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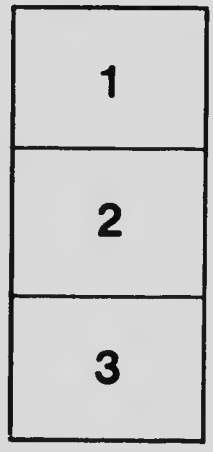

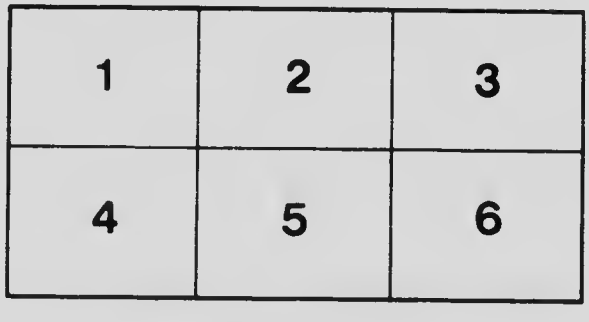

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ON THE DISTRIBUTION OF POTASSIUM IN ANIMAL AND VEGETABLE CELLS.

By A. B. MACALLUM, M.A., M.B., Ph.D. (Plates I and II.)

Reprinted from the Journal of Physiology
Vol. XXXII. No. 2. February 28, 1905

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ON THE DISTRIBUTION OF POTASSIUM IN ANIMAL AND VEGETABLE CELLS.

By A. B. MACALLUM, M.A., M.B., PH.D., *Professor of Physiology in the University of Toronto.* (Plates I and II.)

INTRODUCTION.

THE salts of sodium, potassium, calcium and magnesium are, as is well known, constantly found in the ash of animal and vegetable cells, and they obtain also in the secretions and excretions of such cells when they constitute glands and tissues. They further occur in the proteids and fats derivable from such cellular structures, and even some of the proteids, as, for example, the globulins, remain in solution only in the presence of such salts. Further, experiments with saline solutions show that the activity of not only heart muscle, but also of ordinary striated muscle depends in some way on the presence, in certain proportions, of all these elements except magnesium. In consequence the conclusion has been drawn that these elements are essential constituents of living matter, and attempts have been made to explain the rôle that each and all play in the activities of protoplasm, the more recently advanced explanations being based on the application of the facts and theories of physical chemistry.

A fundamental difficulty in the way of accepting all such explanations lies in the fact that we cannot be certain that the salts of the elements in question are uniformly distributed in the protoplasm of cells, nor do we know for certain that they occur within the cells themselves. And our doubts are strengthened when we consider the analyses of the tissues and of the various physiological fluids, for we find that they do not yield constant results. In muscle and serum the differences in the proportions are striking.

	Na	K	Ca	Mg
Serum (Dog)	100	6·86	2·52	0·81
Muscle „	100	354·00	7·26	25·1

It is evident that between serum and muscle there is on this point a lack of parallelism which cannot be explained on any possible application of the doctrine of ions. The small quantity of potassium in blood plasma, contrasted with the amount of the element in muscle, in which also the calcium and magnesium are more abundant, is difficult to account for on any theory yet advanced. This want of parallelism is, however, based on some fundamental condition, for the perfusion of solutions, in which the elements are in the proportions which obtain in the ash of muscle, quickly brings to an end the activity and irritability of muscle. Perfusion of the frog's heart with a Ringer's fluid in which the sodium, potassium and calcium are in the proportions which obtain in blood plasma causes it to beat with a regular rhythm for hours, but it at once ceases to contract, or it may enter the diastolic phase, if either the potassium or the magnesium or both in the fluid are increased so as to make the proportions parallel to those in the ash of muscle fibre.

These facts make it difficult for us to take any position as regards an explanation except that of reserve.

The difficulty is enhanced when one considers the analyses which have been made of the ash residues of various organs. The data for muscle are very different from those of glands, of connective tissue and of nerve tissue, and the data for the whole organism have been found to vary even in the case of the new-born child, in which there should be found approximately constant results, but the analyses of Camerer and Soldner[1], Hugounenq[2] and de Lange[3] are discordant; and yet the differences are not due to imperfect methods of determination.

What further lies in the way of a solution is the fact that we have never yet determined the proportions of the elements in protoplasm freed from all adventitious material more or less of a degenerative character. If we could prepare a quantity of normal Amœbæ sufficient to furnish ash abundant enough for definite quantitative analyses, it might be possible from the results to formulate some generalization which would assist in the solution of the question.

Such an opportunity, however, is one that is not likely to present itself, and in looking for a method of solving the question the only one which appears feasible is that based on microchemistry. If there were available reactions for the four elements which would reveal infinitesimal quantities of them in the cells of a tissue it would be

[1] *Zeit. für Biol.*, XXXIX. p. 173. 1900.
[2] *Comptes Rendus*, CXXVIII. p. 1419. 1900
[3] *Zeit. für Biol.*, XL. p. 526. 1900.

possible to associate their occurrence with this or that activity in the protoplasm. Unfortunately such reactions are not possible in the case of sodium and magnesium, and those for calcium have not yet been made applicable to microchemistry, but in the case of potassium there is a reaction of great sensitiveness which has given results of considerable interest, bearing not only on the part played by this element in protoplasm but also on the distribution of the other elements in the cell.

This reaction I have employed for over a year in studies on the cells and tissues of both Animal and Vegetable Kingdoms, and I now give, in the following pages, some of the leading facts which have been ascertained in these observations.

The reaction.

When a solution of potassium nitrite (KNO_2) is added to a solution of cobalt, an orange-coloured double salt is precipitated, the composition of which is $Co(NO_2)_3 + 3KNO_2 + nH_2O$. This is known as Fischer's salt, he having first described its preparation in 1849. He found that cobalt, when in so dilute a solution as 1 in 3,000, is almost wholly precipitated on the addition of potassium nitrite.

This reaction was utilized by de Koninck[2] and Curtman[3] as a precipitation test for potassium, and de Koninck, employing a 10 per cent. solution of $NaNO_2$ to which some chloride of cobalt and acetic acid was added, claimed that it was more sensitive than platinic chloride. He further held that, while ammonia gives a like but much less delicate reaction, salts of magnesium, calcium, barium, strontium and rubidium are not affected. He found, however, that when the amount of potassium chloride in solution is so dilute as 1 in 2,000 no precipitate occurs. Curtman also found that the hexanitrite of sodium and cobalt does not give a precipitate with lithium, magnesium, calcium, barium or strontium, and that it does do so with ammonia, rhubidium, cæsium, and particularly with potassium in the presence of sulphates, phosphates, nitrates, acetates and chlorides, and only the presence of iodine is unfavourable to its formation.

More recently Biilmann[4] found that by specially preparing the reagent it would precipitate the potassium when the chloride of the

[1] *Pogg. Annalen*, LXXIV. p. 124. 1849.
[2] *Zeit. für anal. Chemie*, XX. p. 390. 1881.
[3] *Ber. d. d. chem. Gesell.*, Jahrg. 14, p. 1951. 1881.
[4] *Zeit. für anal. Chemie*, XXXIX. p. 284. 1900.

latter is so dilute as 1 in 27568 of a binormal sodium chloride solution while it will show one part of potassium in the presence of 4000 of sodium in a 10 per cent. sodium chloride solution. The cobalt reagent has also been used by Van Leent in the determination of potassium in sea water, the potassium being first precipitated therefrom as the hexanitrite of cobalt, sodium and potassium and the latter element estimated as perchlorate. The results agree very closely with those obtained when the determination was made with platinic chloride[1]. Autenrieth and Bernheim[2], after trials with solutions of potassic chloride of known strength, which gave results exceedingly close to the actual value, also employed the reagent for the estimation of potassium in urine, likewise with results which on the whole were satisfactory.

I have endeavoured to determine how far the reagent is a precipitant of potassium in solutions, using for this purpose one which, so far as the salts were concerned, represented an artificial urine. The results of these determinations[3] go to confirm the statement of Van Leent and Autenrieth and Bernheim on the value of the reagent as a complete precipitant for potassium from its solutions.

The reagent, as I employed it, is a modification of the one recommended by Erdmann[4] and is made by dissolving 20 grams of cobalt nitrite[5] and 35 grams of sodium nitrite in 75 c.c. of dilute acetic acid (*i.e.* 10 c.c. of glacial acetic diluted to 75 c.c.). A vigorous evolution of nitrogen peroxide results. If the preparation of sodium nitrite used contains traces even of the potassium salt, a precipitate of the triple[6] salt will be found to have separated out after some hours, and this may be removed by filtration. The filtrate is diluted with water to 100 c.c. and is then ready for use. When a small quantity of this preparation is added to a solution of a potassium salt there is immediately produced an orange-yellow precipitate of the triple salt, consisting of crystals of

[1] *Zeit. für anal. Chemie*, XL. p. 569. 1901.

[2] *Zeit. f. physiol. Chemie*, XXXVII. p. 29. 1902.

[3] The amount of potassium in 50 c.c. of this solution was 0·1324 grm. Three determinations of the potassium by the hexanitrite-perchlorate method gave 0·1302, 0·1312 and 0·130 grm.

[4] *Anorganische Chemie*, 1898, p. 630. The reference is given by Autenrieth and Bernheim.

