature of the sensations which are experienced after its removal.

The temperature sense is not equally acute over the body. Many factors influence the acuity but in general it may be stated that clothed parts are more sensitive than unclothed, with the

exception of the temples, the lower eyelids and the cheeks. The midline of the body is relatively insensitive. The sensivity increases from the far extremity of the limbs towards the trunk.

Experiment 73.—Using test tubes containing warm or cold water, proceed to confirm the above statements.

SENSE OF TOUCH.

This is likewise mediated by spots (touch spots), which are distributed independently of those of temperature, and are most numerous to the "windward" side of the hair follicles.

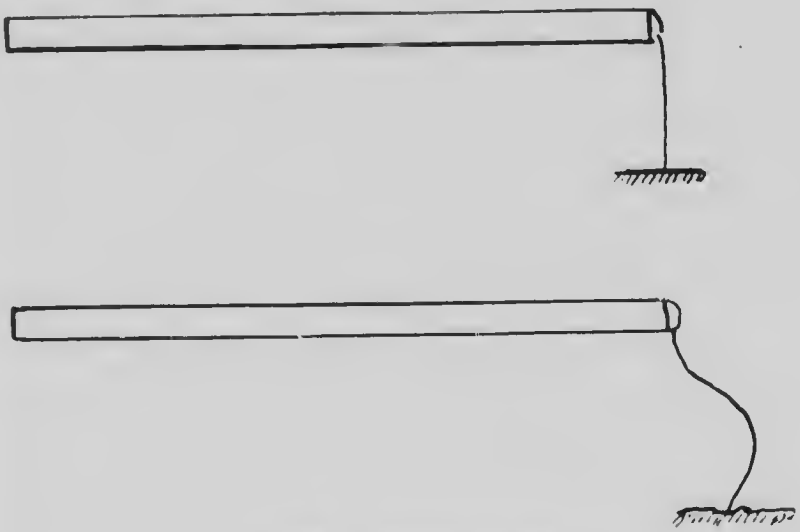


FIG. 60. Hair æsthesiometers.

Experiment 74.—Using the same area of hand as was employed for determining the temperature spots, or a part of it, make an exact chart indicating the position of the hairs (use a lens for this purpose). Now cut the hairs flush with the skin, by means of a sharp scissors, or shave the part. Take a series of hair aesthesiometers made by mounting straight hairs of varying thickness at right angles on wooden holders (Fig. 60). The exact weight at which each hair bends when pressed on one scale pan of a balance is marked on the handle. Select a hair which provides a stimulus of such a strength that it will call forth the

peculiar sensation due to a touch spot. Having made certain that the subject who should be blindfolded, can recognize this sensation (and does not confuse it with one of pain, which is of longer duration), proceed, systematically, to mark the touch spots. Transfer the results to the chart, and note the relationship of the touch spots to the hair follicles. By touching a hair the sense of touch is accentuated. Repeat these observations on the calf of the leg, where the hair follicles are less numerous.

Experiment 75. Select an aesthesiometer which can produce a distinct sensation of touch when its hair is pressed to the bending point on the end of one of the skin hairs. Apply it to a hairless part of the skin and note that it elicits no sensation.

It is important to distinguish between the sensations of deep pressure and touch.

Experiment 76. Touch the skin lightly with a blunt point; then gradually increase the pressure and note the occurrence of the deep pressure sensation. Note that this occurs only after there has been decided deformation of the surface.

The threshold value for touch (touch acuity) varies in different parts of the body. Several things, besides the presence of hairs, determine this (thickness of skin, rate of application of stimulus, previous friction of skin, etc.). In general, however, the order of touch thresholds is as follows: lips, finger tips and forehead; dorsal aspect of finger; palm, arm and thigh; forearm; while much less sensitive are the extensor surfaces of the forearm and the loins.

Experiment 77. Using various hairs, determine the exact threshold value for touch for the above regions by finding what thickness of hair must be used to elicit the sensation of touch at the point at which the hair just bends. Make a table of the results* giving the "bending weight" of the hairs for each region.

Besides touch acuity it is important to study the LOCALIZATION of touch sensations. This may be done either by asking the (blindfolded) person to point with his finger to the exact place where he was touched (absolute localization) or by ascertaining the distance by which two points must be separated in order to

*In many of these experiments on touch a fine camel's hair brush can also be used

be discriminated (touch discrimination). This "local sign", as it is called, varies in different parts of the body.

Experiment 78. With a pair of calipers, determine this distance for the tip of the tongue, the palmar surface of the third phalanx of the finger, its dorsal surface, the tip of the nose, the back and upper arm; make a table of the results. Note that the order of sensitivity for touch discrimination is not the same as that for touch acuity.

SENSE OF PAIN.

Any stimulus will cause pain when it is very intense. This does not mean, as has been thought, that pain is merely due to over-stimulation of any receptors. There are special receptors for pain, just as there are for touch and temperature. One of the main proofs of this is that pain spots are more extensively and regularly distributed than the others.

Experiment 79.—Using the same area of skin as was employed for the location of temperature and touch spots, proceed to mark out the pain spots by pricking with a stout horse hair or a bristle. Note that the sensation is more lasting than that for touch and that the pain spots are distributed in a different manner.

Another proof of the separate existence of pain spots is that they alone are found in the cornea; on the conjunctiva they exist along with temperature spots.

Experiment 80.—Verify the above statement, using medium hairs.

The skin sensations are not at all influenced to the same extent when the blood supply is interfered with. This fact becomes of interest on account of its analogy to the results which have been observed during the regeneration of severed nerves.

Experiment 81. By means of a blood pressure cuff and bulb applied to the arm, determine the systolic pressure, then release the pressure and raise the arm so as to empty it of some of its blood, after which again apply the pressure (but not above the systolic value). Proceed to compare the sensations of touch, temperature and pain in exactly corresponding skin areas of the two hands. Differences may take some time to become evident. Note particularly for which sensations they first become so.

TASTE.

Closely allied to the skin sensations are those of taste and smell The following experiments on taste are important.

Experiment 82.—(a) Dry the tongue and apply a crystal of salt or sugar. Note that it is not tasted.

(b) On the dry surface of the tongue apply by means of a camel's hair brush a solution of quinine (1 to 1,000), sodium chloride (1 to 200), cane sugar (1 to 50), and sulphuric acid (1 to 1,000). Determine at which part of the tongue the strongest sensation is produced by each.

(c) Prepare a series of solutions of gradually increasing strengths of quinine, salt, sugar, and sulphuric acid, beginning in each case at 1 in 5,000, and apply to that portion of the tongue most sensitive to the respective substances. Determine what strength of solution is necessary to stimulate taste.

(d) Apply with a brush a little of the sugar solution of the strength which just stimulated taste, and follow with a drop of salt solution of the same taste value. Note that the salt neutralizes the effect of the sugar and neither is tasted.

(e) Take a solution of strong salt water into the mouth and wash out with distilled water. Note that the water tastes sweet (negative after-stimulation).

SECTION VI.

DEMONSTRATIONS.

In the foregoing chapters experiments have been described which the student, either individually or in small groups, can perform for himself, but there are other experiments of equal importance from an educational viewpoint, in which, for obvious reasons this is impossible and it is necessary that these be demonstrated. The value of demonstrations of more or less complicated experiments in physiology depends on two factors, first, that the student is familiar with the general technique of the methods employed, and secondly that the precise application and bearing of the results of each experiment are appreciated by him. To stage an experiment with the sole object of demonstrating results is valueless, however important and fundamental these results in themselves may be; the demonstration becomes of value only when the student knows exactly how the result is obtained and is able to place it in relationship with previous knowledge gained by other methods of study.

The particular experiments which it may be deemed advantageous to demonstrate will necessarily vary, not only in different laboratories, but also from year to year in the same laboratory. It is believed, however, that it will be useful to describe briefly the fundamental experiments that the authors are accustomed to demonstrate to their classes, partly to assist the students to understand what is being done, and partly as a guide to others. The experiments that have been selected may also be performed by advanced students and are described in sufficient detail for this purpose.

The demonstrations are grouped in chapters, each chapter giving in practicable sequence the experiments which can most conveniently be performed on a single animal.

CHAPTER XXV.

THE CIRCULATION OF THE BLOOD AND LYMPH, AND THE RESPIRATION.

The Reflex Nerve Control of the Circulation and of the Respiratory Movements.

In Exp. 31, p. 74, it has been demonstrated that the heart beat is controlled largely through the vagus nerve and that the degree of resistance to the blood flow through the splanchnic vessels depends on impulses carried by the splanchnic nerves. The vagus and vaso-motor nerves arise in the medulla oblongata. The efferent nerves of the respiratory muscles (phrenic and intercostal nerves) arise in spinal nerve centres, which are dominated by a higher centre. This chief respiratory centre, as it is called, is also situated in the medulla. The present experiment is devised to throw light on the afferent pathways through which the above centres become stimulated.

Demonstration 1. Simultaneous tracings are taken of the carotid blood pressure and of the respiratory movements (p. 88), in an etherised and tracheotomised dog. The sciatic nerve in one leg and the vagus nerves on both sides of the neck are exposed and loose ligatures placed around them. A hot iron is then applied to the skin of the foot while the recording drum is revolving at such a speed that the cardiac pulsations can readily be counted. Observe the effects on: (1) the pulse rate (2) the mean arterial blood pressure and (3) the rate and depth of the respirations. What general conclusions may be drawn concerning the influence on the centres of over-stimulation of skin nerves?

After moderately tightening the ligature, the sciatic nerve is cut peripheral to it and the hot iron reapplied to the skin. What conclusion do you draw from the results?

The central end of the cut sciatic is then stimulated with the faradic current, of a strength which just produces some change in

ne or other of the tracings, the observations being repeated with tronger stimuli. The respirations are invariably increased in lepth and rhythm (hyperpnœa) and the heart rate is often reduced ut the effect on blood pressure as a rule is not readily measurable, ecause of the exaggerated respiratory oscillations which the racing shows. Afferent fibres to the vagus and respiratory centres re demonstrated in this experiment, and those to the vasomotor entre can be inferred in cases where the blood pressure rises or emains constant, while the pulse becomes slower. Why is the onclusion permissible?

One vagus nerve is cut after moderately tightening the ligature round it and the central end is stimulated. Are the results similar o those of sciatic stimulation?

The results of the foregoing experiments indicate that the entres of respiration and of vagus and vasomotor control are imultaneously stimulated through afferent nerves. In order to etermine the relative importance of each effect it is necessary o eliminate one or both of the other afferent influences. With his object in view, the remaining vagus is cut so as to remove the eart from vagus control. (What drugs could have been employed o effect a similar denervation of the heart? Why is there a rise n blood pressure?) The sciatic and vagus nerves are again stimuated. What difference is there in the results? Explain the cause.

Frequently, by using induced shocks that are relatively slow nd feeble, the reflex effects on arterial blood pressure are the pposite of these observed when shocks of ordinary frequency and trength are employed. What conclusions do you draw?

Even the elimination of reflex vagus effects may not suffice to emonstrate the pressor or depressor influence because of the preominance of the respiratory effects. It may be necessary to liminate the latter, which can be done by injecting through the emoral vein a saturated solution of curare in physiological saline.* nder the influence of curare the respirations cease, and to keep the nimal alive it is necessary to connect the respiratory pump to the racheal cannula, with the anæsthetic bottle inserted in the tubing.

*The curare solution should be prepared several days previously and should be ested on a frog before using in the above experiment. The curare at present on market is very unreliable.

(Etherisation must be maintained, otherwise the animal might suffer pain. Why?)

While maintaining the artificial respiration the sciatic and vagus nerves are stimulated as before. What conclusions can be drawn from the results?

The effect of *asphyxia* on the arterial blood pressure is then studied, the asphyxia being induced by suspending the artificial respiration. How do the results compare with those observed during asphyxia with the vagi intact? After the blood pressure has finally fallen to zero the thorax is opened and the heart examined. Extreme venous engorgement is observed. Note the behaviour of the heart when the engorgement is relieved by puncturing the right ventricle. What conclusions do the observations warrant?

CHAPTER XXVI.

The Direct Demonstration of Vasoconstrictor Fibres by the Plethysmographic Method.

Demonstration 2.—The abdomen is opened in an etherised, tracheotomised dog and the intestines retracted to the left side under towels wrung out in warm saline solution. The great splanchnic nerve is isolated and a ligature placed around it on the right side (see p. 76). The right kidney is freed from its bed and the folds of peritoneum running to its upper end are cut after applying mass ligatures. The pedicle is carefully freed by blunt dissection and the plexus of nerves isolated from it and a ligature placed around them. An oncometer, with cotton wool well mixed with vaseline laid in the groove for the pedicle, is then placed under the viscus and more cotton and vaseline placed in the groove until this is filled (without any compression of the vein or ureter) after which the glass lid is applied and clamped down. (There must be no strain or kinking of the vessels during the manipulation). The side tube of the apparatus is finally connected by moderately thick walled tubing with a bellows recorder, the writing style of which is accurately adjusted in the same perpendicular with the writing style of a mercury manometer connected with the carotid artery. A respiratory tracing should also be taken. On a moderately fast drum tracings are taken to show the relationship of the oscillations of the oncograph to the arterial and respiratory tracings. (What is the relationship?)

The effect produced on the kidney volume and the arterial blood pressure is then observed: (1) during (electrical) stimulation of the vagus in the neck; (2) during stimulation of the great splanchnic nerve on the right side (Fig. 61), and (3) during stimulation of the renal nerves. How do you interpret the results? Why is the arterial blood pressure essential in the interpretation?

A small amount of physiological saline containing 1-10,000 adrenalin chloride is now injected into the femoral vein. What conclusions do you draw from the result?