[5] The cobalt nitrite I employed for this preparation of the reagent was obtained from the Baker and Adamson Chemical Co., Easton, Penn., U.S.

[6] The term "triple" salt or compound will, on account of its convenience, be employed in this paper to designate the hexanitrite of cobalt, potassium and sodium, while the expression "double" salt will comprehend only the reagent, the hexanitrite of cobalt and sodium.

pentagonal dodecahedra of varying microscopic sizes and of chrome-yellow colour.

The composition of this precipitate varies with the composition of the original solution of the salts of potassium. Fischer's salt, made by adding a solution of a cobalt salt to one of potassium nitrite, has the composition given by the formula $CoK_3(NO_2)_6 + 2\frac{1}{2}H_2O$. If, however, sodium salts also are present, sodium enters into the precipitate, but the amount obtaining in this latter depends on that in the original solution. In Fischer's salt free from water the potassium is 25·93 per cent., but in the precipitate produced by adding the double salt, $CoNa_3(NO_2)_6$, to a solution of a potassium salt, the potassium was found by K[illegible]lbert[1] to range from 16·31 per cent. to 8·21 per cent., and that th[illegible]fference betw[illegible]en these percentages and that found in Fischer's sal[illegible] was made up by the sodium which varied between 4·46 and 5·77 per cent. As a consequence of his observations Gilbert proposes for the precipitate caused by the $CoNa_3(NO_2)_6$ the formula $Co(NO_2)_3 3(K/Na)NO_2 + nH_2O$, the value of n being either $1\frac{1}{2}$, 2 or $2\frac{1}{2}$.

This precipitate is appreciably soluble in cold water, and it is soluble in sodic nitrite solutions even when sodic acetate is present. It is insoluble in dilute solutions of the precipitating reagent which may be used to wash the precipitate, and it is also insoluble in 80 per cent. alcohol, which removes from the precipitate traces of the precipitating double cobalt salt. Alcohol of this strength was used by Gilbert, and others, for the purpose of washing the precipitate. So far as known there is no other way of purifying it, although it is stated that it is insoluble in solutions of potassic nitrite, but the latter can only be employed after all traces of the sodium compound, $CoNa_3(NO_2)_6$, have been removed, as its presence would merely add to the quantity of the precipitate.

The precipitating reagent is itself extremely soluble, and in consequence it is quickly removable from the precipitate by washing with water. If the latter is ice-cold the amount of the precipitate dissolved thus is extremely small, and consequently very cold water may be used to remove traces of the cobalt salt not united with potassium.

The hexanitrite of cobalt and sodium also precipitates, but less readily, ammonium from its solutions, the precipitate containing, doubtless, ammonium in place of potassium. The crystals of this precipitate are in form and size very like those of potassium salt. It is, however, much more soluble, and it is, therefore, incompletely precipitated while

[1] *Die Bestimmung des Kaliums nach quantitativer Abscheidung desselben als Kalium-natriumkobaltinitrit.* Inaugural Dissertation. Tübingen, 1898.

the crystals are, even in ice-cold water, very soluble. It is possible in this way to distinguish between the triple salt of cobalt, sodium and ammonium on the one hand, and that of cobalt, sodium and potassium on the other, and in the case of tissue to dissolve out the former and to leave the latter where its precipitate occurred in the first place.

The form of the crystals and their orange-yellow colour make it very easy to demonstrate the presence of the triple salt where the potassium was present in abundance. Where, however, it obtains in very minute quantities, or traces, as is the case with many tissues, the crystalline form is absent, and even a yellow tinge may not be found. Consequently some other method of bringing out the presence of the cobalt salt must be resorted to. The one which I found always of service requires the application of a solution of ammonium sulphide which reacts immediately on the cobalt of the salt and forms with it the black sulphide of cobalt. Where the latter, therefore, obtains after this treatment in a preparation of tissue, potassium salts must also have been present and as the black reaction is so readily observable in a cell or tissue, it is consequently very easy to ascertain the distribution of even traces of potassium. It must of course be understood that, before the sulphide reaction is applied, all the superfluous cobalt reagent should be removed by washing the tissue with cold water. The quantity of the cobalt salt in a tissue not combined with potassium is very soluble in water, and several washings of the preparation of tissue remove the reagent so readily that in about half-an-hour the last washing employed is not coloured after ten minutes. If it is left in water for from two to six hours the water may be tinged yellowish, but only when the tissue is bulky and contains a comparatively large quantity of the precipitate. When even the preparation has remained for days in distilled water, the latter fails to remove all the precipitate; and I have kept teased-out medullated nerve fibres for a week in a large quantity of water and found that the potassium cobalt precipitate is but very little diminished. In observing the tardiness with which the triple salt is removed from tissues and cellular elements, one cannot resist the conclusion that tissue structures, while offering no resistance whatever to the removal of the precipitating reagent, are very retentive of the precipitate.

The fact that ammonium compounds give the triple salt also as a precipitate suggested the possibility of the amido acids and acid amides reacting similarly with the cobalt solution, and a study of their behaviour in this respect was carried out. It was, however, found that neither glycin, taurin, leucin, tyrosin, sarcosin, aspartic acid, glucosamin

nor glutamic acid precipitates with the reagent, and, therefore, their presence in tissues offers no difficulty in the determination of the presence of potassium. Further, neither urea nor asparagin combines with the reagent to form a precipitate, and the same must be said of alloxan, allantoin, guanidin and the purin bodies. Only one compound, creatin, was found to react differently, as a 0·4 per cent. solution of it gave at once an orange-yellow precipitate with the cobalt reagent, while in a 0·2 per cent. solution one appeared after a few seconds. The precipitate is a triple salt and consists of crystals in every respect like those of the triple potassium salt. Creatinin, on the other hand, does not, even in concentrated solutions, give a precipitate. This property of creatin is of some importance as the precipitate may be mistaken for that of the potassium cobalt compound, and in consequence one must, if it is at all possible, exclude the occurrence of creatin in animal tissues. It is always present to the extent of 0·21—0·39 per cent. in frog's muscle[1], and of 0·4 per cent. in rabbit's muscle and, therefore, it is abundant enough to complicate the problem of determining the localization of potassium in muscle fibre. According to Valenciennes and Fremy[2] creatin is present in but appreciable quantities in the muscles of the Mollusca, and they found it also in the muscles of several species of Crustacea, but Krukenberg[3] claims that it does not at all occur in the muscle of any Invertebrate, and I have been unable to detect even traces of it in extracts of the muscles of the lobster and crab. It is of course possible that Valenciennes and Fremy's results were due to defective methods, as otherwise their findings are inexplicable.

The creatin triple salt is much more soluble than the corresponding potassium compound, and consequently one may remove by frequent washing in ice-cold water the greater part of the creatin salt from muscle fibre, but it is not necessary to resort to this, as both creatin and potassium are found similarly distributed and localized inside the fibre. Apart, however, from this consideration, whatever difficulty there is in studying the localization of potassium in Vertebrate muscle fibre does not obtain in the case of that of Crustacea and Insecta, in which, if creatin is present, it occurs only in infinitesimal traces and, therefore, in the last resort in this matter it is to these or other Invertebrate forms that we must go to get unquestioned data on the point.

[1] F. Nawrocki, *Zeit. für anal. Chem.*, IV. p. 330. 1865.

[2] *Comptes Rendus*, XLI. p. 735. 1865.

[3] *Vergleichend-Physiologische Vorträge*, p. 316, Heidelberg, 1886. Also Krukenberg's papers in *Untersuchungen a. d. Physiol. Inst. der Univ. Heidelberg*, III. and IV. 1880-1.

Creatin is present in other tissues than muscle fibre, as, for example, those of the nervous system and the testicles, but in such small quantities as to offer no difficulties. It does not occur at all in smooth muscle fibre[1].

Oxalic acid and its salts in solution give a precipitate almost immediately on addition of a cobalt salt and, as oxalates are present in the juices and saps of vegetable forms, it is sometimes necessary to distinguish whether a precipitate in a vegetable tissue is the triple potassium salt or the cobalt oxalate. The form and colour of the crystals of the two precipitates are quite different. One may further control the preparations by treating some of them with a simple solution of cobalt acetate when the localization of the oxalates only is revealed, and by contrasting the two sets one may determine where the potassium salts are distributed and where the oxalates are found. As a rule, however, it was found that the presence of oxalic acid or oxalates offered no difficulty, for they are infinitesimal in amounts in the cells, while the potassium is nearly always in abundance relatively, and even of the sap in the fibrovascular tissue the same statement may be made. In consequence the presence of oxalates was disregarded, as not offering any difficulty in the investigation.

It was found that nerve fibres gave, when treated with the cobalt reagent in the usual way, rather remarkable results, and at first these were attributed to very minute traces of cholin. The latter, in its behaviour towards platinic chloride, resembles potassium and ammonium[2], and it was suspected that this resemblance would be emphasized by giving a precipitate with the cobalt reagent. On examination no precipitate occurred in solutions of cholin even when these were concentrated and after standing for hours. On the other hand the addition of the faintest trace of a potassium salt to a solution of cholin containing a quantity of the cobalt reagent produced at once a precipitate.

To get the best results with the reagent it was applied directly to the fresh cellular elements, and when at all possible these were in the teased-out form or, in the case of animal organs, as sections made from the fresh tissues by means of the freezing microtome. The freezing of the tissue was done rapidly and for this purpose the spray from liquid carbon dioxide was used. The teased-out material and the sections

[1] Krukenberg, *loc. cit.*

[2] Ammonium compounds are present in cells and tissues only in infinitesimal traces and it would appear that their distribution is in no wise different from that of the potassium salts.

were at once placed in the undiluted reagent for twenty minutes, after which they were washed with ice-cold water several times till the washings were, after remaining on the preparations for five minutes, wholly uncoloured. The preparations were then, without further delay, mounted on the slide in a mixture of equal parts of glycerine of 50 per cent. strength and of concentrated ammonium sulphide solution. Such preparations have been found to keep in a fairly good condition for a couple of months, but there is after this period a progressive deterioration, especially in those made from animal forms, doubtless due in large part to the fact that the cellular elements are only very imperfectly fixed, and, in consequence, are affected by the gradual change in the composition of the mounting fluid.

Preparations from vegetable forms keep in good condition for a considerably longer period. Vegetable tissues and structures are also much more readily prepared. In the case of the Protophyta, the objects were placed at once in the reagent where they were left for about twenty minutes, after which they were washed in ice-cold water several times and treated further as described above. Wherever the structures permitted their being obtained from fresh tissue, such were employed and prepared in the same way.

Except where chlorophyll is absent the orange-yellow colour of the precipitate, when the latter is very scanty or infinitesimal in amount, is not of much service in determining the localization of potassium in vegetable cells, for the natural pigment masks more or less the occurrence of the precipitate. A further complication results in higher vegetable forms as a consequence of the nitrous acid in the reagent acting on a constituent of many cells and producing a reddish colour or a deep reddish-brown stain, sometimes causing the section to have a distinctly coloured appearance as if it were stained with a brown aniline dye. As a rule also the nuclei of all vegetable forms, with the exception of the Fungi, give a faint reddish reaction (Fig. 17), which seems to be due to the presence of some compound, or compounds, different from those which obtain in the cytoplasm, and in fact the nuclear reaction may be the only one found in a section. That it is caused by the nitrous acid of the reagent follows from the fact that it can be obtained sometimes with a solution of pure nitrous acid.

Where these special reactions obtain the orange-yellow colour of the potassium precipitate may be masked, and then of course the only decisive test lies in the production of the cobalt sulphide reaction.

The results.

Potassium, as revealed by the hexanitrite method, is found in three conditions of distribution in cells and tissues. One of these conditions is that of infiltration or impregnation, and a second is due to a precipitation, apparently of a physiological rather than of a physico-chemical character, while the third condition arises from the activities of the cytoplasm localizing and circumscribing within itself solutions of compounds of this element.

As an infiltration or impregnation product it occurs more or less uniformly diffused through extra-cellular structures, connective tissue fibrils, the matrix of articular cartilage and it is found also in the walls of many vegetable cells. In the fibrovascular bundles of young vegetable stems the walls seem to be, if not saturated with potassium salts, at least impregnated with them to a remarkable degree. For example, in transverse sections of the stem of *Lilium*, *Tulipa* and *Iris*, the bundles, after the application of the reagent, give an intense yellow reaction, due, as shown under the microscope, to triple salt crystals, which, in large part, are grouped in the immediate vicinity of the walls of the fibres and cells (Fig. 20). In the parenchymatous cells of the same sections the walls give a deep potassium reaction, even when there is no cytoplasm in their cavities.

In older fibrovascular bundles, and especially in lignified tissue, the potassium is less abundant and it is not uniformly diffused. In pine wood, for example, the medullary rays and the "intercellular substance" alone constantly give a reaction, while the sieve tubes and scalariform vessels only here and there give distinct evidence of the presence of the element. In suberin-holding tissue (cork) no potassium salts occur.

These variations in the extent and degree of impregnation are due to differences in the supply of sap or potassium-holding fluid obtaining access to the affected parts, and where the supply is constant and is diffused uniformly, the result is a uniform distribution of potassium throughout all inert material.

The simple condition of impregnation also is seen in the Animal Kingdom wherever there are not only inert structures but also degenerating cells and tissues. In the developing exoskeleta of Arthropoda, and especially in the chitinogenous layers of the exoskeleta of Crustacea (*Homarus* and *Oniscus*), the cells in the last stage of degeneration are highly charged with potassium salts, but in the

undegenerated layers there is very little potassium except that disposed in the intercellular structures (bridges) and spaces. The more marked degree of impregnation is due to the fact that the cells, dying or dead, offer no resistance to the infiltration of potassium-holding fluids, and the salts of the element become fixed in the altered cytoplasm. The abundance, however, at times of potassium in chitiniferous tissue seems to indicate that altered cytoplasmic products have a special affinity for potassium salts. How the potassium is held in this inert material it is not possible to say, but it may be due to the formation of a firm organic compound. In this case the condition may be closely allied to, or may in fact be the same as, that illustrated in the physiological precipitates to be referred to below.

Related to this disposition of potassium is that to be observed in the intercellular spaces of epithelial tissue of smooth muscle fibre and of the constituent elements of the walls of blood-vessels generally. In the epithelial tissues the disposition is at times, not so much in the interspaces, as in the very walls of two adjacent cells, shown by the double rows of granule crystals (Fig. 25), but both interspaces and walls may be, as it were, saturated with potassium salts, a condition illustrated in the epithelium of Descemet's membrane in the frog. Where, on the other hand, no interspaces exist, as, for example, in the epithelium lining the oviduct in the frog, the walls impregnated with potassium salts, while the cytoplasm gives no evidence of its presence.

In the living cell itself potassium may or may not occur. That it may be normally absent can be seen in *Vorticellæ*, examples of which may, at times, be found absolutely free from potassium, except in the invaginated food masses and in the water vacuoles. These are not isolated instances, for other Infusoria (*e.g.*, *Paramœcium*) may be found to illustrate this fact, and the leucocyte, especially the lymphocytic variety of it, may give no reaction for potassium, while the nerve-cell is always free from it.

Where potassium is present it is only in the cytoplasm that it occurs. *The cell nucleus, whether of the Animal or of the Vegetable Kingdom, is absolutely free from potassium.* It never gives a reaction for this element, and, further, this evidence on the negative side is strengthened by the fact that there is not an organic compound of potassium in which the presence of the latter is "masked" as that of iron is in chromatin, haemoglobin or the ferrocyanides. All the organic compounds which contain potassium, including the "albuminates" and nucleates, yield immediately their potassium to the hexanitrite reagent and it is, more-

over, on theoretical grounds, impossible to believe that there exists or can be formed a compound of potassium which will not so react. Consequently, the failure to obtain a reaction for potassium in the nucleus is evidence of a comprehensive kind in support of the generalization enunciated.

The facts in detail, gleaned from preparations made from a host of forms, both animal and vegetable, leave no doubt on this point and when, therefore, an apparent exception is found the evidence must be questioned. For example, when normal nuclei are found, after treatment with the hexanitrite reagent and ammonium sulphide, to give the cobaltous sulphide reaction, it may at once be assumed that the preparation has not been sufficiently washed with ice-cold water to remove from the nuclei the sodium cobalt nitrite, which has penetrated them but has not encountered there any potassium to form the triple salt. As, further, the nuclei are slightly less readily lavable than the cytoplasm there is, unless care be taken, some of the double salt in nuclear structures, while not a trace of it may be found in the cell protoplasm. In the earlier stages of my studies on the localization of potassium in the cell I attributed the complete absence of a reaction for potassium in nuclei to the defective penetration of the reagent, but this explanation will not hold, for, curiously, the nucleus is as readily penetrated by the reagent as is the cell body itself, and one may demonstrate this to one's own satisfaction with any ordinary preparation of fresh tissues treated for a few minutes with the reagent and put, without further treatment, in the sulphide reagent.

Careful washing for about twenty minutes, with the wash water frequently changed, is necessary to demonstrate the absence of potassium salts from the cell nucleus. One must also avoid using any but very minute pieces of tissue, for, though the reagent penetrates readily the individual cells with which it comes in contact, it does not diffuse through these, in the usual time required for its action, in sufficient amounts to attack properly the more remote elements. The reagent precipitates immediately any potassium it meets, but, if rapid penetration of it does not obtain, the potassium compounds in the remoter parts may undergo redistribution before the reagent reaches them. There is, further, the disadvantage that when a tissue is rich in potassium salts the penetrating reagent becomes weaker or more dilute the more it penetrates. All these points have to be borne in mind in estimating its effect on the cell nucleus in tissues.

On this account a very clear demonstration of the freedom of the

nucleus from potassium can be had, perhaps, best and most readily in the case of unicellular animal and vegetable forms. In those species of *Spirogyra*, for example, in which the number of turns of the chromatophor spiral is few, while the cell is long and of considerable transverse diameter, this result is easily obtained, for when the reagent penetrates the cell wall it at once attacks the nucleus and one can observe the penetration of the latter. Such a preparation is quickly washed free from the uncombined portion of the reagent and then it may, without further treatment, be examined under the microscope to determine the distribution of the yellow triple salt. Under the best light, and with the aperture of the substage iris diaphragm increased or diminished, as desired, not the slightest shade of yellow can be observed in the nucleus, although there may be considerable quantities of the triple salt in the immediate neighbourhood of the chromatophor. That there are not even traces of this compound in the nucleus in such preparations may be made certain by allowing some solution of ammonium sulphide to run in under the cover-glass.