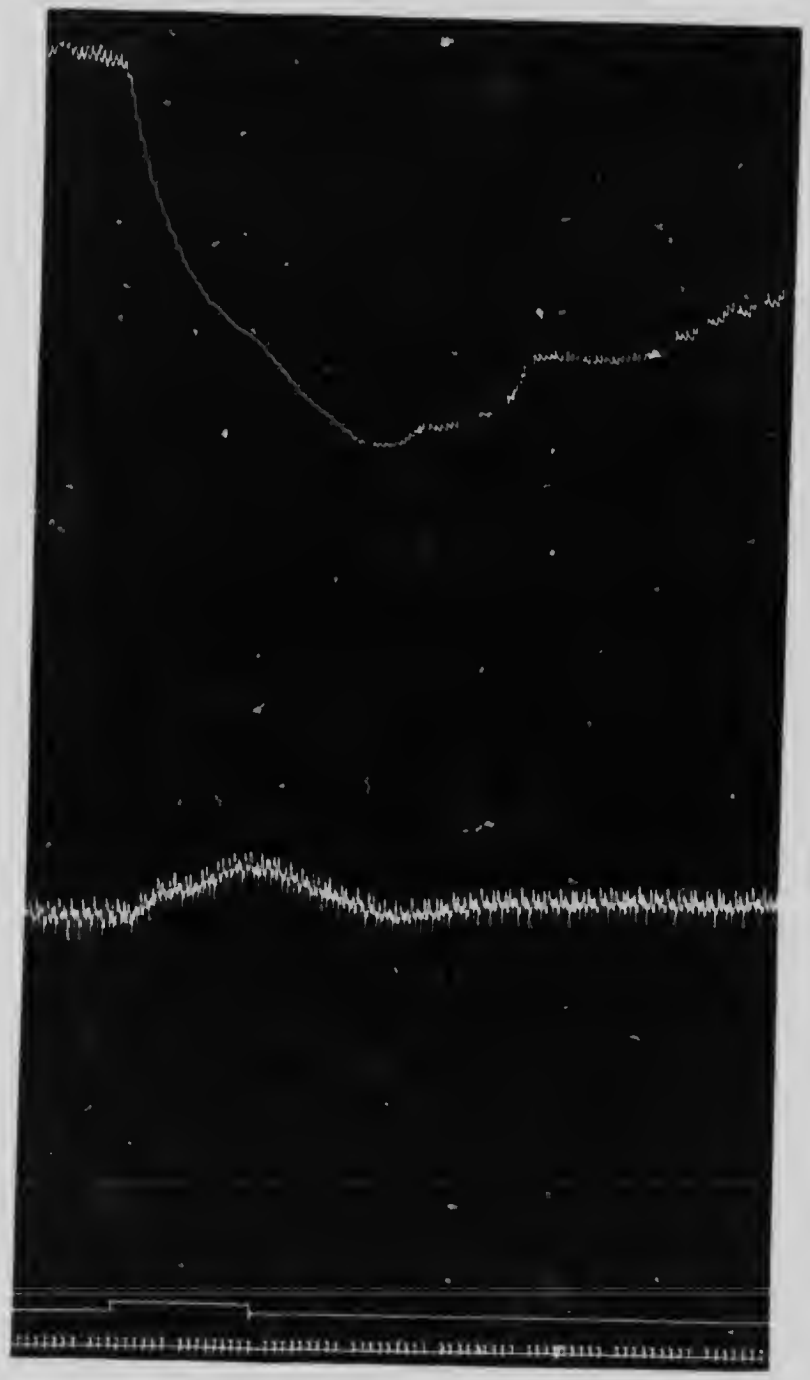


FIG. 61. Tracing of volume of kidney and of arterial blood pressure during stimulation of the splanchnic nerve.

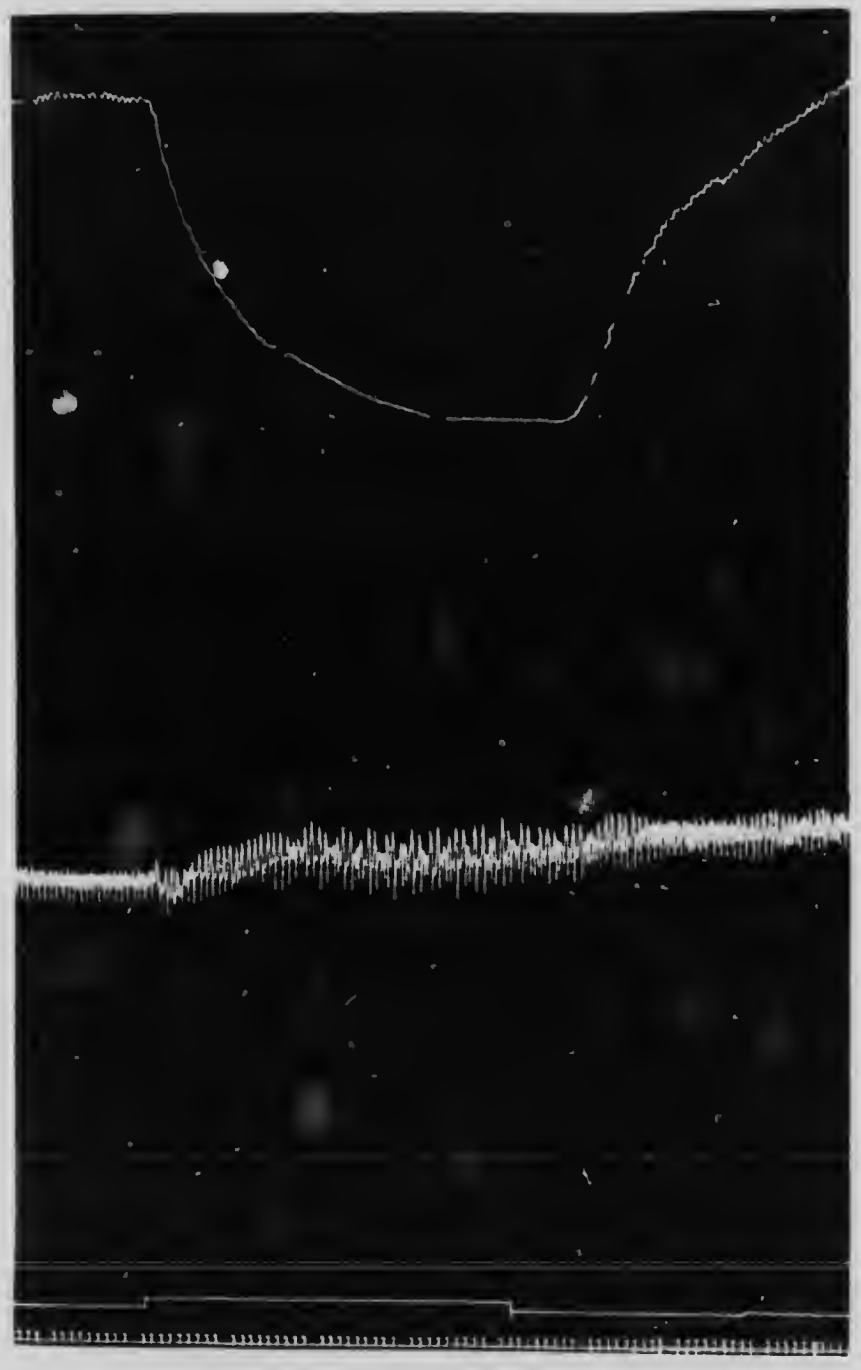


FIG. 62. Tracing of volume of kidney and of arterial blood pressure during asphyxia. Note slight rise in blood pressure accompanied by marked diminution in the kidney volume.

Curare is injected into the femoral vein until the respirations cease (see p. 183). Asphyxia is allowed to supervene by suspending the artificial respiration (Fig. 62), but this is re-established before the final fall in blood pressure sets in. What evidence does the result furnish concerning the cause of the asphyxial rise in blood pressure? (If satisfactory curare is not available, the asphyxia may be induced by clamping the trachea. The results are, however, less clear because of the respiratory convulsions).

Finally, the renal nerves are cut, and, the immediate effect on the kidney volume having been observed and explained, several of the foregoing experiments are repeated. How do you explain the alterations in the results?

The animal is killed by haemorrhage while the various tracings are being taken.

In one or other of these demonstrations the opportunity may present itself of attempting the **resuscitation of the animal by intra-arterial transfusion with epinephrin.** To effect this a cannula, inserted in the central end of the carotid artery, is connected with a burette containing warm isotonic saline solution, which is allowed to flow into the artery. Artificial respiration is also performed and after some saline has transfused a few cubic centimetres of adrenalin chloride is injected into the tubing which connects the artery with the burette and the thorax is vigorously massaged over the region of the heart. In a successful experiment the blood pressure, recorded from the carotid on the opposite side to that through which transfusion is being made, will be observed to rise, at first passively because of the thoracic movements, and later because of resumption of the heart beats. Once these have started the blood pressure quickly recovers and the cardiac massage may be suspended. Explain the mechanism of the resuscitation and particularly how the epinephrin acts. Would intravenous transfusion be of any avail in attempting the resuscitation after complete stoppage of the heart beat?

CHAPTER XXVII.

PERFUSION OF THE EXCISED MAMMALIAN HEART.

If a solution containing certain of the inorganic constituents of blood plasma and saturated with oxygen gas be perfused under a certain pressure through the coronary arteries, the heart will beat with perfect regularity for several hours outside the body. This surviving heart preparation is particularly useful for investigations of the influence on the heart of variations in the saline constituents of the nutrient fluid and of the effect of drugs. The nutrient fluid is caused to circulate through the coronary arteries by introducing it into the aorta, where it closes the semilunar valves and is forced into the openings of these arteries. The observation also demonstrates most convincingly the remarkable recuperative power of the heart, and it affords evidence that resuscitation methods should be persistently applied after drowning accidents and when death has occurred from other forms of suffocation.

The most convenient apparatus to use is that depicted in Fig. 63. The perfusion fluid (Ringer-Locke's or Tyrode's) is contained in the bottle A, and it is kept constantly saturated with pure oxygen by bubbling the gas through it. The fluid then passes to the cannula in the aorta through a warming device which consists of a wide tube surrounded by a water jacket kept at the desired temperature by a thermo-siphon system, as shown in the diagram. In order that the fluid may be thoroughly warmed it is caused to flow in a thin film on the walls of the tube (B) by placing in this a somewhat smaller tube (C) closed at both ends. Although it is convenient to use an apparatus in which the free end of the cannula is ground on its outer surface so as to fit the lower end of the inner tube of the warmer—an apparatus devised by the late T. G. Brodie—the simpler apparatus devised by Gunn, which is also shown in the figure, is perfectly satisfactory. In Brodie's apparatus a thermometer (T) is inserted into the aortic cannula through a side tube; in Gunn's apparatus the thermometer is made to serve as the inner tube of the warmer.

The heart is prepared for perfusion as follows: A rabbit is killed by a blow on the back of the head and is immediately laid supine on the table. A longitudinal incision is made through the skin of the thorax, extending for a short distance on the abdomen. The skin is retracted to both sides of the incision and the abdomen opened at its upper end. One blade of a stout pair of scissors is then passed through the diaphragm under the cartilages of the ribs on one side

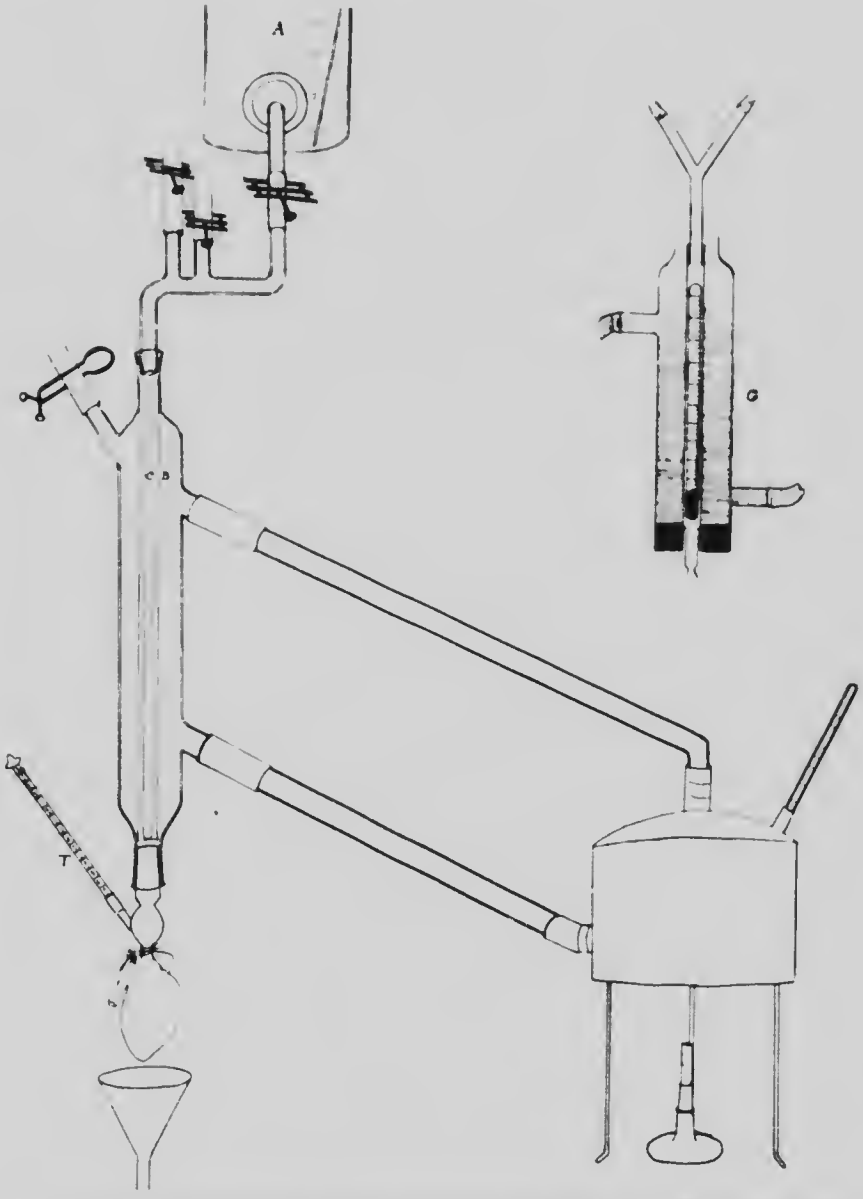


FIG. 63. Apparatus for perfusion of the mammalian rabbit's heart. The apparatus connected with the heating device is that of Brodie. Apparatus of Gunn is shown in insert.

and these are cut from below upwards, care being taken to keep the blade away from the heart and lungs. The cartilages are similarly cut on the other side of the sternum. The isolated piece of sternum is bent upwards and cut across at the upper end. The exposed pericardium is picked up with a forceps and opened with fine scissors, after which the animal is placed on the left side, so as to expose the structures at the root of the right lung. These are cut through and the operation is repeated on the other side. The aorta must now be made out and cut across just proximal to where its first branch (the innominate) comes off, after which the superior vena cava is cut a short distance from the auricle. A snip is made in the apex of the right ventricle, using a small sharp-pointed scissors for the purpose: this as to permit of free escape of the fluid from perfusing the heart. Finally a thread attached to a packing needle is inserted in the inferior vena cava, and cautiously guided upwards so that it emerges from the superior vena cava. The needle is unthreaded, and the thread tied in a loop. This thread is introduced to serve as a guide to the venae cavae into which later a tube is to be inserted. The heart is removed, by cutting the inferior vena cava below the thread, and placed in a dish containing cold Locke's solution. Any blood remaining in the right auricle and ventricle is then washed out by introducing Locke's solution from a large pipette through the superior vena cava. The special cannula is now tied into the aorta, care being taken that no air bubbles are entrapped. (This part of the technique is more conveniently performed with Brodie's apparatus than with Gunn's, but it is perfectly simple using the latter). A bent pin hook is passed through the outer coat of the right ventricle and a thread attached to it is carried over a pulley to a heart lever arranged to write on a drum. A similar hook and thread may also be attached to the auricle so that simultaneous tracings of auricle and ventricle may be taken. In order to steady the heart it is well to pass a thread through the apex of the left ventricle and tie it below to a glass rod fixed to a stand.