The result is not peculiar to *Spirogyra*, for one may obtain it in *Cladophora*, *Oedogonium*, and *Chara*, in all of which the nuclei are less favourable objects for this purpose, but in *Zygnema* the demonstration is as readily made as in *Spirogyra*.

Protozoa also yield decisive results (*Amœba*, *Vorticella*, *Paramœcium* and *Stentor*). In all the binucleate forms of Infusoria the macronucleus, as well as the micronucleus, is free from potassium. In order to obtain preparations of all such forms they were heated, in a thin film, on a cover-glass to 110° C. for two to three minutes and then treated directly with the reagent.

This method and the result are open to the objection that in the few minutes of fixation there may occur a complete redistribution of the potassium salts. This objection can be met in the case of *Vorticellæ*, for these may be found attached to *Confervæ*, and the latter can be readily removed from their media and with their attached organisms put, without further treatment, into the reagent. After twenty minutes the tuft of *Confervæ* is removed, thoroughly washed with ice-cold water, and portions of it examined under the microscope for *Vorticellæ*. These when found may contain traces of potassium in the cytoplasm only Such preparations sometimes show, after the application of ammonium sulphide, a brownish-red tinge of colour diffused throughout the cell, due to a reaction dependent on the presence of some unknown organic compound or compounds.

In the cells of higher animal forms which can be obtained in a condition which permits, readily and at once, the application of the reagent, the result, so far as the nuclei are concerned, is the same. In the ova of Insecta and Crustacea and in the oogenous cells of the ovary of *Rana*, *Necturus* and *Amblystoma*, as well as of various fishes, the nucleus remains unaffected by the reagent, although there may be found at times abundance of potassium in the cytoplasm. If the ova are large and the cytoplasm rich in the element, the absence of a reaction in the nucleus may not be evident, but in the smaller and immature cells it is otherwise. Neither is the result doubtful in the testicular cells. The testes of *Oniscus*, *Julus* and *Lithobius* can be removed directly from these forms, and, owing to their very minute size, placed without further manipulation in the reagent. These cells are particularly favourable objects, because when they are carefully washed free of the uncombined reagent the nuclei and a large part of the cytoplasm of each cell manifest a clear silver-white colour in marked contrast to the yellow portions of the cytoplasm which contain the triple salt. On the addition of ammonium sulphide there is not the slightest trace of blackening ever to be found in the nuclear elements. In the testicular cells of the frog and *Necturus*, the rat and guinea-pig, complete absence of potassium from the nucleus may be established, although there are often in the cytoplasm of the spermatogonia of the rat, guinea-pig and other forms, large masses seemingly saturated with potassium salts.

The other animal tissues yield as definite results as one obtains in the case of the ovary and testes, although the manipulation to this end is often less easy. The most favourable for this purpose are those which permit a penetration of the reagent and whose cellular elements are rich in potassium salts. Examples of these are found in the intestinal epithelial cells, for these have, in their activities, to dispose of considerable quantities of the salts and in consequence their cytoplasm is always more or less rich in potassium. In *Oniscus* and *Julus*, for example, this is specially the case during the summer season, but the nucleus is absolutely free from potassium, however much there may be of it in the cytoplasm in its immediate vicinity. In the intestinal epithelial cells of Vertebrates, cells which are always excreting potassium compounds, the nuclei alone are always free from them.

The nuclei of the salivary, gastric and pancreatic glands, of the liver cells in the rabbit, guinea-pig, and dog, of the renal and vesical epithelial cells in these animals as well as in the frog and *Necturus*, have all been found to be wholly free from potassium compounds. The same

must be said of the nuclei of muscle fibres, smooth and striated, in the frog, *Necturus* and guinea-pig, and further of the nerve cells of the retina, spinal cord and spinal ganglia of these animals, and the rabbit.

These and a number of isolated observations on the nuclei of various cells in other forms have made it quite clear and definite that potassium is an element wholly foreign to the nucleus, and that in this there is no difference between animal and vegetable cells. In other words the nucleus, so far as potassium compounds are concerned, is a structure in the cell wholly apart from it.

The first explanation of this remarkable fact which suggests itself is that the affinities of the cytoplasm for potassium must be satisfied before any of the element reaches the nucleus, and that, as these affinities may be extensive, it happens rarely, if ever at all, that the cytoplasm attains the condition of complete saturation, thus arising the apparently universal freedom, from potassium, of nuclear structures. This explanation will not suffice, for in the dividing cells of the developing ovules of *Lilium* and *Tulipa* the free chromosomes, surrounded by a cytoplasm sometimes abounding in potassium salts, give no reaction whatever for that element. Further the discs of the red blood corpuscles in Amphibia are rich in potassium, particularly the zone in these immediately adjacent to the nucleus (Fig. 23), and yet the latter is absolutely devoid of potassium. In such there can be no question of affinity, for the very much greater part of the disc of the corpuscle consists of hæmoglobin which does not unite directly or indirectly with potassium compounds. But the clearest evidence is furnished in the results of observations on spermatozoa. The head of the spermatozoon, as well known, represents the nucleus of the spermatic cell which gave rise to the organism. Around the head there is a membrane, but no cytoplasm to take up the infiltrating potassium salts which are in solution in the semen. Now in cover-glass preparations of fresh spermatozoa of *Oniscus*, *Julus*, *Necturus*, frog, guinea-pig, rat and man, there cannot be obtained the slightest trace of the triple salt reaction in the heads, although in those of man there may at times be seen, in the membrane, a slight shadow which suggests the occurrence there of a trace of the triple salt. The result is not due to imperfect penetration by the reagent, as is clear, first from the fact that when preparations made with the reagent, after slight washing with water, are placed in the sulphide reagent, the heads are all rendered black, and in the second place, from preparations which after the application of the hexanitrite

reagent are carefully washed with water and afterward treated with the sulphide reagent, the heads then showing often the curious faint reddish tinge that one finds in the nuclei of vegetable cells, referred to above.

In the Cyanophyceæ (blue-green Algæ) there is no nucleus, but there is a structure which contains all the active chromatin of these forms and which is known as "the central body" (Centralkörper). The relationship of this is a moot question which I have fully discussed elsewhere[1], and I am inclined to regard the central body as a structure *sui generis*, which, in so far as it contains the analogue of the nuclear chromatin of higher forms, performs some of the functions of a nucleus. As regards the absence of even traces of potassium the central body is like the nucleus. In *Oscillaria princeps*, *O. tenuis*, and *Tolypothrix lanata*, potassium compounds are present, but only in the cytoplasm outside the central body. Though there is no membrane, as in the case of true nuclei, there obtains some process in the central body which acts as a barrier to the penetration of the latter by potassium compounds. On the other hand the bodies known as the "red" granules of Bütschli which contain, or are formed of, a chromatin-like substance and are, further, situated on the outside, or on the periphery, of the central body, are usually impregnated with potassium salts and are, therefore, to be regarded as elements apart, not only from the central body, but also from nuclear structures.

The cytoplasm of animal and vegetable cells is, except in Infusoria and in the case of nerve cells, never wholly free from potassium which is rarely in a diffused condition in the cell body. The element occurs here either (1) as a local precipitate or a series of localized and circumscribed precipitates, or (2) in the form of a biochemical condensation at one or more points or in one or more structures of the cytoplasm. The term precipitate is not the most satisfactory one to apply here, for, apart from the compounds it forms with platinic chloride, cobalt hexanitrite and one or two other precipitating reagents, none of which occurs outside the laboratory, the salts of potassium are extremely soluble and it is, consequently, quite unlikely that any purely physical precipitate of potassium obtains in the cytoplasm of living cells. This, however, does not exclude the possibility of there being purely physiological "precipitates" of potassium whereby compounds of the element, though still in solution in the cellular protoplasm, are rendered,

[1] "The Cytology of Non-nucleated Organisms." *Trans. Can. Inst.*, VI. p. 439. 1900.

through their relation with the colloid material containing them, as inert and indiffusible as if they were converted into the physically insoluble form. Such an explanation is the only one to offer in the case of confervoid Algæ which, growing in media rich in potassium salts, permit the entrance of the latter but localize them in that part of the cytoplasm immediately under the membrane. In Diatoms (Fig. 15) the potassium-holding compounds are confined to points immediately under the test in such a manner as to suggest at once the character of the precipitate for the localization. A somewhat similar condition is found in higher forms, animal or vegetable. In the guard cells of stomata in the tulip (Fig. 18), in the mycelial fibres of *Agaricus* (Fig. 6), and in the testicular cells of *Oniscus* and the frog, the potassium salts are very definitely localized, and the potassium-holding structures are strictly circumscribed bodies in the cytoplasm of the spermatic cells of the rat and guinea-pig. Also in the ovaries and salivary tubes of Crustacea and Insecta the precipitate-like character of the localization is very pronounced at times. Further, at circumscribed points in the medulla of nerve fibres are found, not infrequently, considerable quantities of a potassium compound, while elsewhere in their neighbourhood there may be slight or no traces of the element.