Demonstration 3. Having secured a tracing of the heart and noted the temperature of the perfusion fluid, the following observations may now be made:

1. The influence of changing the temperature of the perfusion fluid. This can best be done by adding hot or cold water to the upper vessel connected with the water jacket.

2. The influence of the local application of heat and cold in the right auricle. To demonstrate this, pass a thin-walled glass tube through the superior and inferior venae cavae using as a guide the string previously placed in the vessels. The upper end of this tube is connected by rubber tubing with a funnel, and either cold or hot water is made to flow through it while a tracing of the beat is being recorded. The temperature of the perfusion fluid is meanwhile kept exactly constant (cf. Sherrington).

3. The effect produced on the heat by increasing the relative proportion of the calcium or potassium in the perfusion fluid.

This is very conveniently accomplished in the Brodie apparatus by closing one of the side tubes on the inflow tube leading to the upper end of the warmer with rubber tubing through the wall of which is inserted the needle of a small hypodermic syringe containing the solution. For more continuous addition of greater dilutions of the foreign solution the side tube may be connected with a burette. In Gunn's apparatus a Y tube is provided for these operations. By thus injecting the foreign solution at a distance from the heart it is mixed thoroughly with the perfusion fluid and is properly warmed before the heart is reached. Observations should be made using first of all a few c.c. of a 1% solution of calcium chloride. When the beat becomes very small and the ventricle is almost in calcium rigor (see p. 57) inject a few cc. of a 1% solution of KCl. If the beat does not become restored to the normal in a few minutes or so, inject a little more KCl solution. Then add still more KCl until the heart again almost comes to a standstill (in diastole, p. 57), when it may again be restored by injecting CaCl solution.* It is, of course, evident that this observation does not in itself prove that the two cathions are mutually antagonistic to each other. To prove this it would be necessary to show that without the addition of the antagonistic cathion the beat is much more slowly restored.

4. The effect of alteration of the H-ion concentration of the perfusion fluid. This is done by cautiously adding, by the above procedure, 0.9 per cent. solution of NaCl containing either some HCl or some NaOH.

5. The effect of epinephrin. For this purpose about 1 c.c. of a 0.002 per cent. solution of adrenalin chloride solution should give definite results.

6. The effect produced by applying a tetanising electric current to the ventricle. Whenever the fibrillation becomes marked discontinue the stimulation and see whether the normal beat becomes restored by continuing the perfusion. Observe carefully the behaviour of the auricles.

It is usually necessary to employ more than one heart for all of the above observations. Sometimes, however, all can be done on one preparation.

*It is not possible to state precisely what amount of the two solutions should be used, because this will vary with the rate of the main perfusion. It may be necessary to repeat the observations several times before the effects are obtained

CHAPTER XXVIII.

MEASUREMENT OF THE BLOOD FLOW THROUGH THE HANDS AND FEET, BY THE CALORIMETRIC METHOD OF G. N. STEWART.

Because of their relatively great exposed surfaces the hands, and to a lesser degree the feet, quickly give off heat to their environment. They are excellent radiators. The muscles of these parts when at perfect rest, contribute but a very small quota to this heat, most of which therefore is carried to them by the blood from other parts of the body. From these considerations it follows that the amount of heat given out in a given time must be proportional to the amount of blood flow, or expressed in precise terms: the amount of heat given off in calories, divided by the difference in temperature between the blood flowing to and away from the part equals the volume flow of blood in cubic centimetres.*

If Q be the grammes of blood per minute, H the gram calories in m minutes, T the temperature of the blood entering the hand and T′ that of the blood leaving it, S being the specific heat of blood then:

$$Q = \frac{H}{m(T-T')} \frac{1}{S}$$

The calories are measured by placing the hand in a water calorimeter; the temperature of the entering blood may be taken, as that under the tongue† and the temperature of the leaving blood T′, as the mean temperature of the calorimeter during the observation.

The calorimeter (see Fig. 64) consists of a tinned inner copper vessel capable of holding somewhat over 3000 c.c. of water. This vessel is placed within a wider vessel, the space between the two being packed with broken cork. To the open upper end of the inner vessel is soldered a lid of copper with a hole cut in it towards one side of the centre and large enough and shaped so as to allow the

*A small or gram calorie is the amount of heat required to raise the temperature of 1 gm. of water through 1° C.

†Because of the liability of the mouth temperature to vary, it is more accurate to take for T the rectal temperature less 0.5° C.

bend to press into the vessel. About an inch beyond the edges of this hole a strip of copper, about 1 inch wide, is soldered at right angles to the lid. Beside the hand hole, and on the opposite side of the centre, three small circular holes are made in the lid each about one inch in diameter and provided with a copper tube of similar diameter, projecting upward. A piece of insulating cork is cut so as to form a lid large enough to fit the outer vessel, and holes are made in it to fit the tubes round the openings in the copper lid of the inner vessel. After fitting the cork cover in place, it is covered with varnish. The three small holes are used, one of them for a thermometer, and the others for long feathers to serve as stirrers. A piece of thick saddler's felt is cut to fit the hand opening in the lid. Several collars of the same material are also cut, 1 inch wide, and of various sizes to fit wrists of different sizes. The felt lid and collars rest on the copper ledge between the hand opening and the ridge of copper. A thermometer with a scale which reads between 0° C. and 50° C. divided into 1 10ths is sufficiently accurate. By using a telescope the temperature can be read to 1 50th or 1 100th of a degree.

Fig. 61. Stewart Calorimeter.

Besides the calorimeter, a large bath of 20 or 30 litres capacity (garbage can) is necessary. It is also convenient to have an adjustable stool (like a music stool) on which to place the calorimeter.

Demonstration 4. To make the measurement, the first step is to draw a line with a fat pencil on the skin at the lower border of the styloid process of the ulna of the hand chosen for observation. A felt collar of proper size is then applied to the wrist with its lower border corresponding to the pencil line, and a second line is drawn on the skin opposite the upper border of the collar. This upper line will serve as a mark to show that the hand is not changed in position

while in the calorimeter. The hand of the subject, who is sitting on a high chair, is then placed in water at about 30·32° C., contained in the large bath (the temperature of the water is taken by an ordinary thermometer) and it is left there for ten minutes, or so, in order that the skin and other tissues of the hand may be brought to the same temperature as the water. By so doing, conditions are established which are analagous to those which would exist if the blood vessels were alone suspended in the water bath. The opposite hand is covered with a glove, or placed in the pocket, so that it may not become cooled and so cause reflex vaso-constriction in the observed hand. While the hand is being brought to the correct temperature, 3,000 c.c. of water is removed from the large bath and placed in the calorimeter, the hand opening of which is then closed by the felt lid. The water is occasionally stirred by the feathers, the thermometer is placed in position, and the telescope adjusted so that an exact reading of the temperature may be taken.

When the ten minutes is up, the hand is withdrawn from the bath, the wrist quickly wiped dry with a towel, the hand with the fingers extended carefully passed through the hand opening into the water in the calorimeter, and the subject instructed to keep the fingers abducted and extended and not to move them. He must also be warned not to touch the thermometer. The felt collar is placed round the wrist and the height of the calorimeter is adjusted so that the subject does not require to strain his body in order to keep the hand in the correct position (or with the upper skin line at the edge of the felt collar).

The observer sits on a low stool behind the subject, and having noted the time, and stirred the water in the calorimeter, proceeds to record the temperature. The stirring is maintained throughout the observation, which lasts 10 minutes, and the thermometer is read every 2 minutes. When time is up, the felt collar is removed, the hand carefully withdrawn, and the hand opening covered by the felt lid. It is necessary to observe the temperature in the calorimeter for a further period of 10 minutes, with constant stirring, so as to determine the extent to which it is losing heat to the air (the self-cooling of the calorimeter).

The volume of the hand is determined by placing it, while still wet, in water which completely fills a lipped thick-walled beaker of

suitable size. The hand displaces an equal volume of water, which overflows into a basin in which the beaker is standing. When no more water overflows, the hand is slowly withdrawn, and water is added from a graduate to the beaker until it is again full; the amount of water required gives the volume of the hand.

The temperature of the mouth or rectum is finally taken and the temperature of the room, read from a thermometer hanging from the chair on which the subject sits, recorded.

To apply the above formula to calculate the blood flow it is necessary to introduce several corrections to allow for differences in the specific heats of the hand tissues, of the metal of the calorimeter and of water. These corrections are expressed as the water equivalents of the hand and calorimeter. The water equivalent of the calorimeter is determined experimentally for each calorimeter, and for one of the above dimensions will be about 100 c.c. The water equivalent of the hand is obtained by multiplying its volume by 0.8, this factor being the product of the specific gravity and the specific heat of the hand.

Suppose in an experiment that the temperature of the calorimeter during an observation lasting 10 minutes had risen from 31° C to 31.5° C and the self-cooling of the calorimeter during the subsequent 10 minutes was 0.1° C, the mouth temperature being 37.5° C, and the volume of the hand 450 c.c., then (applying the above equation):

$$Q = \frac{(3000 + 100 + 360)\text{ c.c.} \times (0.5 + 0.1)^{\circ}\text{C}}{37.5 - 31.25\dagger} \times \frac{10^{*}}{9} = 36.9 \text{ or in 1 minute and per 100 c.c.}$$

The measurement should now be repeated under the following conditions:

1. When the opposite hand, instead of being carefully protected from cooling, is placed in cold water. A marked curtailment of blood flow, due to reflex vaso-constriction, will be observed. This observation may be made in continuation of the previous one, i.e., the unobserved hand kept covered for 10 minutes and then placed in cold water for the next 10 minutes. It is particularly important in these observations to read the calorimeter temperature at frequent intervals, because the vaso-constriction that is immediately induced gives place later to a dilatation, even while the opposite hand is still in the cold water. The blood-flow result for each two minutes should be plotted on coordinate paper to show this phenomenon.

2. After the hand and arm have been used to perform work, such as dumb bell exercise, until almost fatigued.

*This is the reciprocal of the specific heat of blood.

†This is the mean temperature in the calorimeter during the observation of the hand $\frac{(36.9)}{(10 \times 4.5)} = 8.2$ gms.

CHAPTER XXIX.

LYMPH FORMATION.

Demonstration 5.—A large dog is given a meal containing an excess of fat (lard) several hours prior to the experiment. After anaesthetising with morphine and ether and inserting tracheal and carotid cannulae the thoracic duct is exposed as it enters the left subclavian vein at the root of the neck. This operation is rather difficult and should be performed as follows: after extending the incision through skin and subcutaneous tissue down to the sternal notch, the sterno mastoid and sterno hyoid muscles are cut as low down as possible on the left side and reflected upwards. The external jugular vein is then followed downward till it joins the subclavian, which is traced inwards to its union with the internal jugular. In the fork at the union of these two veins the thoracic duct is sought for by very careful dissection (see Fig. 65), great care being exercised so as not to wound the pleura which lies immediately beneath the vein. Just before entering the vein the thoracic duct, curving forwards and outwards, is joined by the somewhat smaller neck lymphatic. The duct is rendered visible by the white creamy fluid it contains, the neck lymphatic being similarly injected to a lesser degree. (Usually it is advisable to ligate the neck lymphatic, but when possible a cannula should be placed in it (pointing upwards) since the lymph flow from this lymphatic does not behave exactly like that from the thoracic duct). To introduce the cannula into the thoracic duct two ligatures are placed under the latter, the one next the subclavian vein being tied, and a slit is made with a fine pointed sharp scissors in the duct; the edge of the slit is then caught in a fine pair of forceps and a glass cannula (with an outside diameter of about 1/32 inch) inserted and tied in by the second ligature. If the creamy lymph escapes so freely from the slit in the duct that it fills the wound its flow should be controlled by pulling gently on the free ligature. Usually a certain amount of lymph flow is desirable since it facili-

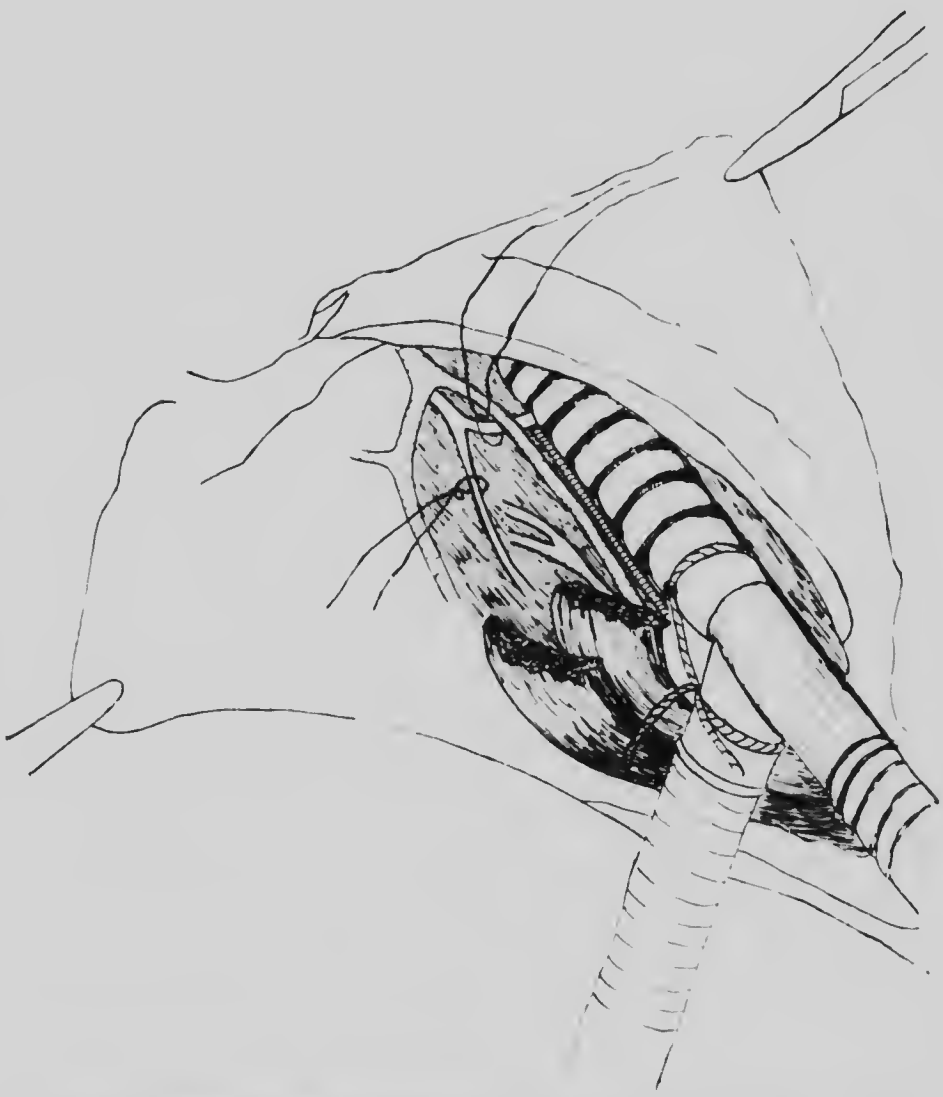


FIG. 65. Dissection necessary for exposure of thoracic duct and neck lymphatic. Ligatures are placed around these structures. (After Jackson).

tates insertion of the cannula. It should be remembered that the lymph clots readily, and if this occurs in the neck of the cannula, the clot will require to be broken up by means of a fine hair. The cannula is connected by a short piece of flexible rubber tubing with a bent glass tube, from the end of which the lymph is allowed to drop.

A cannula is also inserted in the femoral vein and connected with a burette.

There are two types of experiment which may be demonstrated on lymph flow. One of these concerns the stimulating effect of certain chemical substances when injected into the general circulation—the lymphagogues—and the other, the effect of alterations of the circulatory conditions in the intestines and liver.

The **lymphagogues** are of two classes, saline and colloid. To illustrate the former a strong solution of glucose (20 per cent.) is injected intravenously a few c.c. at a time until an increase in the number of drops of lymph is produced. The amount of injection is then increased. If the neck lymphatic flow is also being observed, particular regard should be given as to whether it behaves similarly to that of the thoracic duct. Repeat the experiment, using a 10 % solution of sodium chloride. Explain the results.

In the foregoing experiment it will be observed that the arterial blood pressure either remains unaltered or rises slightly. To illustrate the colloid lymphagogues, small quantities of a solution of commercial peptone are injected. Note that besides affecting the lymph flow there is a decided drop in arterial blood pressure. (Care must be taken not to allow this to reach the danger limit). Explain the result.

Finally it may be shown that certain drugs and hormones also act as lymphagogues. To illustrate this, pituitrin may be used (1 c.c. for a dog of average size, 8 kgm.).

The influence of **circulatory changes** in the splanchnic region may be illustrated as follows: after opening the abdomen in the linea alba a loose ligature is placed around the portal vein near the hilus of the liver. When the normal rate of lymph flow (from both ducts) has been ascertained the vein is closed by pulling on the ligature; a decided increase in flow is observed.

If the ligature be tightened for long the lymph will become turgid with blood. How do you explain the results?

Finally the lymphatics which accompany the portal vein are ligated. This is readily done by separating the vein by blunt dissection from the accompanying structures, which are then mass ligated. A loose ligature is next placed around the inferior vena cava just above the diaphragm, by making an opening at the posterior end of the 6th or 7th intercostal space on the right side, prying the ribs apart with a strong pair of retractors (meanwhile maintaining artificial respiration) and threading the ligature around the vein by a long aneurysm needle. After applying the ligature, the opening in the thorax should be closed by artery forceps, when it will often be found that artificial respiration can be discontinued. Having observed the rate of lymph flow the ligature is tightened for a few moments. Is the effect similar to that of the previous experiment? How is it explained?

CHAPTER XXX.

EXPERIMENTS TO DEMONSTRATE THE PUMPING ACTION OF THE HEART AND THE ACTION OF THE VALVES.

Demonstration 6. A wide glass cannula is tied into the superior vena cava of the excised heart of a dog or sheep and the cannula connected by rubber tubing with a funnel. A glass tube is also tied into the pulmonary artery, the upper end of the tube, at a distance of about 50 cm. from the heart end, being bent double and arranged so that the opening lies over the funnel which is connected with the vena cava. The inferior vena cava is tied and the preparation and tube are held by suitable clamps in a vertical position; water is poured into the funnel so that it distends the right auricle and ventricle. Some of the water escapes through cut vessels (left azygos vein) which are now tied. When the ventricle is rhythmically compressed by the hand the water, which has meanwhile risen in the pulmonary tube to the level of the water in the funnel, rises higher and higher with each compression, and remains up between them, until it reaches the bend and flows back into the funnel. This illustrates the circulation of the blood through the heart.

It is interesting to study the effect produced by DAMAGING THE SEMILUNAR VALVE. The fluid still rises in the tube with each compression, but leaks back into the ventricle between the "beats". To raise the fluid in the tube high enough so that it overflows into the funnel it is now necessary to compress the ventricle much more rapidly. This illustrates in a rough way how the heart may compensate for a valvular inefficiency by more energetic action.

The OPERATION OF THE TRICUSPID VALVES is also readily shown by removing the tube and cutting away most of the right auricle. When the ventricle is filled the water flaps are floated up into position, a narrow chink, however, remaining in the centre. This can be temporarily closed by allowing the water to drop in the

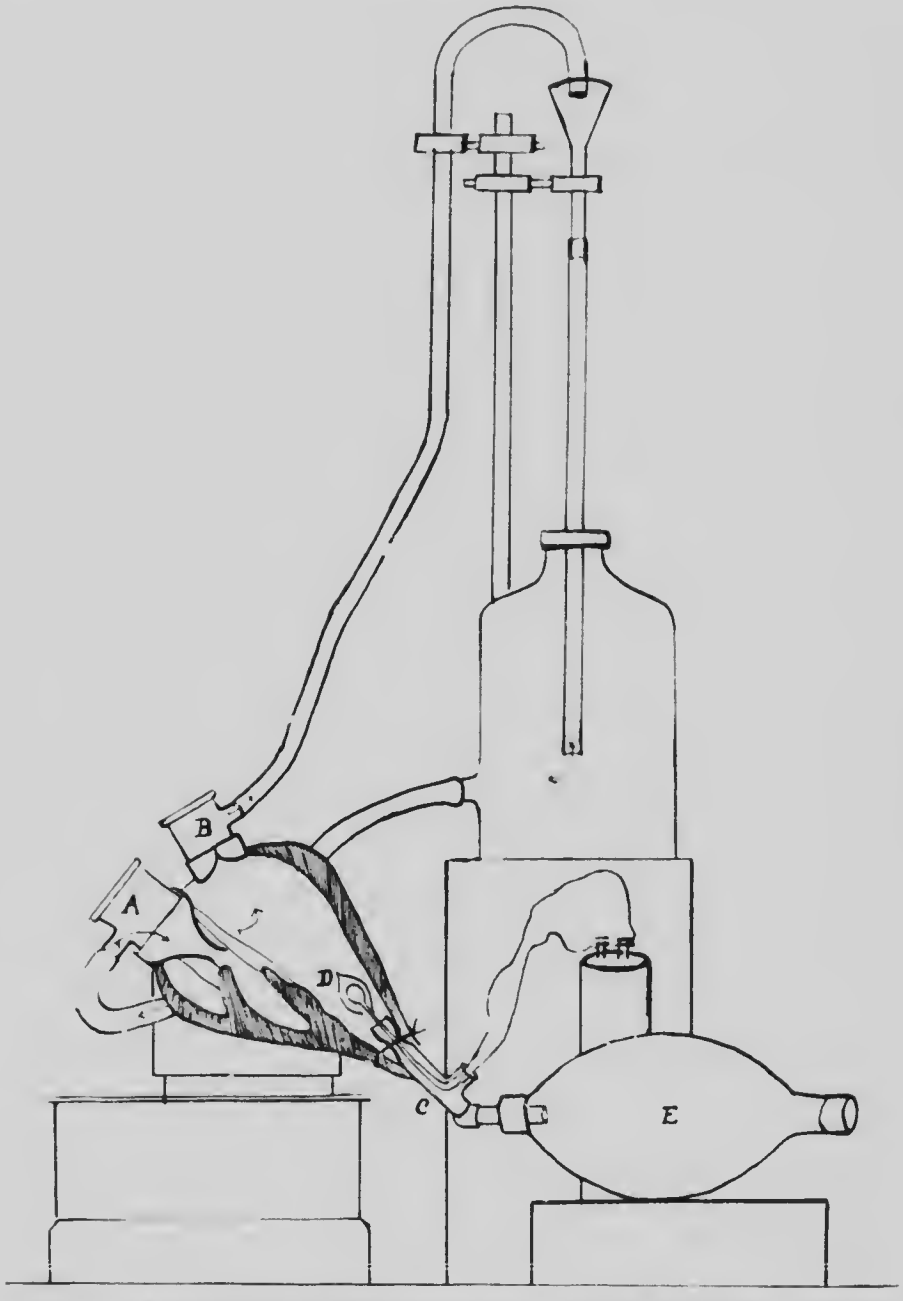


FIG. 60.—Gad's arrangement for observing the action of the cardiac valves. (After Figerstedt.)

centre of the chink; following each drop the flaps come accurately together because the waves of pressure produced by each drop are reflected onto the under surface of the flaps from the walls of the ventricle. What significance may this observation have in connection with the mechanism of the closure of the valves in the normal heart?

To illustrate the efficiency of the tricuspid valves, ligate the pulmonary artery, fill the ventricle with water, and hold the ventricle upside down; the water stays in.

The operation of the valves can be very clearly shown by observing them through windows inserted in the right auricle and pulmonary artery. This is known as **GAD'S HEART PREPARATION.**

Demonstration 7.—The general arrangement, using an ox heart, is shown in Fig. 66. A brass tube A (40 cm. diam.) closed by a glass window at one end and with a narrow tube soldered into its side is tied into the right auricle, and another similar, but narrower tube B (25 cm. diam.) into the pulmonary artery. The side tube of A is connected by rubber tubing with the outflow of an irrigation bottle, to the mouth of which leads a tube from B. Through a narrow cut in the apex of the ventricle a brass tube C connected at its free end with a rubber bulb and having a side tube closed by a small rubber stopper, which is pierced by waterproofed insulated wires, is inserted and tied in. The wires are connected with a small electric lamp D which projects into the ventricle. By rhythmically compressing the bulb E, to simulate the ventricular contractions, water is transferred from the auricle to the pulmonary artery, and with each pulsation the tricuspid and semilunar valves can be very distinctly seen to open and close in obeyance to the pressure changes.

SECTION VII.

THE DIGESTIVE SYSTEM.

CHAPTER XXXI.

THE INNERVATION OF THE SALIVARY GLANDS.

The general nature of THE NERVE CONTROL OF SECRETORY GLANDS is most readily studied on the submaxillary gland of the dog or cat. The responses are not exactly alike in the two animals, but the results typify glandular innervation in general.

Demonstration 8. A dog is anaesthetized preferably by urethane (p. 72) and tracheal and carotid cannulae are inserted. An incision is made along the inner border of the ramus of the lower jaw extending from the mouth to the angle of the jaw. The digastric muscle is exposed and is strongly retracted outwards so as to expose the mylohyoid muscle, the fibres of which run transversely to the wound. The hypoglossal nerve is seen extending backwards behind the posterior edge of the mylohyoid. A probe or blunt dissector is pushed under the latter muscle and its fibres cut in the same line as the main wound. This exposes the ducts of the submaxillary (and sublingual) glands with the lingual nerve crossing them. A ligature is tied on this nerve below the ducts and the nerve cut peripheral to the ligature, after which it is traced up under the ramus of the jaw, the small branch which arises from it and proceeds forwards being cut. Just above where this branch comes off another branch passes backwards and downwards to join the ducts a short distance from the place of crossing of the lingual. This is the chorda tympani nerve. It can be prepared for artificial stimulation by tying a second ligature round the lingual nerve above where the chorda arises and cutting above the ligature. By holding up the piece of lingual by the two ligatures the electrodes can be readily applied to the chorda.

Before attempting to insert a cannula in Wharton's duct the chorda should be stimulated with a feeble tetanizing current after

placing bull dog forceps on the two ducts well forward of the point where the lingual crosses them. This causes the ducts to distend and into the one which does so most markedly a small cannula is inserted by the same manipulation as for a vein (p. 77).

The skin wound should now be carried back and the submaxillary gland carefully freed by blunt dissection from the neighbouring tissues, care being taken not to injure the veins. The exposed gland should be kept moist with warm Locke's solution.

The cannula is connected by suitable rubber tubing with a bent glass tube so arranged that the drops of secretion may fall from its free end. Having ascertained the rate of secretion (by counting the drops) for a normal period of one minute the chorda is stimulated for a few moments with a feeble interrupted current. When the secretion has returned to normal, stimulation is repeated with a stronger current.

The relationship between the response and the strength of the stimuli should be carefully noted. Those near the preparation can usually see that the gland becomes flushed and apparently swollen by the stimulation, this effect being usually most pronounced during the first-applied stimuli. What conclusions are warranted from the results? The observations are repeated during stimulation of the central end of the vago-sympathetic in the neck.

The secretion pressure is now measured by connecting the cannula, by moderately thick-walled, but yet flexible tubing, with a mercury manometer, the tubing being filled with physiological saline. The carotid blood pressure is also observed, and it is advantageous to arrange the writing styles of the two manometers so that they write in the same perpendicular on a drum. When the chorda is stimulated with a current which gives a maximal secretion, the pressure in the duct manometer steadily rises until it overtops that in the artery. Explain the significance of this result.

The manometer is now removed, and, through a cannula previously inserted in the femoral vein, 10-15 mgm. of atropine sulphate dissolved in physiological saline is injected. When the drug has developed its full action on the heart (how is this tested?) the chorda is again stimulated with a maximal current, and the effect on the secretion and the vascularity of the gland observed. What has been the action of the atropine? Of what significance is the experiment?

CHAPTER XXXII.

THE CONTROL OF THE PANCREATIC SECRETION. THE SECRETION OF BILE.

As an example of the control of glandular function through hormones it is most convenient to take the pancreas. The hormone for this gland is derived from the duodenal mucosa where it is produced by the action of the acids present in the chyme on a constituent of the epithelial cells, and it is then carried to the pancreas by the blood.

Demonstration 9. An anaesthetized dog that has been starved for 24 hours is prepared for registration of the arterial blood pressure, and a cannula is inserted in the femoral vein. The abdomen is opened in the linea alba and the duodenum along with the adherent pancreas pulled out of the wound. The lower (and larger) duct of the pancreas is then exposed, and a ligature placed under it. The position of this duct is indicated approximately by a small lobe of pancreas, accompanied by blood vessels, which extends to the duodenum at a distance of about 20-25 mm. above the point where the head of the pancreas leaves the duodenum. It must be remembered that the duct begins branching very close to its insertion into the duodenum so that only a small piece of it can be dissected free. To insert a cannula into the duct the duodenum is opened by a longitudinal incision along its free border opposite the duct, the opening of which is then visible in the centre of a small papilla of somewhat paler tissue than the remainder of the mucosa. A blunt probe should be inserted into the duct and gently guided along it so as to ascertain the exact direction of the duct and the position of the first branch. As the probe is withdrawn a suitable cannula with a well-marked neck is inserted and tied in position by the ligature previously applied outside the duct. A cannula is now placed in the common bile duct, which is readily found accompanying the portal vein.