The condition which I have indicated by the term biochemical condensation admits of no doubt, although its character is very difficult to explain. Examples of this condition may be found in *Spirogyra* (Figs. 7 and 8), in which the potassium compounds seem to be localized in the immediate neighbourhood of the margin of the chromatophor, the other cellular structures as well as the cell sap being wholly free from them. The substance that appears to contain the potassium compound is a colourless plasma-like material in immediate contact with the chromatophor and probably a very considerable factor in the carbohydrate anabolism, which is supposed to be, in part at least, the function of the chlorophyll-holding band. The absence of potassium elsewhere in the cell, not only from the nucleus but also from the radiating strands of protoplasm which connect with the potassium-holding plasma, shows quite clearly the power living matter has of localizing, even in a soluble form, of condensing, as it were, the potassium salts required for metabolism.

One of the most striking illustrations of this "condensation" in the Animal Kingdom is to be found in striated muscle fibre, the dim bands of which are charged with potassium, while the light bands are devoid of even a trace of it (Fig. 30). The disposition of the element as it

obtains here and in *Spirogyra* shows that condensation is due to biochemical forces.

It is sometimes very difficult, nay impossible, to determine whether a quantity of potassium localized in a cell is a condensation or a precipitate. Examples which illustrate this may be found in *Spirogyra* threads entering the stage of plasmolysis (Fig. 11), in the chlorophyll-holding cells of the lily and tulip (Figs. 17 and 20).

It is of course possible that there is no sharp distinction between what is a precipitate on the one hand and what, on the other, is a condensation, and one can only say that a typical instance of one is in character quite different from the other. It is also possible that a precipitate and a condensation product may occur together, and this explanation would appear to serve in the case of some vegetable cells in which aggregates of potassium-holding material are in close relation with the cell nucleus (Fig. 17 *d*).

Of one point there is apparently no doubt, and it is that by precipitation the cell gets rid of, or renders inert all excess of dissolved and diffused potassium salts. This is the case specially with those of leaves and other vegetable transpiration organs. The current of water from the roots always carries in solution inorganic salts to the leaves, and those of potassium are by no means the least in quantity. On evaporation they are left in the leaves, and of course quantities accumulated there depend on the soil in which the organism grows, on the quantity of fluid transported to the leaves and on the degree of humidity of the atmospheric medium; but in every case it is considerable, and more potassium must reach the cells than they require or than is favourable to the biochemical activities of the cells. This excess is disposed of by them, it being packed away or "precipitated" in an inert form in portions of the cytoplasm, as illustrated in Fig. 17 *a*. This would explain why it is that the ash residues of many vegetable forms vary so widely in their composition, and especially in regard to the percentage of the potassium found. It explains also why some plants grow equally well in soils rich or poor in potash salts, and whose ash residues vary accordingly in the amount of the potassium present. It is in this way that vegetable forms acquire the capacity to tolerate the presence of large quantities of potassium.

Neither in the nucleus nor in the cytoplasm of the nerve-cell, as already stated, is there a trace of potassium. Preparations of the ganglia of the posterior spinal roots and of the sympathetic in the frog, rat and guinea-pig, show potassium, often in abundance, in the

tissue elements between the nerve-cells and someti. s is specially the c.se with those of the sympathetic, on th' surface, but the hexanitrite leaves their cytoplasm absolutely .ected. The superficially deposited potassium in the sympathetic . 'ls of the frog may be in such quantities in some instances that the triple salt which it forms may wholly obscure the view of the interior of the cells, but this difficulty may be overcome by pressure on the cover-glass and so displacing the superficial precipitate. In the nerve-cells of the retina such a superficial deposition of potassium does not obtain, nor is it found in the nerve-cells of the cerebral cortex or of the anterior cornua of gray matter in the spinal cord in the guinea-pig and frog. To show this, thin sections from fresh material frozen with the carbon dioxide spray v -re treated with the reagent for ten minutes, washed carefully and n..unted in a mixture of glycerine and ammonium sulphide. In such preparations the nerve-cells appeared as white or absolutely unshaded structures in a more or less shaded (dark) gray matter.

This freedom from potassium is found also in the dendrites and axons. As regards the former, the demonstration is not readily made, for these, being very minute structures, are difficult to bring into view or to isolate for the purposes of examination. They form, however, the by far greater part of the outer and inner molecular layers in the retina; and these layers in the frog give no reaction for potassium, although, at times, one may see other structures which present a curious resemblance to the synapsis of dendrites (Fig. 32*a*). In the case of the larger nerve-cells of the spinal cord of the frog the polar processes, as far as they can be traced in the gray matter, show no evidence of the occurrence in them of any potassium compounds.

The case of the axons is much clearer. In preparations of the sympathetic in the guinea-pig and frog the non-medullated fibres may show a faint potassium reaction in their neurilemmas, but not in the axons themselves. Non-medullated fibres from Insecta and Crustacea are not less decisive on this point and are favourable objects for its demonstration. The fact is rendered clear also from preparations of medullated nerve fibres. In these latter the fat-holding sheath offers some resistance to the penetration of the reagent, for in the case of single isolated fibres from the sciatic of the frog somewhat less than half-a-minute is required for the passage of the reagent to every part of the axis cylinder, the paths which it takes being the trabeculæ of neurokeratin and through the nodes of Ranvier. The result, however, is the same even when the reagent obtains immediate access to the axon, as,

when on isolating the fresh fibres by teasing, the medulla happens to be torn away, leaving the axon uncovered. Also in transections of the fresh spinal cord of the frog in which there is ready penetration of the reagent to every part, the axis cylinder gives no reaction.

The medullary sheath, on the other hand, contains potassium, sometimes in abundance, but not usually uniformly distributed. It is found in the neurokeratin trabeculæ, but often more in those parts of the latter which are immediately adjacent to the axon, and in consequence the latter may appear in such preparations as a merely negative image, or, to state it differently, as a white shaft or strand enclosed in a coloured tube (Fig. 38 *c*). The two thus appear in contrast, the distinction between the innermost portion of the neurokeratin framework and the axon being of the sharpest degree. When the axon does appear coloured it is found due to the colour in the sheath through which it is seen.

In addition to the potassium occurring more or less diffusely in the neurokeratin framework, quantities of it are found in minute masses or in bizarre structures to be observed here and there through the sheath. These are sometimes immediately adjacent to the axon, sometimes immediately beneath the neurilemma (Fig. 38*b*). In the nerve fibres of the rat and in some of those of the frog one observes structures like those represented in Fig. 34 *b*, oval or circular rings, formed of granules constituted of potassium-holding substance and sometimes a central mass of the same material. These rings are, in the rat, regularly distributed along the fibre, which has a remarkable appearance in consequence (Fig. 39 *a*).

Frequently under the sheath and in contact with the axis-cylinder are minute potassium-holding masses, sometimes in groups but more often isolated and distributed at irregular intervals in the extent of the fibre between the nodes of Ranvier. The size of these varies very much, but they may be but minute granules. Sometimes also the largest of them may be seen to be composed of aggregations of minute granules. The variability in size and density is found also in the potassium-holding material observed at the nodes of Ranvier or in their neighbourhood. Here often there may be what appears to be a dense mass surrounding the fibre, the shape of the mass varying, but partaking sometimes of the appearance given the silver method employed to demonstrate the nodes. In nerve fibres from the frog the potassium reaction is sometimes a diffuse one in the neighbourhood of the node, where also the potassium-holding material may be a collection of fine granules situated between

the axis-cylinder and the sheath and extending for some distance on either side of the node (Fig. 37). It may even happen, though rarely, that the part of the fibre in the immediate neighbourhood of a node gives no evidence of the presence of potassium, but further down the fibre is a granular precipitate of it (Fig. 36 *a*)[1].

That even at the node the axon, though completely surrounded by, or immediately in contact with, potassium-holding material, is free from traces of the element may be clearly demonstrated by carefully pressing the cover-glass down on the preparation so as to bring into view the part of the axon covered or surrounded by the potassium-holding ring. Also in the course of teasing out the fibres, after they have been treated with the reagent, it happens now and then that the sheath is torn away at the node, where, in consequence, the potassium-holding material is displaced sufficiently to expose the underlying reactionless axon.

The marked and constant potassium reaction at the nodes indicates that there is at these points an unusual distribution of inorganic compounds. Potassium salts, as already pointed out, are not confined to the nodes, being at other points distributed in the sheath and between it and the axon, but it is characteristic of the nodes to display an abundance of material which reacts orange-yellow with the hexanitrite reagent. The potassium present is, in large part, perhaps united with chlorine, for I believe that the silver reaction, as employed by Ranvier and others to demonstrate the nodes, is due to the formation of chloride of silver which undergoes its characteristic change under the action of light. The view usually accepted is that there is some organic material in the nodes which also fixes the silver nitrate in itself and which reduces the latter in sunlight. This cannot be readily accepted for the reaction is, as I observed it in the frog, often of a minute granular character, and in some cases also the node itself gave no reaction while one was obtained on either side of it for some distance along the axon. The confinement of the reaction to the nodes and their immediate neighbourhood is not evidence that chlorides are not present elsewhere in the fibres, for the reagent does not penetrate them very readily, except at the nodes and, of course, here the chloride precipitate, once formed, would be a barrier to further penetration. If, however, the reagent contains free nitric acid its penetrating power is increased somewhat and then one may sometimes see, both in the sheath and between it and the axon in some fibres, minute granules distributed

[1] So far as my observations go, nerve preparations from summer frogs do not contain as much potassium as those from the winter frog show.

more or less uniformly between the nodes. These are probably due to the presence of chlorides and especially of chloride of potassium.