The following observations on the secretions from the two ducts are now made.

1. Having observed the normal rate of secretion, a small piece of cotton soaked in a weak solution of hydrochloric acid (less than 1%) is placed in the duodenum. Whenever the secretion from the pancreatic duct becomes decidedly increased (not an invariable result) the cotton is removed, and the duodenum washed with physiological saline rendered faintly alkaline by sodium carbonate. Sometimes an increased secretion of bile occurs. From what sources may this bile be derived? What experimental steps would you suggest in order to ascertain this?

2. The exciting influence of the acid might of course be due to its absorption into the blood. To test this possibility inject some of the acid into the femoral vein. A negative result is obtained even when massive doses are injected.

3. Pieces (about 2 feet long) of the upper end of the jejunum and of the lower end of the ileum are then removed, by cutting between previously applied ligatures, and the contents washed out by tap water. Each piece is then slit open and the mucosa scraped off by the blunt edge of a scalpel and collected in separate watch glasses. One half of each scraping is thoroughly ground in a small mortar with fine quartz sand and about 30 c.c. of 0.6 per cent. hydrochloric acid (2 c.c. HCl (Con) in 100 c.c. water.) The extracts are filtered through fine muslin and nearly neutralised (but left distinctly acid towards litmus). About 5 c.c. of the extract of jejunum is then injected into the femoral vein and observations made on the arterial blood pressure, the secretion of pancreatic juice and the secretion of bile. (Prior to this, however, the cystic duct should have been tied off. Why?) The blood pressure usually falls considerably and care must be taken so to regulate the rate (and amount) of injection that the fall is not allowed to go too far. What conclusions regarding the mechanism of the increased secretion can be drawn from the results? To obtain satisfactory results it may be necessary to repeat the injection using double the amount and injecting more quickly.

The observation should then be repeated using a similarly prepared extract of ileum. A much feebler response, if any response at all, is observed, but the fall in blood pressure is as pronounced as before. What conclusions do you draw from the result?

4. Having demonstrated the secretagoguary action of the duodenal extract, the question arises as to the general nature of the exciting substance. The remaining portion of the acid extract of jejunum is therefore boiled (in faintly acid reaction) and filtered through the filter paper. The filtrate is found to be still active when injected. What conclusions do you draw? Has any change occurred in the vaso-depressor effect? The secretion of bile is often observed to respond to the injections in the same manner as the pancreatic juice, and its behaviour therefore should be carefully watched.

5. Having established, by these experiments, that acid extracts a pancreatic hormone from the mucosa (secretin) the question arises as to whether the hormone is present therein as such or as a precursor which the acid activates. To throw light on this question the unextracted half of the mucosal scrapings is ground in a mortar with quartz sand, and 0·9 per cent. sodium chloride solution, and after filtering the extract through muslin, about half of it is injected intravenously. The result is negative. The remaining portion of the extract is then rendered faintly acid with HCl, boiled and filtered. On injection a slight, but yet definite increase usually occurs in pancreatic secretion. What conclusions are permissible from these results?

6. Finally the influence of the intravenous injection of a solution of bile salts on the outflow from the bile duct should be observed.

CHAPTER XXXIII.

EXPERIMENT ON THE NORMAL SECRETION OF SALIVA AND GASTRIC JUICE.

In order to study the secretion of the digestive glands in an unaesthetized normal animal it is necessary by surgical methods to establish permanent openings or fistulæ on the surface of the body, through which the secretions may escape. This method has been employed with great profit in the case of several of the glands, the general nature of the experiments being adequately illustrated by observation on the parotid gland (representing a gland with a definite duct) and the stomach (representing a gland which secretes directly on to a mucous surface). The operations necessary to establish the fistula are performed by some competent assistant, and the operated animals are carefully tended so that the wounds heal without suppuration. For successful observations it is furthermore of importance that the animal should become used to the person who is to demonstrate the experiments.

The Normal Secretion of Saliva.

Method for Making Fistula of the Parotid Gland. A dog is anæsthetised with morphine (morphine hydrochloride 0.01 gm. per kgm. body weight) and finally with chloroform. The mouth is held open by means of a suitable gag and the ductus Stenonianus located (on the mucosa of the cheek opposite the second molar tooth). A small blunt probe is pushed into the duct and the mucous membrane around it sponged with sterile surgical gauze. A circular incision is then made through the mucosa around the duct, the area of the circle being a little less than one sq. cm. It will probably be necessary at this stage to suspend further operating for a minute or so in order to administer some more chloroform, and throughout the remainder of the operation similar pauses will occasionally be necessary. The circle of mucosa is then quickly dissected from the underlying tissue up to the duct, after which a stab is made by a fine scalpel through the skin of the cheek opposite the opening of the duct, the edge of the skin incision being trimmed by a scissors so as to make the wound elliptical in shape. By means of a surgical needle a fine silk ligature is passed through the anterior edge of the circle of mucosa and its free ends pulled out through the skin wound. By gentle

traction on the ligature and by bending the free end of the probe out through the wound the duct and encircling mucosa is brought out to the skin, care being taken not to stretch or twist the duct. The edges of the mucous circle are now stitched by a fine silk or chromacised catgut ligature to the edges of the skin wound. The wound in the mouth is similarly stitched and without the application of any dressing the animal is allowed to come out of the anæsthetic. The wound should be bathed daily with physiological saline and a free secretion of saliva occasionally stimulated by giving the animal some dry food (bread crumbs).

When the wound has healed, it will be necessary to accustom the dog to being strapped into a suitable holder and it is most important for the success of the

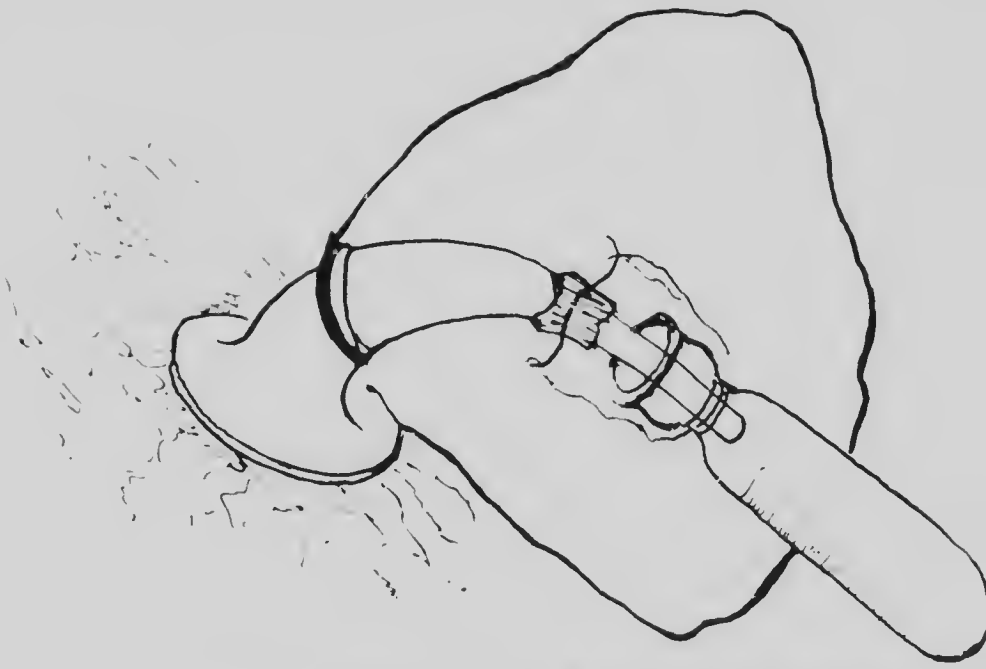


FIG. 67.—Funnel applied to skin of animal for collection of [illegible] (Pavlov).

observations that the animal should be trained to submit to the harnessing without fear or excitement. The knowledge that feeding is to follow soon makes the animals eager to participate in the proceedings. To collect the secretion Pavlov uses a funnel, made out of wax and resin* with its edges flared out so as to form a flat surface to apply to the skin (Fig. 67). To apply the funnel the hair around the duct is clipped close by a scissors and thoroughly dried. The funnel is then moderately warmed and the flared edge firmly pressed on the cheek to which it adheres, after which the narrow end is attached to a light graduated test-tube. In removing the funnel care must be taken not to abrade the skin. Holding a warmed metal rod near the edges helps to loosen the attachment to the skin

*50 parts, rosin; 40 parts, ferric oxide (Fe_2O_3) and 25 parts yellow wax.

Demonstration 10. The secretion should be observed during the following conditions:

1. When various foods are fed to the animal.

2. When acid solution or dry bread crumbs are thrown into the mouth.

3. When the animal is teased with a bone (which should afterwards be given to him).

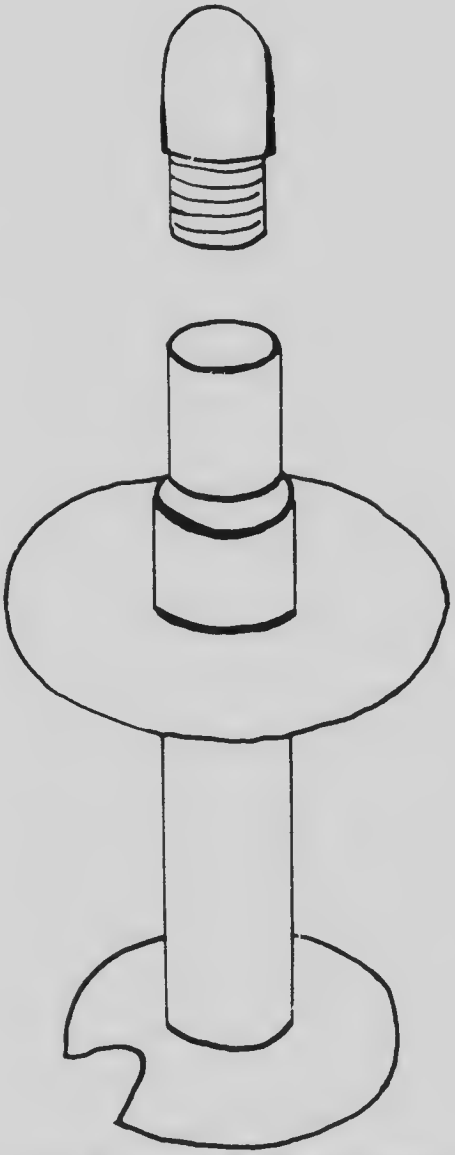


FIG. 68.—Stomach cat fistula. See text for description.

It is also profitable to train the animal so that he learns to associate a certain sound or visual impression with the subsequent stimulation of salivary secretion, as by giving him bread crumbs. The experiments on conditioned reflexes are however more striking when the fistula is one involving the submaxillary or sublingual ducts. The exact nature of the demonstrations and experiments on material of this type must naturally vary with circumstances. Advanced students may profitably devote considerable time to this work.

The Normal Secretion of Gastric Juice.—Satisfactory demonstration of the secretory activities of the gastric glands requires the establishment of both gastric and œsophageal fistulæ on the same animal. This can be undertaken only when there is someone who can devote considerable

time every day to the proper care and attention of the animal. A few observations may, however, be made on an animal with a gastric fistula alone.

Method for Making the Gastric Fistula.

Through an incision 6-7 c.m. long in the linea alba just below the sternum the stomach wall is caught by a forceps and after pulling it somewhat over to the

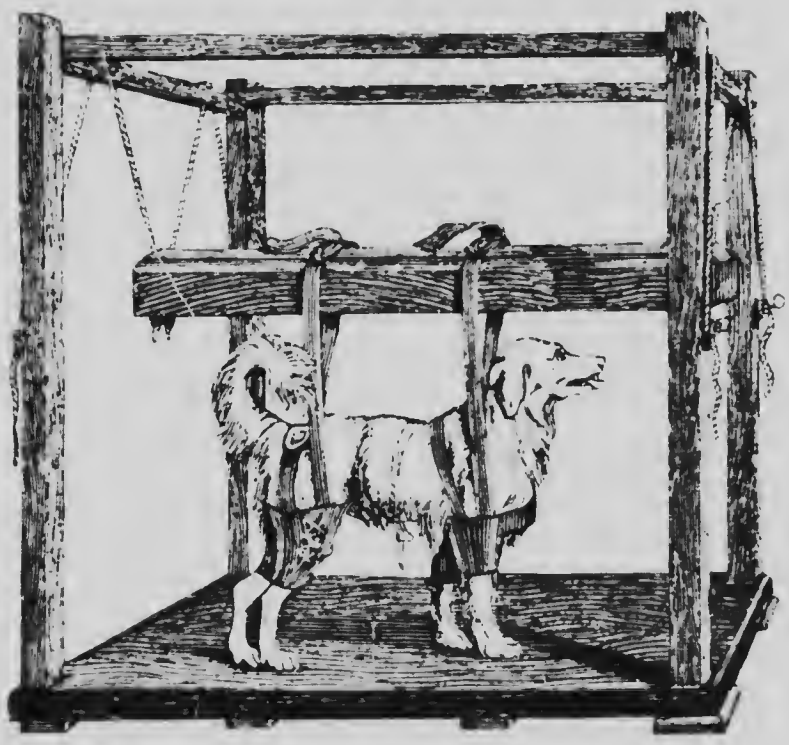


FIG. 69. Stand for holding animal on which fistula operation has been performed. (From Tigerstedt's "Practical Physiology".)

right, the serous coat is attached to the edge of the skin wound by a few discontinuous sutures. An incision is then made through all the coats of the stomach wall, bleeding being carefully controlled by ligatures. The incision is just large enough to admit the notched flange of the gastric cannula (Fig. 68). A cromicised catgut ligature is now stitched like a purse string a short distance from the edges of the incision and the cannula inserted and tied (not too tightly) in place by the ligature. It is well to apply a second purse string ligature. The operation is completed by closing the skin wound up to the cannula with discontinuous sutures.

the wound is then dressed with cotton and collodion and the outer shield of the annula screwed on, but not so far as to press on the wound. The tube of the annula is closed by the screw-in stopper.

Demonstration 11.—In about a week, when the wound will usually have healed, the dog is placed on the observation holder (Fig. 69) and the following observations are made. A pledget of absorbent cotton attached to a hæmostat is passed into the stomach and gently moved over the mucosa; the nature and reaction of the secretion which adheres to it after removal is observed. A thin-walled rubber bag attached to a glass tube is carefully passed into the stomach through the fistula and filled with warm water. The tube, leading out of the fistula, is connected with a water manometer, the free limb of which is attached by tubing to a sensitive tambour arranged to inscribe its movements on a drum. Observations are now made on the hunger contractions. (It may be necessary to wait some time before these appear). When satisfactory records have been secured the animal is teased by tempting it with appetising food and the effect on the contractions noted.

The rubber bag is now removed and the reaction of the secretion adhering to it tested by means of litmus paper.

These observations on hunger contractions in a dog should be supplemented by similar ones on man. For this purpose a thin rubber bag is firmly tied on a narrow stomach tube and passed down the œsophagus until the bag lies in the stomach. The bag is then distended by air (cf. Carlson) and the outer end connected with a water manometer and tambour. The observation should be made on a person who has not taken food for some hours previously.

CHAPTER XXXIV.

THE MOVEMENTS OF THE ŒSOPHAGUS* AND INTESTINE.

Demonstration 12. A rabbit is narcotised by means of urethane (p. 72 †) and the œsophagus is exposed. This is accomplished partly by drawing the trachea to the right by a stout silk ligature passed around it and partly by pulling the œsophagus to the left. The vagus nerve is then followed up to where the superior laryngeal leaves it, and this branch is cut after tying a thread round it as near to the vagus as possible. Stimulation of the laryngeal nerve by a Faradic current excites the act of swallowing and it can be seen that this consists, in order, of a movement of the floor of the mouth, of elevation of the larynx and of the passage of a peristaltic wave along the œsophagus. A record of the peristaltic wave can be obtained by inserting into the lumen of the œsophagus, through a small incision, a thin-walled rubber bag (finger cot), which is connected by tubing with a water manometer and tambour, after distending it with warm water.‡

It is now important to ascertain whether the peristaltic wave can be more readily set up by mechanical irritation of the œsophagus or of the pharynx. The stimulation can be produced by stroking with a feather. The œsophagus is finally cut across and a bent pin connected by a thread with a muscle lever attached to the peripheral end of the cut tube. The superior laryngeal nerve is again stimulated. From the results of this observation what conclusions can be drawn concerning the manner of transmission of the œsophageal peristaltic wave?

---

* The action of the base of the tongue, etc., in swallowing may be readily studied in the decerebrate cat, as described on p. 227.

† For the success of the observation the anæsthesia must not be too deep.

‡ It is best to tie the finger cot to a rubber catheter so that the end of the catheter reaches to the end of the cot. This facilitates insertion of the cot into the œsophagus. It is important to see that the cot becomes uniformly distended with water before connecting with the water manometer. The edges of the incision in the œsophagus should be stitched together so as to hold the instrument in place.

THE MOVEMENTS OF THE INTESTINE.

Demonstration 13. The same animal may now be used for observations on the intestinal movements. For this purpose a tracheal cannula is inserted and artificial respiration established.* The thorax is then opened and the vagus and splanchnic nerves quickly cut, this procedure being necessary in order to secure pro-

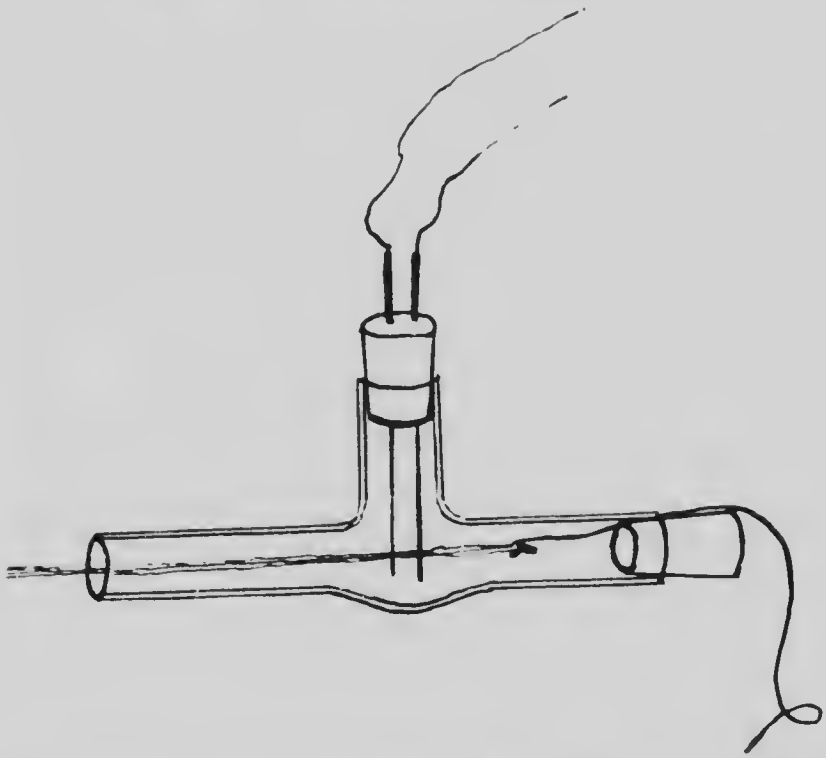


FIG. 70. Sherrington Electrodes. The nerve is pulled through the tube by means of a thread attached to it, of it is arranged so that it lies between the platinum electrodes inserted through the side tube. The nerve must on no account be stretched when being pulled into the electrode.

nounced intestinal movements. One of the splanchnic nerves is connected with Sherrington's electrodes (Fig. 70) the wires of which emerge from the wound. The thoracic wound is closed by sutures, and the abdomen opened along the linea alba. The animal is then

* It is advisable also to place a cannula filled with saline in the central end of the jugular vein. Through this cannula drugs can subsequently be injected

placed in a bath of physiological saline at about 33-35° C. contained in a tank of suitable size and the intestines allowed to float out in the saline. The pendular movements are very conspicuous and the loops should be closely examined with the object of ascertaining which of the muscular coats are contracting. If no peristaltic waves are observed, they may be set up by pinching the intestine. Observe closely the characteristics of these waves.

The effect produced on the movements by stimulation of the peripheral end of the splanchnic nerve is now studied.

Records of the movements may be secured by the balloon method already described for the œsophagus.

Finally, if the animal is still in suitable condition, the effect produced on the intestinal movements by injections into the jugular vein of epinephrin (1-10,000 adrenalin chloride) and of atropine are observed.

SECTION VIII.

THE CENTRAL NERVOUS SYSTEM.

CHAPTER XXXV.

REFLEX ACTION IN THE MAMMALIA.

Although certain basic facts concerning the physiology of reflex action can be learned by observations on the spinal frog (p. 90) the variety and complexity of the reflex movements exhibited by the preparation are too limited to enable us to understand much about reflex action in the higher animals. Practically the only reflex movement elicitable in the spinal frog is the flexion reflex, which is of a type quite different from that of the reflexes that are concerned in maintaining such animals as the dog or man in the erect posture (postural reflexes), or in enabling movement to occur from place to place. The functional unit of the nervous system is the reflex arc and the ability to perform complex co-ordinated movements depends on the integration of the various reflex arcs among one another — the integrative action of the nervous system (Sherrington). In order that we may study the principles that govern this integration it is necessary to simplify the conditions from those obtaining in the intact animal, which is done by breaking the connection between the higher brain centres and those of the lower portion of the spinal cord (the spinal animal).

Demonstration 14.—The most satisfactory preparation to use for a study of the spinal reflexes is one in which the spinal cord has been cut some weeks previous to the observation. Spinal shock will have been recovered from and the following reflex movements can readily be demonstrated:*

*These can readily be demonstrated with the animal lying on his side, but if graphic records are desired, as for measuring latent periods, etc., it is best to suspend the animal in a suitable stand with the posterior extremities hanging free. Threads attached near the paws are carried over directing pulleys (or glass rods)

1. THE FLEXION REFLEX by pricking the skin of the paw with a pin or applying a moderately strong electric shock. The flexion at knee and hip is accompanied by extension of the leg of the opposite side, THE CROSSED EXTENSION REFLEX. By taking simultaneous tracings of the two legs after attaching the feet to suitable levers by threads, the correspondence in time between the two reflexes is demonstrated.

2. THE KNEE JERK by giving a sharp blow with a ruler or the handle of a scalpel to the ligamentum patellæ. Note that the leg swings limply back to its flexed position after the tap: compare with the behaviour of the jerk in a decerebrate animal (p. 91).

3. THE SCRATCH REFLEX by moving the finger or a pencil backwards and forwards on the skin of the body. The skin area from which the reflex can be elicited is very extensive and the paw usually is directed approximately to the place of stimulation ('local sign'). Sometimes this property of 'local sign', however, is very imperfect. An electrical stimulus through the stigmatic electrode will also elicit the reflex, but much less satisfactorily than the mechanical one.

4. THE EXTENSOR THRUST by pushing the blunt end of a pencil between the pads of the paws. The corresponding leg makes a sudden extension movement.

The following **properties of reflex action** may now be studied using one or other of the foregoing reflexes.

1. THE LATENT PERIOD OR UNCORRECTED REFLEX TIME. To determine this it is necessary to employ electrical stimulation and to insert a signal magnet in the primary circuit. A fast rate of drum

to be attached to the long arm of a right angled lever, from the short arm of which a second thread runs to a straight muscle lever. The threads are adjusted so as suitably to diminish the amplitude of movement at the writing points.

A large electrode made out of a strip of copper covered with a pad of surgical gauze, which is moistened with salt solution, is tied on to the middle of the back (indifferent electrode) and a much smaller electrode of the same type is tied to the foot of one side (stimulating electrode). A third (stigmatic) electrode composed of a stout copper wire covered at its tip by a piece of surgical gauze is also required. The wires leading from the electrodes are connected with the secondary coil of an inductorium, the indifferent electrode with one pole and the stimulating and stigmatic electrodes with the other. A well insulated simple key should be inserted in the wire leading from the attached foot electrode.

must be used, the exact relationship of the signal pointer to the recording lever being indicated by alignment marks (see p. 85). A time tracing, preferably of 1/100 sec., must also be taken. Several observations on the flexion reflex with stimuli of varying strength will show the latent period to vary from about 0.03 to 0.12 sec. The latent time of the jerk is much shorter. The signal for the application of the stimulus is afforded in this case by the slight movement of the lever produced by the blow to the tendon, the attachment of the threads to the levers being adjusted so as to make them sensitive enough to record this.

The latent period of the scratch reflex using the stigmatic electrode is relatively very long and it varies with the strength of the stimulus. Taking these results with those already attained on the palpebral reflex (p. 95) into consideration, what conclusions do you draw concerning the latency of reflex action in general? What corrections must be made to determine the true or reduced reflex time?

2. GRADING OF INTENSITY. This is investigated for the flexion and scratch reflexes by varying the strength of the electrical stimulus and for the extensor thrust by applying varying degrees of pressure to the paws. How do the results obtained from these two types of reflex compare with those already obtained for the palpebral reflex?

3. SUMMATION. Using the flexion reflex, a strength of current is found which is just ineffectual when single shocks are applied, a simple tapping key being inserted in the primary circuit of the inductorium in place of the vibrating hammer. The shocks are then applied frequently. Is the summation more pronounced than you found it to be for isolated nerve?

4. AFTER EFFECT.—By recording the movement of the flexion reflex on a fairly rapid drum, the degree of after effect in relationship to the strength of stimulus is observed. Both single shocks and tetanizing shocks are employed. In what respects do the results differ from those obtained on a nerve?

5. REFLEX FATIGUE.—The effect of maintained stimulation is studied in the flexion and scratch reflexes, using a slow drum. How does reflex fatigue differ from fatigue in an isolated nerve-muscle preparation? After the leg has been thoroughly fatigued for the

flexion reflex, the scratch reflex is elicitated by mechanical and electrical stimuli. What do the results indicate as to the locus of fatigue in the reflex arc?

6. IMMEDIATE INDUCTION.— The end of the handle of a scalpel or of a small ruler is pressed against the skin of a scratch area, but there is no response. If the same object be moved on the skin, however, the scratch movement is likely to be set up. What is the explanation of the result? What is the analagous experiment on vision?

7. SUCCESSIVE INDUCTION.— Using a slow drum, record a considerable number of knee jerks produced by regularly applied taps of as nearly as possible equal intensity to the patellar tendon, and when there is approximate equality in the heights of the contractions throw the corresponding leg for a few seconds into the flexion reflex, meanwhile continuing the patellar taps. When the flexion reflex subsides the tendon jerks are greatly exaggerated. Explain why this shows successive induction. Demonstrate the same phenomenon for the flexion reflex and extensor thrust, and for the crossed extension reflex and flexion reflex. What is the analagous experiment on vision?

CHAPTER XXXVI.

CEREBRAL LOCALIZATION, DECEREBRATE RIGIDITY, RECIPROCAL INNERVATION, FUNCTIONS OF SPINAL ROOTS IN THE MAMMAL DOG.

Demonstration 15.

A tracheal cannula is inserted in an etherized dog, and ligatures placed loosely around the carotid arteries on both sides. With the animal in the prone position the head is raised by placing a pad under it, being careful to see that there is no kinking of the tube leading from the tracheal cannula to the ether bottle. To serve as a landmark for the crucial sulcus of the cerebrum (which corresponds to the Rolandic fissure on the human brain) two threads are stretched across the head, one of them joining the outer canthi of the eyes and the other, the condyles of the lower jaw. The crucial sulcus lies a little behind the mid point between the two threads. An incision is made along the mid-line of the scalp and by blunt dissection the temporal muscle is separated from the bone far enough to make room for two trephine holes one in front of the other and with their inner margins at least 3 mm. from the mid line so as to avoid wounding the superior longitudinal sinus. To manipulate the trephine, the steel point is first of all adjusted so that it projects beyond the sawing edge, and this point is bored into the skull in order to afford good fixation. When the sawing edge reaches the bone, care is taken it cuts uniformly, and when a good start has been made the central point is pulled up so that this may not puncture the brain. The trephining is continued until the inner table of the skull is cut through around most of the circle, the trephine is then removed, and the disc of bone pried up by means of a pericostal elevator or a stout pair of forceps. There is apt to be considerable hæmorrhage at this stage, but it can usually be controlled by applying for a few minutes a pad of gauze thoroughly wrung out with hot isotonic saline. When the two trephine holes have been made they are connected together by bone forceps, taking care not to wound the brain and controlling bleeding with hot gauze. A curved surgical needle is passed through the dura and by means of it the latter is raised sufficiently so as to be able to cut it with a sharp pointed scissors. The exposed brain should be covered with gauze soaked in isotonic saline.

In order to stimulate the cortex a large plate (indifferent) electrode is placed on the moistened skin of the lower dorsal region and connected with one terminal of the secondary coil of an inductorium with the other terminal of which a blunt-pointed (stigmatic or diagnostic) electrode is connected.