Potassium compounds are also found in the medullary clefts or imbrications of Lanterman and in Frommann's rings. The former may in some fibres be brought into view very distinctly by the potassium reaction, while in others the external portion only of the imbrication may be revealed (Fig. 40), and then one may see that there is no break in the continuity of the external border of the sheath.

The material constituting the membranes of the imbrications is continuous with the neurokeratin of the adjacent portions of the medulla and is formed of that substance. This explains the occurrence of potassium therein, for, as it is inert material, it undergoes impregnation with compounds of the element.

The rings of Frommann were only occasionally seen, and when observed they were usually found remote from, or, at least not adjacent to, the nodes of Ranvier. The parts of the rings revealed by the reaction were very thin bands around the axon and disposed at regular intervals along the fibre. They appeared to be independent of the axon and to be formed of neurokeratin. Wherever they occurred there were no other precipitates or collection of potassium-holding material between the sheath and the axon, but the neurokeratin of the former was impregnated with compounds of the element and these were also present in the nodes and their immediate vicinity.

In striated muscle fibre there is a remarkable disposition of the potassium compounds. This is most distinctly shown in preparations from Insecta and Crustacea, particularly on account of the ease with which the muscle fibrils in these may be isolated for the purpose of bringing the reagent into intimate contact with them, and because also of the large size in them of the constituent parts of the fibril. The value of these and other Invertebrate preparations also consists in the fact that they do not, as pointed out above, contain creatin, which is a constituent of Vertebrate muscle and which reacts with the hexanitrite reagent to a considerable degree like potassium. In the wing muscles of beetles and the claw muscles of the crayfish the very clearest results were obtained, the dim band alone giving the reaction, the whole of the light band, including the doubly refractive material of Dobie's line, maintaining, even after long application of the reagent, its pristine clearness. In the wing muscles of the scavenger beetle, in the resting or relaxed condition, the reaction as a rule appears most marked in zones near, but not immediately adjacent to, either end of the disc, the two

zones in each disc being separated by one in which the reaction is often much less distinct. At the extreme ends of the disc the reaction is less intense and there is, sometimes, no very sharp line of demarcation between the light and the dim bands (Fig. 30 *a*).

In the contracted fibril there is a slight redistribution of the potassium, for the deepest effect is given in the centre of the dim band, which however may not be detected owing to the marked reaction of the dim band as a whole. This reaction gives also a sharp border to the light zones which, as in the uncontracted or resting fibril, are unaffected (Fig. 30 *b*).

That these reactions in the dim bands are not due simply to impregnation of the doubly-refractive material with cobalt salt, rather than to the triple salt reaction, may be made evident in carefully washed preparations, but the clearest indications regarding this are furnished by muscle preparations from certain Vertebrates. In these the fibres are too thick to permit of rapid penetration of the reagent, and they cannot be resolved into fibrils without altering their chemical character and thus redistributing their inorganic compounds to some extent. When applied directly to the fibres the reagent consequently affects only their more superficial parts, that is, those immediately underneath the sarcolemma. Here the reaction in normal preparations is found to be a uniform one throughout the substance of the dim band in the higher Vertebrates (*e.g.*, the guinea-pig), but in the frog it consists of a more or less granular precipitate, the granules being of irregular size and some also of semi-crystalline form (Figs. 29, *a*—*c*). These may not be due to potassium alone, for creatin, instead of the latter, may enter into the composition of some of them, in which case they would be indistinguishable from those of the potassium-holding kind; but the important point is that if there are kinds, so varying in their composition, they are confined to the dim bands, yet do not occupy the whole area or volume of the latter, whereas if the results were due to simple impregnation it would not be localized, as in this case, to portions only of these bands.

There are other facts which indicate that the reaction obtained is not due to impregnation. Solutions of cobalt acetate applied directly to muscle fibre either from the guinea-pig or the frog will not give results like that described above, but will rather tend to impregnate both light and dim bands alike, and when, with great care, its distribution is limited to the dim bands, it is not confined to portions of the latter, as is the case with the hexanitrite compounds in frog's muscle fibre. Further, when the penetration of the fibre is tardy or when the preparation, previously to treatment, is allowed to remain untouched some time before

bringing the reagent to act on it, the disposition of the granular precipitate may be quite different from that obtained in normal preparations. I have in such preparations found the granules, sometimes in the light bands alone and sometimes along the line of separation between two adjacent light and dim bands. In other cases again the reaction is uniform throughout the whole sarcoplasmic "network," and granules are not shown.

The explanation, which, with some reserve, I advance for these abnormal results is that in the preparations illustrating them there had occurred in the fibres, before they were subjected to the action of the reagent, a redistribution of the potassium compounds, and possibly also of the creatin, and, as a consequence, different structural elements were rendered visible by the reaction. This may also be to a certain extent the true explanation of the conflicting results of observers regarding the finer structure of muscle fibre, for the parts in question are exceedingly minute and thus, even in normal cases, make the interpretation of what is observed very difficult, but the difficulty would be greatly increased if there were a shifting of the soluble constituents of the fibres from one minute point to another, thereby making the results of the treatment with simple staining reagents more or less different from what they are in the case of normal or unaltered tissue.

I have not once succeeded in obtaining a satisfactory demonstration of the distribution of potassium in the cardiac muscle fibre of the frog, but in that of mammals, and specially of the guinea-pig, such a demonstration has been often obtained. The potassium found is confined to, and uniformly distributed throughout, the dim bands, which, through the cobaltous sulphide reaction, appear in as quite a marked contrast to the light bands as in the case of the fibres of the scavenger beetle. There is no evidence of any concentration of the potassium salt in the neighbourhood of Hensen's line, but this may have due to the absence of contracted cardiac fibres from my prepara I have not observed in such cardiac muscle fibre any tendency for the reaction to develop a granular precipitate confined to portions of the dim band, instead of the uniform distribution described.

General remarks.

Although a large number of observations, on a wide range of forms, animal and vegetable, regarding the distribution of potassium, have been made, it is not yet possible to formulate with certainty conclusions as to

the rôle or rôles played by the element in living matter. This can only be done when the range of forms examined for this purpose is much more extensive than I have employed to this end, and when the cells of all types, and showing all species of activity, have been carefully studied with the aid of this method.

It is, nevertheless, possible now to discuss certain general features which the results already obtained make manifest, and it is also of advantage to do so, in order to indicate the lines which further investigation must take.

There is, first of all, no difficulty regarding the potassium salts of impregnation, and especially in those cases where the material affected is inert. This is a purely physical phenomenon in which vital processes play no part, except when the inert material is situated in, and surrounded by, cytoplasm. Then the potassium present represents a portion of that excreted or disposed of by the active living matter.

There is, further, little difficulty regarding the potassium of precipitation. Its abundance in the cells of vegetable forms, whose media are rich in salts of the element, or in whose transpiration currents they are present in considerable quantities, makes it apparent, as previously indicated, that this is a method which living matter has of disposing of the quantity of potassium in excess of what it requires for its functions. It is, as already pointed out, very difficult to distinguish in some cases between a precipitate and a condensation, that is, a quantity of potassium salt concentrated at a point in the cytoplasm to assist the metabolic functions of living matter. There are, however, enough facts regarding the latter condition alone which enable us, in discussing the rôle of potassium, to eliminate the processes due to precipitation.

It may also be regarded as certain that potassium does not subserve either the generation or conduction of nerve impulses. If potassium is so employed it must be in amounts infinitesimal, or so minute, that they are beyond the limit of detection by a reagent and a method sensitive enough to precipitate 99 per cent. at least of the potassium in solution. Such infinitesimal amounts of potassium would, in ordinary quantitative chemical analysis, be either unweighable, or, if within the limits of the ponderable, be ascribed to impurities derived from the adventitious tissue elements quite, or practically, inseparable from nerve cells and axons. To postulate such an attenuated dilution of the potassium ions as serving, for example, for the conduction of nerve impulses along an axon, is to make it open to question whether we can derive much

assistance from the electrolytic theory in trying to understand the conductivity of nerves.

It is possible that such conductivity depends on the presence of a sodium compound, but that compound cannot be the chloride, for, in preparations appropriately made with nitrate of silver, used as a microchemical reagent, chlorides cannot be demonstrated in either the nerve cell or its axons, although it may occur at the nodes of Ranvier and at other points in the fibre exterior to the axon. The sodium cannot occur as a phosphate, for by the nitric-molybdate method the nerve cell and its axons are shown to be free from inorganic phosphorus-holding compounds. Are we then to suppose that sodium occurs as a sulphate, or a carbonate, or both, the anions as well as the kations of which are not demonstrable by any microchemical method at present at our disposal?

The evidence is also clear regarding the freedom of the nucleus from potassium. Here also, as in the case of nerve cells and their axons, one could perhaps postulate the presence of potassium in such minute quantities as would defy detection; but it may, very properly, be held that with a very delicate reaction, giving negative results, it is not of much service to resort to refined explanations on the point. Further, the negative result is corroborated by the absence of chlorides and inorganic phosphates from the nucleus, as demonstrated by the silver nitrate and the nitric-molybdate methods. Is it possible that, potassium compounds and all phosphates and chlorides being absent, the sulphates and carbonates of sodium alone obtain in the nucleus? The negative answer to this question would practically involve the conception of the nucleus as a structure in which only organic compounds are present. Potassium being absent, does there occur in it such a compound as sodium nucleate? Assuming the absence of all inorganic salts[1] the latter compound would constitute the only electrolyte which the nucleus might contain.

[1] So far as the microchemical reactions for calcium are serviceable they do not show its occurrence in the nucleus. These reactions are of course not nearly as sensitive as those for potassium or iron are, and consequently one cannot regard them as establishing the absence of calcium salts from the nucleus, but the sulphate and carbonate are insoluble, the phosphate largely so, while chlorides and inorganic phosphates do not occur in the nucleus. What salt of calcium could then obtain in the nucleus except a nucleate or further, one in which the occurrence of the calcium is masked? Loew holds also that the magnesium serves for the assimilation of phosphates or as a carrier of the latter to points where such assimilation occurs. This would exclude magnesium from the nucleus except in infinitesimal quantities.

With the freedom of the nucleus from potassium salts and, inferentially also, from other inorganic compounds, it would follow on morphological grounds that the head of the spermatozoon should be lacking in such compounds, and the facts so far obtained indicate clearly that it does not contain potassium. If fertilization of the ovum, as has been suggested, is due to transference of inorganic salts to the female cell, those of potassium cannot be amongst them, unless such salts are taken up by the head just previously to its penetration of the ovum.

The facts regarding localization of potassium in the cytoplasm demonstrate the power which living matter has of shifting to this or that point, or even excluding from itself altogether, salts of the element. Most instructive on this point are preparations of the corneal and vesical epithelium of the frog, in the cytoplasm of which there may not be a trace of potassium while the intercellular substance and spaces may be saturated with the element. There is thus in living protoplasm a directing force controlling the distribution of potassium salts, a force apparently opposed to, or, to express it with a due degree of caution, more refined than, the ordinary physical force comprehended by the term osmosis. This force, or, perhaps, group of processes, must be exercised in a more marked degree in the case of the nucleus to preserve it free from potassium salts.

There remain the metabolic processes in which the presence of potassium may be a necessary factor, and here the data are not sufficiently numerous to permit the formulation of exact conclusions[1]. The facts so far ascertained point definitely to a participation in some way of potassium salts in the processes of assimilation and perhaps also in those of dissimilation. In *Zygnema*, and particularly in *Spirogyra* the close association of the potassium salts with the neighbourhood of the chromatophor suggests that compounds of potassium enter into the synthetic processes concerned in the formation of carbohydrates. Even in the higher vegetable forms potassium salts accompany chlorophyll, although the association is not as striking as it is in *Spirogyra*.

In the growth and formation of vegetable cell walls potassium seems to play a part. In the spores of *Equisetum arvense* (Fig. 16) the cell which gives rise to the primary root hair is, from the first, very rich in potassium salts which, as the root hair begins to develop, collect at that point where the hair is to arise, and while the growth continues the

[1] Loew (*Arch. d. ges. Physiol.*, xxii. p. 510, 1880; also *Die chemische Energie der lebenden Zellen*, 1899, pp. 32, 33), holds that potassium salts exercise several functions, but that one of these concerns the condensation processes of organic synthesis.

inner surface of the hair membrane remains in association with the salts. This appears to obtain also in the formation of the pollen tubes in *Lilium*, the potassium observed in the active cell of the grain appearing, when the tube develops, to accompany its extension downwards through the tissues of the capitate stigma. As the tube becomes very long the quantity of potassium that may be at any one point within its wall is very minute, or in traces only, but when the tube first begins to grow the amount at the point of growth is sufficient to make a clear demonstration of the fact.

In several preparations of *Spirogyra* which illustrated the process of conjugation there was found, within the tips of the outgrowing processes, and almost immediately adjacent to their terminal walls, a marked potassium reaction, quite distinct from, and much more marked than, that of the adjacent cytoplasm. When the terminal walls of two processes were in contact the reaction was most decided, but when the double wall was absorbed no potassium was found at the point. Whether the potassium salts are associated with the outgrowth of the processes alone, or with this and the absorption of the walls in contact, cannot be determined, but it is at least possible that there is here a function of potassium salts like that illustrated in the formation of the primary root hairs of *Equisetum arvense*.

The exceptional distribution and abundance of potassium in striated muscle fibre must indicate a special function. That contractility is not necessarily due to this function follows from the fact that smooth muscle fibre contains but little potassium, which is uniformly diffused through its cytoplasm. This function may be concerned in the generation of contractions of very short duration but not in the production of tetanus, for the same distribution, as pointed out, is found in cardiac muscle fibre. It is, however, well to remember that, if the distribution of potassium in striated fibre is exceptional, the structure and composition of the latter are equally so. In the dim bands where the potassium obtains are found glycogen, myohæmoglobin, a nucleo-proteid and apparently also, although this is not certain, the myosinogens. Such compounds do not occur in close association in any other tissue element, but their occurrence together in those parts of the muscle fibre, the dim bands, which are believed, if not universally recognized, to be the active elements in bringing about contraction, gives them an importance which is enhanced by the fact that potassium is associated with them. It is to be noted also that in the ash of striated muscle potassium greatly exceeds in proportion every other basic element present, even sodium, an excess

that is not found in the case of any other tissue. This points to its having a special rôle in striated muscle fibre, but what that is, further than that it is probably metabolic, it is impossible to say.

The distribution of potassium salts in the secreting cells of the pancreas of the rabbit and guinea-pig suggests the idea that such compounds take part in some way in the act of secretion. They are not at all found, even in traces, in the "protoplasmic" zone of the cell and they are most abundant in that part immediately adjacent to the lumen of the tubule. It is not possible to say whether in the living cell they are in the granules, or in the cytoplasm surrounding these, or in both, for the granules quickly dissolve in the reagent and are not seen in the preparations of the gland made with it. The condensation of potassium shewn in the cell border of the lumen (Fig. 33), as well as its presence in the walls between the cells, would seem to indicate that it occurs also during the life of the cell in the cytoplasm of the granular zone, in which case it would participate in the elaboration of the secretion.

The avidity of some moulds, fungi and bacteria, for potassium salts postulates some metabolic rôle for the latter in these forms. This does not imply, of course, that large amounts of potassium salts are absolutely necessary to the life of these forms, for in the case of *Saccharomyces Ludwigii* some of the apparently most active cells may show only traces of them, and young budding cells rarely give a reaction for potassium (Fig. 5). But there is one form in which this avidity obtains in a remarkable degree. It is parasitic on *Spirogyra*, into whose cells and their cytoplasm it sends mycelial threads (haustoria) which absorb from the more or less plasmolysed protoplasm all the potassium salts which it contains (Fig. 14), and a part at least of the potassium so taken up finds its way, or is removed, to the spore-like body from which the haustorial threads are derived (Fig. 13). After a full development of the haustorial filaments is reached all the potassium compounds are in the threads whose course throughout the disorganized cytoplasmic mass can be clearly followed through the distinct reaction which they give. Wherever these parasitic forms prevail they illustrate this fact, and the constant presence of potassium in them suggests that there is in these organisms a degree of kaliophilism parallel to the sideropilism of *Leptothrix ochracea*, or the thiophilism of the *Beggiatoæ*.

Summary.

The data and the discussion of the foregoing pages may be summarized as follows:

1. The hexanitrite of cobalt and sodium, $CoNa_3(NO_2)_6$, in solution and in the presence of acetate of sodium, is an immediate precipitant for potassium from its solutions, the precipitate being an orange-yellow triple salt, which is, according to K. Gilbert's investigations, the hexanitrite of cobalt, sodium and potassium, $Co\begin{pmatrix} K_x \\ Na_y \end{pmatrix}_2(NO_2)_6$.

2. When the reagent is specially prepared it precipitates, as the triple salt, instantaneously and completely, all the potassium from its solutions. For this purpose it is made by dissolving 20 grams of cobalt nitrite and 35 grams of sodium nitrite in 75 c.c. of water, adding thereto 10 c.c. of glacial acetic acid and diluting the solution with water to 100 c.c.

3. When isolated cellular elements, or fresh tissues, very minutely teased out, are put in quantities of this reagent, the latter penetrates to all parts of these elements in a few seconds, and, in consequence, in that time precipitates all their potassium at all points where its compounds occur. If these are abundant, as, for example, in some vegetable tissues, the precipitate is crystalline, but if they occur only in minute quantities, as is the case in all cytoplasmic structures, the precipitate is of such a character that its constituent particles are not individually visible.

4. In order to determine the distribution of the precipitate in a preparation, the latter must be washed thoroughly with ice-cold water to remove all traces of the precipitant, after which the triple salt formed and left behind will, if it is abundant, reveal itself under the microscope by its light orange-yellow colour. To reveal the presence and distribution of the more minute quantities the preparation may be treated with ammonium sulphide solution, which gives the black cobalt sulphide reaction wherever the triple salt is precipitated, and thus the black reaction at any point is evidence of the presence there of potassium. Such preparations mounted in 50 per cent. glycerine will keep for about two months.

5. As revealed by the method potassium occurs in both the cytoplasm and the extracellular structures. In the latter it is present as a product of impregnation and infiltration and, as a consequence, there are few such structures that are free from it. In intercellular material and in inert or dead matter it is usually very abundant.

6. The cell nucleus does not normally contain the slightest trace of potassium nor does the head of the spermatozoon give the slightest evidence of its presence there, and, further, in the structure known as the central body of the Cyanophyceæ, which is regarded by many cytologists as a nucleus, or as a bod, resembling a nucleus, no potassium reaction can be obtained.