Having made a rough sketch indicating the position of sulci and convolutions of the exposed portion of the cerebrum, the front and hind legs on the side opposite to the opening in the skull are loosened and the tetanizing current applied for short periods and at varying strengths until definite movements are observed to occur. For successful results it is necessary to have the animal as lightly anæsthetized as is consistent with the entire absence of pain

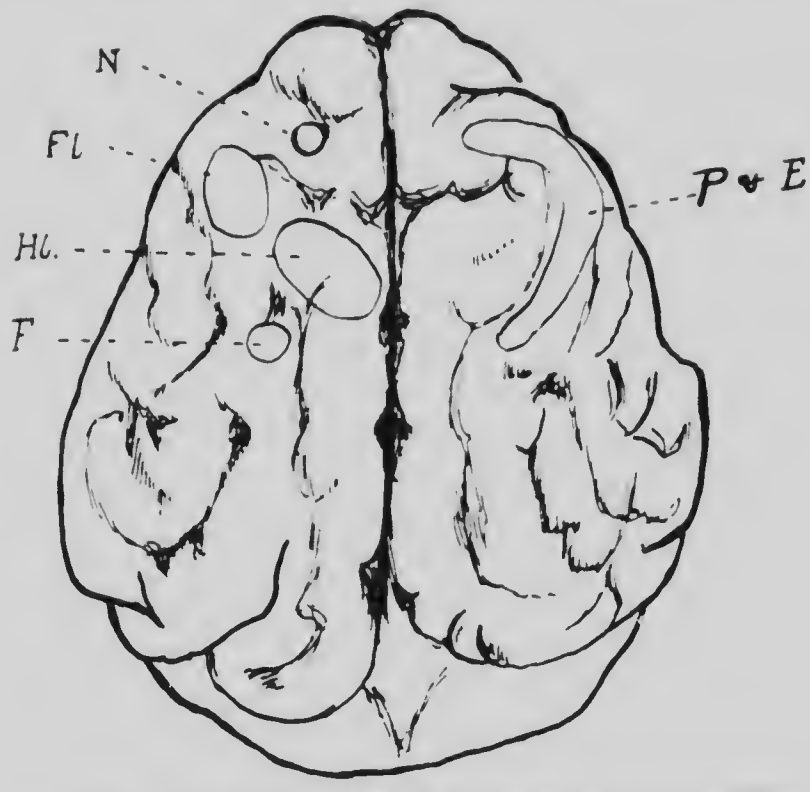


FIG. 71.—Surface of cerebrum of dog, showing on the left side approximate positions of the various centres. n, neck, fl., forelimb, hl. hindlimb, f. face. Movements of the eyes, E, as compared by dilatation of the pupil, P, are also obtained from the portions of the cortex indicated on the right side. (After Stewart).

When a suitable strength of stimulation has been found, the exposed area of cerebrum is systematically explored (Fig. 71), and the observed responses noted on the sketch. The character of the movements must also be carefully noted (i.e., whether there is evidence of reciprocal innervation, etc.). The pupils and eye movements on both sides, movements of the head and ears, changes in the respirations and movements of the tail must also be looked for. The

general results are indicated in the accompanying chart. Finally the effect of the application for some time of a very strong stimulus is studied.

The animal is finally **decerebrated**. For this purpose a large aneurysm needle is passed through the posterior or one of the trephine hole backwards until the tentorium cerebelli is felt. It is then directed downwards and inwards so as to break across the base of the brain stem just in front of the tentorium. Immediately this is done the respirations are likely to cease or to become very irregular so that artificial respiration must be started. The ether can now be discontinued since the animal is incapable of feeling any pain. The muscles of the extremities are carefully observed from time to time for the onset of decerebrate rigidity and for the appearance of reflex movements, such as the knee jerk and the flexion reflex.

For the purpose of studying **reciprocal innervation*** the tendons of the rectus femoris and the semitendinosus are exposed and isolated from adherent aponeurosis on both sides and the tendons are cut after threads have been tied to them. The peroneal nerves are also exposed as they lie under the skin on the inner side of the leg or the dorsum of the foot. After attaching threads and cutting, each nerve is placed in a pair of Sherrington's electrodes attached to an inductorium. The threads on the tendons are connected with muscle levers, by means of pulleys or angle levers, and the writing points are arranged so that they write in the same perpendicular on the drum, signal magnets being inserted in the primary circuits of the two inductoria. When the peroneal nerve on one side is stimulated it will be found that the homolateral semitendinosus contracts, and that the rectus femoris simultaneously relaxes, if it be in a hypertonic condition as a result of decerebrate rigidity.*

If the preparation is still in suitable condition, the experiment should be terminated by exposing the lumbar portion of the spinal cord and studying THE FUNCTIONS OF THE SPINAL ROOTS, noting also the effect produced on the rigidity by their section. To expose the roots, place the animal on its belly with a thick pad or block of wood under the lower portion of the abdomen, and make an incision in the mid line of the back over the spines of the lumbar vertebrae. Separate the muscles from both sides of the spinous processes and

*This experiment succeeds best on the decerebrate cat. If time is limited the experiment should be omitted and that on the spinal roots performed.

retract strongly so that the laminae of the vertebrae are exposed. Much of this can be done by blunt dissection, which will avoid hæmorrhage. Cut the tissues between the spinous processes of the 2nd and 3rd lumbar and also between the last lumbar and 1st sacral and amputate at their bases, the spinous processes between the two cuts, using a strong bone forceps. Remove the spinous processes and after bleeding has been controlled by the application of cloths wrung out with hot water proceed to open the spinal canal by cutting through the laminae of the exposed vertebrae with bone forceps, taking care that the point of this instrument does not go deeply into the spinal canal. The spinal cord enclosed in the dura is now exposed, but it is necessary in order to expose the roots properly that the articular processes between neighbouring vertebrae be picked away.* (The so-called hawk's bill bone forceps are useful for this process).

The posterior root ganglia came into view when the articular processes have been removed. After again stopping hæmorrhage, which is likely to be considerable at this stage, the roots are prepared for stimulation.

Lift the posterior root of the 6th nerve carefully on a strabismus hook or small aneurysm needle, and tie a ligature round it as near to the ganglion as possible, cutting the root distal to the ligature. Loosen the leg on the corresponding side and stimulate the central end of the root with a tetanising current, using ordinary electrodes. Note the character of the movements of the leg and watch for any changes in the respirations.

Ligate and cut the 7th posterior root as near the cord as possible and stimulate the peripheral end.

What conclusion do you draw from the results of these experiments?

The functions of the anterior roots are then determined by a repetition of the same procedure as for the posterior. It is somewhat difficult to prepare these roots for stimulation, and it often assists to pass a tape round the cord, by which it is cautiously pulled up and to one side. Observe carefully any difference in the type of movement which results by stimulating the anterior and posterior roots.

Draw a diagram showing the functions of the roots.

*It will be observed that the lower (6th and 7th) lumbar roots are larger than those higher up, the level of the 7th being about on a line with the iliac crests.

CHAPTER XXXVII.

THE DECEREBRATE CAT I.

Demonstration 16.

A cannula is inserted in the trachea in a deeply etherized cat (preferably a young animal) and a pair of serres fines is placed on the carotid arteries on both sides of the neck. The animal is then placed on the 'decerebrator' with the pelvis resting on the back platform (P), the hind legs hanging free, and the neck on the neck block (N), care being taken to prevent any kinking of the

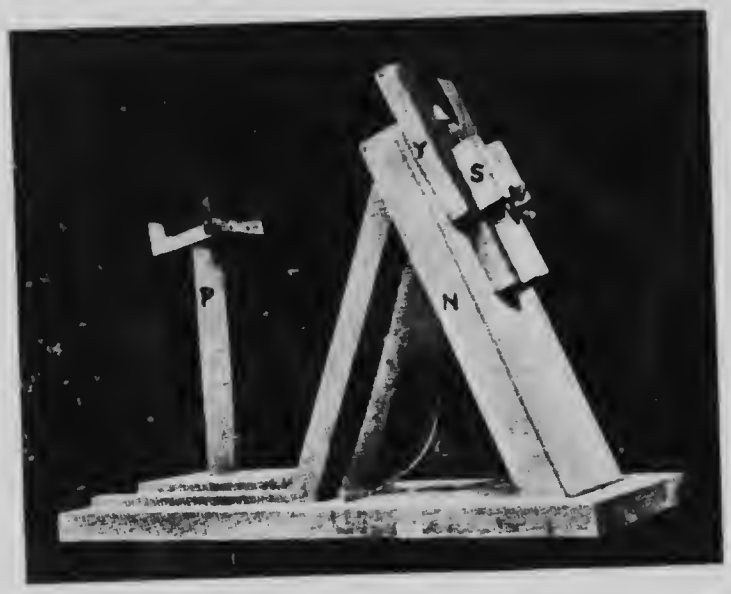


FIG. 72. Decerebrator. For description, see context.

tracheal cannula. The nasal septum is punctured by a scaplel and a cord is passed through to a hole on the neck block, at the lower level of the tongue guard (T). The mouth is opened and by gentle traction on the cord and manipulation of the head the snout is pulled over the tongue guard which lies over the tongue with its point between the fauces and over the epiglottis. The cord is then tied to a hook on the base of the stand and the nose piece (S) is pressed up against the snout and fixed in position by tightening the clamp.

An incision is now made along the mid line of the skull and the skin reflected to both sides. A distance of 30 mm. is measured back from the coronal suture

(which is particularly evident in young animals) and marked by a cut with a scalpel. The preparation is now ready for decerebration. This is best done by using a planing blade (12·5 cm. wide, 9 cm. high and 1 cm. thick) bevelled on one edge and mounted on a wooden handle, but a good axe of about the same dimensions is quite satisfactory. To perform the decerebration, the operator holding the blade in the left hand, places the edge on the notched mark, and holds the plane of the blade at right angles to the plane of the head. By means of a heavy mallet (or hammer, if an axe is used) held in the right hand he gives the blade a light blow so as to make the edge engage the skull, the blade being held accurately transverse to the long axis of the skull and directed so that when the head is cut through the edge will strike a mark made on the metal plate of the base of the tongue guard. The operation is then completed by applying a couple of strong blows and the decerebrated animal is immediately removed from the stand and placed on its back on the table, the neck being grasped with the finger and thumb just below the transverse process of the atlas so as to compress the vertebral arteries. The head is kept elevated to diminish haemorrhage, and etherization is discontinued. In many animals the respirations cease for a few moments, and artificial respiration may be necessary. They usually return spontaneously, but if they do not do so, the vertebral arteries should be momentarily released so as to allow some blood to flow to the respiratory centre. The bleeding from the cut across the head is not usually serious if the vertebrals are properly compressed and when time has been allowed for clotting to occur these may be cautiously released. There is no particular advantage in trying to accelerate the clotting by applying absorbent cotton wool to the wound. It usually takes from 15 to 20 minutes before the vertebrals can be entirely released. Finally, the clamps are removed from the carotid arteries, one at a time—any bleeding point in the cut muscles being ligated—and the head is tied to an upright so as to keep it elevated.

The resulting preparation is suitable for many experimental purposes (consult Sherrington's Mammalian Physiology, The Clarendon Press, Oxford.)

The blood pressure, although it may rise considerably while the vertebrals are being compressed (because of partial asphyxia) soon returns to about the normal level, and the respiratory centre responds to changes in C_H of the blood much more promptly than in anaesthetised animals. Why should this be the case? It is particularly in the study of reflex actions, however, that the preparation is of value, although certain of them are masked by the decerebrate rigidity which develops. Since the section if properly made goes through the mesencephalon just behind the anterior corpora quadrigemina, reflexes involving the head end of the animal can be demonstrated. The following are to be elicited and studied:

1. THE PINNA REFLEX. Pinch or slightly twist the tip of the pinna between the finger and thumb. The response consists of

a quick retraction of the pinna accompanied often by a folding back of its free end.

2. THE ACOUSTIC REFLEX.—Pricking up of the ears when a sharp sound is made as by clapping the hands. This reflex occurs only when the section is forward of the anterior corpora quadrigemina.
3. THE DEGLUTITION REFLEX. Swallowing movements when water is dropped on the base of the tongue. Note the behaviour of the respiratory movements during the swallows. Repeat the observation by dropping the water on various parts of the tongue. Observe the effect on swallowing produced by pressing a moistened camel's hair brush on the pharynx. In order to do this, it is necessary to slit the soft palate in the mid line. The movements of the vocal cords can readily be observed by using a laryngeal mirror.
4. THE HEAD-SHAKE REFLEX.—Rapid shaking movements in rotary direction when air is blown into the external auditory meatus. The reflex may also be elicited by squirting cold water from a syringe into the ear.
5. THE FLEXION REFLEX.—By painful stimulation of the paw — flexion at knee and hip occurs.
6. THE KNEE JERK.—Quick extension (kick) at knee produced by tapping the patellar tendon. Note particularly that the return of the leg after the contraction is not complete, and that if the tendon be tapped at short intervals the knee becomes more or less permanently extended. This result depends on a contraction remainder, which is a feature of the 'rigid' postural muscles in decerebrate preparations. In the spinal animal there is no postural hypertonus so that the leg passively falls back to its previous position after the jerk.

The scratch reflex is not present as a rule.

The most striking reflex condition produced by the decerebration is the rigidity, which it can readily be seen affects particularly the extensor (postural) muscles. The rigidity is not of the same nature as the tetanic contraction produced by continuous stimulation of the motor neurone, for the muscle yields to a slowly applied pull and does not spring back to its old position when the extending force is removed. This is tested by bending the knee or

elbow joints. The condition has therefore been styled 'REFLEX POSTURAL TONUS' and it can be shown that the weight required to counterbalance the rigid muscle is practically the same whether muscle is lengthened (knee in extension) or shortened (knee in flexion).*

If the preparation is still in suitable condition, the experiment should be terminated by exposing the lumbar portion of the spinal cord and cutting the sensory roots of the nerves of the hind limbs (see p. 93). What effect has this procedure on the decerebrate rigidity? What conclusions do you draw from the results?

*The flexor muscles acting on the joint should be paralysed by cutting their nerves in testing these points.

CHAPTER XXXVIII.

THE DECEREBRATE CAT II.

THE DECEREBRATE PIGEON.

There are certain fundamental reflex reactions which can be studied only after considerable operative preparation of the animal. One of these is RECIPROCAL INHIBITION which the following experiment devised by Sherrington clearly demonstrates. In it reflex inhibition is demonstrated of the plastic tonus of the extensor muscles of the knee in decerebrate rigidity and of the same muscles while actively contracted as a result of reflex stimulation.

Demonstration 17.

Whenever bleeding has stopped from the neck stump of a decerebrate preparation (cat) this is placed on its back on a well-heated operating table and the hind limbs tied in an extended position. With the left leg well abducted, an incision is made about 5 cm. long down the thigh towards the outer border, and then curved across the thigh towards the inner border. The bluntly V-shaped skin flap is reflected along with the subcutaneous fat towards the median line, so as to expose the femoral artery and vein. About 8 cm. outside the artery will be seen the psoas muscle with the femoral nerve emerging from it. There are three branches of the nerve. Of these the outermost (a small branch) runs to the sartorious muscle and is cut, the middle (a large branch) runs to the quadriceps extensor and is left intact and the innermost (a small branch) is the saphenous nerve, which also is cut. Finally, the psoas muscle is cautiously cut across piece by piece, using small scissors for the purpose. By these operations the flexor muscles of the hip joint are rendered incapable of acting on this joint. The operations are repeated on the right leg.

The sciatic nerves on both sides must now be exposed, cut low down and the central ends placed in Sherrington's electrodes, the branches of them that run to the hamstring muscles being severed. By this latter operation the knee joint is rendered incapable of active flexion. To accomplish these objects an incision is made down the mid line of the back of the thigh, starting above from a point midway between the tuber ischii and the great trochanter and continuing down to the outer condyle of the femur. From the

upper and lower ends of this incision, short incisions are made across the limb and the skin flap is reflected forward. At the upper end of the exposed wound the lower edge of the relatively small gluteus maximus muscle is made out (a small vein running along its lower border) and is cut at its lower end and reflected upward. It is now possible by retracting the muscles between which it lies to separate the sciatic nerve and to make out a large branch passing backwards from it. This is the hamstring nerve and it is cut. The sciatic nerve is next followed downwards to where the two divisions of the trunk (peroneal and tibial) diverge. A ligature is tied around the nerve at this point, the nerves cut distal to the ligature and their central ends placed in Sherrington's electrodes. It will be noted that the above nerve sections render practically all muscles in the thigh incapable of reflex contraction except the extensor muscles.

It is now necessary to arrange for graphic records of movements at the knee joint. For this purpose the leg is amputated near the ankle joint. After tying a stout mass ligature (threaded by a pack needle through the skin and muscles around the stump so as to hold it in place) a thread is also attached to the skin of the stump, and connected with a suitable muscle lever, using pulleys if necessary. The femur must also be immobilized at its lower end so that the knee joint may be held in a flexed position and the slightest movement at this joint recorded without disturbance by any other movements of the preparation. For this purpose it is necessary to insert a threaded steel pin into the condyles of the femur on their median aspect. To insert the drill pin, an ordinary drill, procurable at any hardware shop, is used. The drill head is unscrewed from the pin after this has been screwed into the bones and a brass rod, threaded at one end so that it fits the thread of the screw pin, is connected to the pin with its outer end held by a clamp to an upright stand.

These preliminary operations being completed, the preparation is placed on its right side, and the femur of the left thigh arranged so that it is supported by the drill pin and brass rod in a nearly vertical position, with the hips flexed almost to a right angle. In cases in which the decerebrate rigidity is marked, the left knee will be nearly extended. If it is not so, it may be placed in this position by passive extension (the shortening reaction—what does it depend on?)

With everything ready to take a graphic record of the movements of the left leg, the Sherrington electrodes are connected on both sides, each with a separate inductorium, and the recording drum having been started the left sciatic is stimulated momentarily with a tetanising current of moderate strength. The leg drops into flexion because of reflex inhibition of the postural tonus. Does the leg go back to its extended position when the stimulus is removed?

A similar stimulus is applied to the right sciatic, when a reflex contraction of the left extensor muscle will occur (crossed extension reflex (see p. 218).

Finally it can be demonstrated that this reflex contraction is inhibited through stimulation of the contralateral sciatic nerve. To do this the right sciatic is stimulated, and whenever the tracing shows that the left extensor muscles are decidedly contracted, the left sciatic is simultaneously stimulated for a brief period. If the proper strength of stimulus is employed, it will be observed that the extensors relax. These observations should be repeated with stimuli of varying strengths and duration. When the stimulation of the left sciatic is discontinued while still maintaining that of the right the muscle goes back to the contracted state. Draw a diagram to show the probable arrangement of the reflex pathways in the spinal cord.

DECEREBRATE PIGEON.

Demonstration 18. While under the influence of ether the skull is exposed by a transverse incision of the skin. A piece of bone just large enough to expose the cerebrum is quickly removed by sharp scissors. By means of a glass tube connected with a suction pump*, the cerebrum is sucked up. Great care must be taken to avoid injury to the cerebellum, as there is no tentorium in the pigeon. The advantage of this method of decerebration is that the blood is sucked away, thus allowing a clear view of the field of operation. The cavity is plugged with absorbent cotton, and the skin sewn up. The actual decerebration should take but a few seconds.

*The tube should be slightly drawn out with an opening 2 or 3 mm. in diameter. This tube is connected by rubber tubing to a filter flask so that the brain tissue will not be drawn into the pump.

The operation should be performed at least two or three hours before the observations are to be made in order to permit complete recovery from the ether.

A decerebrate pigeon has no memory, therefore no sense of fear. It is not conscious of painful stimulation. It sleeps unless stimulated. It starves unless food is placed back in the throat so that the swallowing reflex is started.

Stimulate the pigeon in the following ways:

1. Push it in order to get it to move.
2. Produce a loud sharp sound near one ear.
3. Allow it to smell strong ammonia.
4. Stand it on a sheet of metal and gradually heat the latter until too warm for your own hand.
5. If the bird is not too weak, toss it into the air and it should fly.

Make notes of the behaviour of the animal in response to these stimuli and explain the results.

CHAPTER XXXIX.

THE SPINAL CAT (SHERRINGTON'S PREPARATION.)

In this procedure the spinal axis is cut about 4 m.m. behind the point of the calamus scriptorius, and the spinal animal exhibits a considerable number of complex reflex movements, although the arterial blood pressure remains low. Spontaneous respirations, of course, cease entirely so that it is necessary to apply artificial respiration. The control of body temperature also disappears, necessitating artificial warmth.

Demonstration 19.—Anæsthetise a cat deeply, insert a tracheal cannula low down and ligate the carotid arteries on both sides of the neck. Place the animal in the prone position and holding the head in the left hand, make a wide transverse incision through the skin over the occiput and retract the skin downward so as to expose the muscles of the upper end of the neck. Feel for the transverse processes of the atlas and cut the muscles across at the posterior edges of the processes. Cut off the spinous process of the axis with a bone forceps. Thread a large packing needle (at least 15 cm. long) with stout string, and pass it close under the body of the axis (i.e., posterior to the œsophagus) and tie it tightly in the depths of the cross cut. This ligature compresses the vertebral arteries as they pass between the transverse processes of the axis and atlas.

The animal is now decapitated. For this purpose flex the head so as to stretch the occipito-atloid membrane, and thrust the point of a narrow (12 mm. wide) amputation knife through the membrane moving it laterally so as to cut the cord. With the point of the knife resting on the anterior wall of the spinal canal bend the head forcibly to one side, and carry the edge of the knife through the opposite occipito-atloid joint. Repeat this procedure for the other joint and then complete the decapitation by cutting through the remaining tissues. If there is bleeding it can be stopped by raising the stump. When it has ceased, bring the skin flaps together over the stump and lay the preparation on a warmed observation table. Artificial respiration by means of a pump connected with the tracheal cannula must of course be instituted before the cord is

severed, if not before. It is advisable to warm the air from the pump, but whether or not this is done, great care must be taken to see that the temperature of the preparation, observed by a clinical thermometer placed in the rectum, does not fall. For some time after the decapitation little reflex activity is shown by the preparation—why is this the case? In about one hour, however, many complex reflex movements can readily be elicited. Of these the following should be studied: the movements may be recorded by tying threads to the hind limbs and connecting with reducing levers.

1. THE FLEXION REFLEX, by applying stimuli (mechanical, electrical) to the skin of the foot or stimulating the central end of one of the sensory nerves (peroneal) with the tetanising current. The latent time, grading of intensity, summation, etc., may be studied by the procedures already described on p. 219.

2. THE KNEE JERK, by passively flexing the knee joint and tapping the patellar tendon. The prompt and limp-like return of the leg to its original position should be contrasted with the gradual and imperfect return observed in a decerebrate preparation (p. 227)

3. THE SCRATCH REFLEX, by stroking the skin at the side of the neck. The scratching movement of the homolateral hind limb is not so easily evoked as in a spinal dog that has recovered from shock, and it may not appear until the decapitated animal has been partially asphyxiated by discontinuing the artificial respiration for a minute or so. Sometimes the preparation shows a hyperexcitable scratch reflex, but this often depends on inadequate pulmonary ventilation. When it occurs, the respiratory apparatus should be examined and the tracheal cannula cleared of any mucus that may be interfering with the free passage of air into and out of the lungs. If the scratch reflex is marked, its inhibition may readily be demonstrated by stimulating the central end of the peroneal nerves of either leg.

4. STIMULATION OF THE POSTERIOR COLUMNS OF THE SPINAL CORD, by exposing the upper end of the severed cord and stimulating by the unipolar method. This observation is of value because it shows that stimuli descending by the main sensory pathways of the cord—because the fibres transmit in both direction (cf. p. 45)—flow into the collaterals which are adjacent to the point of entry of the fibres into the cord. To make the observation, place an indifferent

electrode on one foot (well moistened with strong saline solution) and connect its wire with one pole of the secondary coil to the other pole of which a fine stigmatic electrode is attached. Clear away any blood clot from the upper end of the cord (using a moistened camel's hair brush) and while holding the neck stump in one hand, stimulate the dorsal columns of the cord, first near the median fissure and then as near as possible to the posterior horn of grey matter. In the former case it is the homolateral hind limb that flexes, in the latter case, the homolateral fore limb. It may be necessary to repeat the observation several times with varying strengths of stimulation in order to secure definite results.

5. THE FUNCTIONS OF THE SPINAL ROOTS, by removing the laminae and articular processes of the lumbar vertebrae. The procedure for this operation is in general the same as that described for the dog (p. 223) with the difference that the spinous processes of the exposed lumbar vertebrae are not cut at their bases, but the laminae are freely exposed by cutting away the muscles which lie over them. The articular processes are then snipped across and while pulling up the lowermost (7th) spinous process with a strong forceps, the laminae are cut through beginning with the 7th lumbar and working upwards. The ganglia of the posterior roots are brought into view by picking away the stumps of the articular processes. The 7th ganglion lies on a line with the iliac crests.

Finally it is important to use the decapitate preparation to study the various conditions which control THE ARTERIAL BLOOD PRESSURE. The technique is the same as that already described for the anaesthetized dog (p. 72) only, of course, small cannulae must be used and the pressure established in the tubing which connects cannula to manometer prior to removal of the clip from the artery, must not be more than about 50 mm. Hg.

There are certain vascular reactions which it is especially valuable to investigate in the decapitate preparation. These are:

1. The effect of stimulation of the spinal cord on the blood pressure.

2. The effect of varying amounts of epinephrin injected into the femoral vein.

3. The effect of pituitary extract similarly injected.

4. The effect of asphyxia.

This last group of observations may be done by advanced students.

APPENDIX.

TABLE I

THE PERCENTAGE OF OXYGEN WHICH IS EQUIVALENT TO THE NITROGEN FOUND IN THE EXPIRED AIR.

To obtain the nitrogen in the expired air, add the percentage of CO_2 and O_2 found and subtract the sum from 100. The table gives the percentage for O_2 corresponding to this figure:

% N_2	78.7	78.8	78.9	79.0	79.1	79.2	79.3	79.4	79.5	79.6	79.7	79.8
	79.9	80.0	80.1	80.2	80.3	80.4	80.5	80.6				
% O_2	20.86	20.88	20.90	20.93	20.96	20.98	21.01	21.04	21.07	21.10	21.12	21.14
	21.16	21.19	21.22	21.25	21.28	21.31	21.35	21.38				

TABLE II

TENSION OF AQUEOUS VAPOUR IN MILLIMETRES OF MERCURY.

To obtain the dry barometric pressure, subtract the mm. Hg. corresponding to the temperature of the air from the barometric pressure at the time of the experiment:

Temp.	15° C.	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
Mm.	12.7	13.5	14.1	15.4	16.3	17.4	18.5	19.7	20.9	22.2	23.5

TABLE III.

TEMPERATURE CORRECTIONS TO REDUCE READINGS OF A MERCURIAL BAROMETER WITH A BRASS SCALE TO 0° C.

Subtract the appropriate quantity as found in table from the height of the barometer. The table is for a barometer with a brass scale, and the values are a little lower (about .2 mm.) than for the glass scale. The corrections for intermediate temperatures can be approximated.

Temp.	700 mm.	710 mm.	720 mm.	730 mm.	740 mm.	750 mm.	760 mm.	770 mm.
15°C.	1.69	1.72	1.74	1.77	1.79	1.81	1.84	1.86
20°	2.26	2.22	2.32	2.36	2.39	2.42	2.45	2.48
25°	2.83	2.87	2.91	2.95	2.99	3.03	3.07	3.11

TABLE IV.

TABLE FOR REDUCING GASEOUS VOLUMES TO NORMAL TEMPERATURE AND PRESSURE.

The observed volume, when multiplied by the factor corresponding to the temperature and pressure, will give the volume of the expired air reduced to 0°c. and 760 mm.

Mm.	15° C.	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
729	.898	.894	.891	.888	.885	.882	.880	.877	.873	.870	.867
730	.910	.907	.904	.904	.897	.891	.894	.888	.885	.882	.879
740	.922	.919	.916	.913	.910	.907	.904	.901	.897	.891	.891
750	.935	.932	.928	.925	.922	.919	.916	.913	.910	.907	.904
760	.947	.944	.941	.938	.934	.931	.928	.925	.922	.919	.916
770	.960	.957	.953	.950	.948	.945	.940	.936	.933	.930	.927

TABLE V.

R.Q.	CALORIES FOR 1 LITER O_2 Number	RELATIVE CALORIES CONSUMED AS Carbohydrate	Fat
		per cent.	per cent.
0.707	4.686	0	100
0.71	4.690	4.4	98.6
0.72	4.702	18	95.2
0.73	4.714	8.2	91.8
0.74	4.727	11.6	88.4
0.75	4.739	15.0	85.0
0.76	4.752	18.4	81.6
0.77	4.764	21.8	78.2
0.78	4.776	25.2	74.8
0.79	4.789	28.6	71.4
0.80	4.801	32.0	68.0
0.81	4.813	35.4	64.6
0.82	4.825	38.8	61.2
0.83	4.838	42.2	57.8
0.84	4.850	45.6	54.4
0.85	4.863	49.0	51.0
0.86	4.875	52.4	47.6
0.87	4.887	55.8	44.2
0.88	4.900	59.2	40.8
0.89	4.912	62.6	37.4
0.90	4.924	66.0	34.0
0.91	4.936	69.4	30.6
0.92	4.948	72.8	27.2
0.93	4.960	76.2	23.8
0.94	4.973	79.6	20.4
0.95	4.985	83.0	17.0
0.96	4.997	86.4	13.6
0.97	5.010	89.8	10.2
0.98	5.022	93.2	6.8
0.99	5.034	96.6	3.4
1.00	5.047	100.0	0.0

(From Lusk.)

INDEX