7. Nerve cells are wholly free from potassium and this freedom extends to the dendrites and axons. External to the axons in medullated nerves potassium compounds obtain chiefly at the nodes of Ranvier and in the neurokeratin framework of the sheath, but at other points also often in minute masses of bizarre form.

8. The potassium obtaining in cytoplasm occurs in two conditions, that of physiological precipitation, and that of physiological or biochemical condensation.

9. The precipitation is not of a physical character but may perhaps be of the nature of fixation, in an inert form, of the potassium in passive colloidal material in the cytoplasm. This precipitation is the process, apparently, by which living, active cells dispose of the excess of potassium salts which may invade them, as in the case of vegetable forms, in very great excess.

10. In the condition of physiological or biochemical condensation potassium salts in solution are concentrated in some particular part or parts of the cytoplasm and excluded from the remainder. This condition of condensation is, undoubtedly, in some cases at least, a factor in the metabolic processes peculiar to the cells, or species of cells, illustrating the condition. That salts can be in solution and at the same time confined strictly to parts of the cell is shown in *Spirogyra*, in which, in the healthy normal form, the potassium is strictly localized to the immediate neighbourhood of the chromatophor, which, in the intact cell, is supposed to function, in some degree, in the synthesis of the carbohydrates.

11. In smooth muscle fibre the potassium found is scant and it is diffused throughout its cytoplasm, but in striated fibre there is a condensation of the potassium in the dim bands, the rest of the fibre being free from the element. When the fibre is in the contracted condition the potassium is most abundant in the middle third of the band, at least such is the case in the wing muscles of the scavenger beetle. It is the doubly-refractive substance of the dim bands that, apparently, constitutes the contractile material, that is, the inogen of Hermann, and its association with potassium suggests some relation of

the latter, not with contraction, for smooth muscle fibre shows that property, nor with tetanus, for in cardiac muscle the potassium is disposed as in ordinary striated fibre, but with rapidity of contraction, which distinguishes striated fibre from smooth.

12. There is in the secreting cells of the pancreas of the guinea-pig and rabbit a remarkable concentration of potassium compounds in that portion of the granular zone immediately adjacent to the lumen, while the remainder of the cytoplasm is free from them.

13. There are organisms which manifest a distinct capacity to absorb potassium and amongst them is one, parasitic on *Spirogyra*, whose mycelial threads exhibit kaliophilism in a special degree.

PLATES I AND II.

Note.—The orange-yellow in the Figures represents the distributing of the triple salt, the black the cobaltous sulphide reaction of the same.

Fig. 1. Protococcoid forms growing on sandstone. In *a* the potassium occurs in a large granule in each cell, but in *b* each cell contains several potassium-holding granules, and in one is an irregular mass in which there is shown a diffused trace of potassium. × 1000.

Fig. 2. *Tolypothrix sp.*, showing potassium present in the granules and in the peripheral zone, the "central body" being free from it. *c*, one of the cells seen on its flat or end surface. × 1360.

Fig. 3. *Tolypothrix sp.* (different from preceding). The lowest cell seen in optical section, the others represented as in superficial view. × 1360.

Fig. 4. *Bacillus subtilis.* × 1360.

Fig. 5. Forms in a culture of *Saccharomyces Ludwigii* in the sap of *Ostrya virginica.* × 1360.

Fig. 6. Mycelial threads from the stem of *Agaricus sp.* × 680.

Figs. 7 and 8. Two different species of *Spirogyra*, prepared from normal and actively growing cultures. × 500.

Fig. 9. *Spirogyra sp.* Preparation from culture kept for several days in the laboratory and more or less in an abnormal condition (*i.e.*, of partial plasmolysis). The potassium now occurs only at points on the chromatophor bands. × 500.

Fig. 10. A fragment of a chromatophor in the same preparation from which Fig. 9 was drawn. In this potassium is found localized in the neighbourhood of some of the pyrenoids. × 1000.

Fig. 11. *Spirogyra sp.* (as in Fig. 8) in a partially plasmolysed condition. The chromatophor bands are displaced and the potassium is found only at points on and in their course, sometimes in the immediate neighbourhood of pyrenoid bodies.

Fig. 12. *Spirogyra sp.*, Zygospore. The view represented shows the distribution of potassium to be almost wholly under the membrane, yet in the cytoplasm. × 500.

Fig. 13. Cell outline of *Spirogyra sp.*, showing the occurrence of a parasite (?Chytridiaceous) rich in potassium. × 500.

Fig. 14. Intracellular filament of same parasite showing its kaliophilous character. × 500.

Fig. 15. *Epithemioid* diatom. The potassium besides being irregularly distributed is found also in parallel arrangement under the test. × 680.

Fig. 16. Germinating spores of *Equisetum arvense*, *a*, earlier, *b* later stage. In both the potassium seems to diffuse in advance of the cytoplasm which forms the primary root-hair. × 250.

Fig. 17. *a*, *c* and *d* cells from the mesophyllous layer of the Easter lily, the nucleus with a faint pink reaction. *b*, collection of chlorophyll corpuscles from a cell which was subjected to the action of the reagent for so short a period as three minutes only, in order that they should be preserved intact for observation. × 680.

Fig. 18. Surface view of leaf of *Tulipa sp.* *a*, cells on a level with the stomatic cells but below the cuticular elements. × 1000.

Fig. 19. Mesophyllum of *Lilium Harrisii*, showing the potassium in connection, not only with the scanty protoplasm, but also with the cell walls. × 600.

Fig. 20. Mesophyllum of *L. Harrisii*, cell showing starch granules but a smaller quantity of potassium. × 600.

Fig. 21. Pollen grain of Tulip. × 680.

Figs. 22 and 23. Corpuscles of frog's blood. Fig. 22 white (? fusiform) corpuscle, Fig. 23 red corpuscles. In all the nuclei are free from potassium. × 1000.

Fig. 24. Bladder epithelium of frog, optical section of the cells. *b*, a cell, of a type occasionally found, in which the cytoplasm was rich in potassium. × 680.

Fig. 25. Cells from intestinal mucosa, frog. The potassium is shown on the periphery of each cell, not in the intercellular spaces, which appear free from it. × 680.

Fig. 26. Cells from the intestinal mucosa of *Ouiscus*. *n*, nucleus. The view represented is in a plane through the nuclei of the cells. × 340.

Fig. 27. Groups of cells from xiphioid cartilage of the frog. The matrix in this preparation was free from potassium salts. The distribution of the latter is indicated by the orange-yellow triple salt reaction. × 250.

Fig. 28. Smooth muscle fibres, frog's bladder. A faint reaction obtains in their cytoplasm. *a*, a superficial view of a portion of a fibre. × 680.

Fig. 29. *a*—*c*. Portions of muscle fibres, gastrocnemius, frog. In *a* the nucleus is free from potassium. *a* and *b*, × 1400, *c*, × 1830.

Fig. 30. Muscle fibrils. Wing muscles of scavenger beetle. *a*, resting, *b*, contracted. × 1380.

Fig. 31. Retina of frog. *a*, cone, free from potassium. *b* and *c*, lateral views of rods, *d*, end views of the rods, the substance of which gives a faint diffuse reaction for potassium. The potassium-holding particles which occur in *b* and *c* are shown in *d* to be between the rods. × 680.

Fig. 32. Retina of frog. *a*, element of the nuclear layer with peculiar potassium-holding arborescences on its surface. *b*, superficial view of rod with potassium in minute elongated granules, regularly disposed. *c*, optical section of a rod showing the occurrence of potassium at definite points and along definite lines. × 680.

Fig. 33 *a* and *b*. Acini of pancreas, guinea-pig, showing potassium limited to the neighbourhood of the lumina, and to a portion of the intercellular walls. × 1380.

Fig. 34. Nerve fibres, rat; *a*, showing the orange-yellow triple salt reaction at the node of Ranvier and also in a mass in the sheath adjacent to the fibre (axon) at an intermediate point. *b*, a portion of a nerve fibre showing the triple salt reaction in a not unusual distribution in the medulla. × 680.

Fig. 35. Nerve fibres, frog. *a* and *b* showing potassium-holding material at a node of Ranvier (*r*) as well as in the sheath immediately under the neurilemma, the free portion of the axon (*n*) showing absolutely no reaction.

Fig. 36. Nerve fibres, frog. In *a* the potassium is on the periphery of the axon (as at *r* and *i*) and in small masses in the medulla. The large aggregation of potassium-holding material at *i* is at a point remote from a node of Ranvier. × 680.

Fig. 37. Nerve fibre, frog. The potassium-holding material distributed along the surface of the axon for some distance on each side of the node of Ranvier and also in the neurokeratin framework of the sheath. × 680.

Fig. 38. *a—c*. Nerve fibres, frog, giving surface view showing the neurokeratin framework of sheath infiltrated with potassium salts as irregular potassium-holding patches and aggregations of material situated in the sheath. × 680.

Fig. 39. *a—b*. Nerve fibres, rat. In *b* the material of Frommann's rings (outside or on the periphery of the axon) is rich in potassium. × 680.

Fig. 40. Nerve fibre, guinea-pig, showing the peripheral portions of the imbrications of Lantermann to be impregnated with potassium. 680.