Acineta tuberosa: *A Study on the Action of Surface Tension in Determining the Distribution of Salts in Living Matter.*

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I. *Introduction: The General Effects of the Action of Surface Tension.*

The distribution of salts in living matter is supposed, in the current conception of the subject, to be on the whole the same as in ordinary fluids. Living matter is generally regarded as a semi-fluid, semi-viscid material in which the conditions, though not fully typical of those which obtain in a fluid like water, are, nevertheless, such as to allow the substances that are dissolved in it to be distributed uniformly throughout it. The only obstacle to this distribution may be presented by a membrane such, for example, as that which encloses or surrounds the cell nucleus. Elsewhere throughout the cytoplasm there is, it is believed, a free play of the force that determines the diffusion of the substance or substances dissolved, until uniformity in their dispersion obtains throughout the volume occupied by the cytoplasm.

This force is that postulated in the van 't Hoff theory of solutions extended to include the Arrhenius theory of dissociation. In this composite theory, as is well known, the material dissolved in a fluid is supposed to be in a state analogous to that of a gas, that is, in as rarefied a condition as if its molecules were isolated from each other and occupying alone the volume filled by the solution itself. The molecules of the solute and their ions, when they are dissociated, are thus supposed to be in translational motion, and the resulting pressure—the osmotic pressure—which they give, acts on the surfaces enclosing the fluid as the molecules of a typical gas act on the walls confining it. At every point in the system there would be, on this

view, the same pressure and, in consequence, the number of molecules per given volume of the solution in any portion of it would be uniform.

On this conception of the force determining the distribution of salts in fluids there were based a number of views which have played a part in explaining physiological processes. Of these the most important is that which postulated that all diffusion, whether in the cytoplasm of a cell or through a living membrane, is due to osmotic pressure acting as a driving force, the ultimate result of whose action would be to equalise the pressure throughout the cytoplasm or on both sides of the membrane. This reduced the processes of secretion and excretion, as well as the diffusion into and from cells, to the operation of gas laws.

This explanation of the force and the conditions that make for the diffusion of solutes, not only in physical solutions but also in living matter, has obtained and still obtains a wide acceptance. The very simplicity of it, the support it derived from a considerable range of experimental evidence, and the unifying effect it appeared to exercise in a large number of phenomena manifested by solutions and gases, told very strongly in its favour, and eventually revolutionised the aspect from which all the problems of osmosis and diffusion were viewed.

Criticism, however, was not silenced. It was seen that there were phenomena which not only could not be explained in that way but also were irreconcilable with the explanation. These were physical and physiological. Of the physiological only is there concern here. In one of these, that observed in renal excretion, the concentration of the urine is much greater, ordinarily, than that of the blood plasma from which it is derived through the activity of the kidney tubules. In other words, the osmotic pressure of the product of renal action is greater than that of the blood. This cannot be explained by the van 't Hoff-Arrhenius theory, the only conceivable result of which would, in such a case, be approximately an equality of pressure or that the urine formed would not exceed in concentration, and, therefore, in osmotic pressure, the blood plasma itself.

The failure of the van 't Hoff-Arrhenius theory to explain this and other physiological results of a like character does not put the theory out of court in explaining many physical phenomena. It still may be regarded as of value in accounting for these, though even in this respect it may be looked upon as beset with limitations. In the physiological sphere its application is of much less service and, were it here the last word in the way of an explanation, the causation of a few physiological phenomena would ever remain an insoluble problem.

In recent years the aid of other factors in explanation of certain physio-

logical phenomena has been sought for, and, as a result, attention has been specially directed to the principle of surface tension. The participation of this force, although considered as a factor in the causation of amœboid and contractile movement ever since 1869, was not suggested as influencing the distribution of salts in living matter till 1910, when the author advanced the view that surface tension plays an all-important *rôle* in determining the localisation of salts and other solutes in cells and tissues, and in controlling the diffusion through living membranes that brings about the formation of excretions and secretions.

It was the observations derived from the microchemical study of the distribution of potassium salts in living cells that led to this view. The compounds of this element are amongst the most soluble of all known salts. Only two of its salts are under certain conditions insoluble in water, and these are the triple salt, the hexanitrite of cobalt, sodium and potassium, and the double salt, the potassium platinum chloride. Neither of these is found in the natural world, and therefore the salts of potassium found in living tissue, when aggregated in masses or layers in a cell, cannot be so localised as a precipitate. Some other explanation for this localisation had to be sought for, and the author, after full consideration of all the facts involved, claimed that the localisation observed is a condensation due to the influence of surface tension.

How such a condensation may develop through surface tension may be recognised on an examination of the results of the action of the Gibbs–Thomson principle of surface concentration. This law or principle may be stated in a few words. It is to the effect that when a substance in solution increases the surface tension of a fluid system (*e.g.*, a drop of water) it is less concentrated in the surface layer than in the rest of the system, while a substance that lowers the surface tension of the system is more concentrated in the surface film than it is in the rest of the system. It has also been found by Lewis and others that solutes which raise the surface tension at a water–air interface as well as those which lower it, also lower it at a liquid–liquid interface and at a liquid–solid interface and undergo condensation there, as a result, it is understood, of the operation of the Gibbs-Thomson principle. At such interfaces the degree of condensation depends on the extent of the diminution of surface tension as well as on the concentration of the solute throughout the fluid system, but, assuming the application of the gas laws to dilute solutions, the concentration as deduced from Gibbs' formula for this value would be

$$S = -\frac{C}{iRT} \cdot \frac{d\sigma}{dC},$$

where S is the surface excess per unit of surface area of the part affected, C the concentration of the solute throughout the fluid, σ the surface tension value, i R the gas constant, and T the absolute temperature.*

The value of S as experimentally ascertained was in a great many instances very small. Forch (3) found that in a normal solution of sodium chloride, which raises the surface tension of water, the deficit in the surface film was 0·024 mgrm. per square metre. Whatmough (16) with a normal solution of acetic acid, which lowers the surface tension of water, determined the surface excess to be 0·2 mgrm. per square metre, and this concentration increases by less than 15 per cent. even when the concentration of the acid throughout the system was increased eight-fold. Milner (13) estimated that in a sodium oleate solution of 0·00204 gramme-molecular strength the surface concentration of the sodium oleate was 0·4 mgrm. per square metre over that of the solution generally, but from the data furnished by Reinold and Rücker (15) regarding the conductivity of films made from a solution of 1 part of sodium oleate in 60 parts of water, Milner estimated the surface excess therein to be 2·4 mgrm. per square metre. The results of Benson (1) obtained with aqueous solutions of amyl alcohol of 0·0375 molar value gave a considerably higher value, the surface excess of amyl alcohol reaching a concentration of 0·0394 molar value, involving an increase of about 5 per cent.

Were these the only values to come into consideration, surface concentration, as a result of the action of surface tension, would be negligible, except for the solution of certain problems of very limited interest. There are, however, other experimentally determined values which make it plain that surface concentration is, under certain conditions, a very great factor in influencing the distribution of salts in solutions.

These values were recently determined by W. C. M. Lewis† (6, 7, 8) who, to ascertain them, employed ingeniously devised methods. The surfaces on which the condensations were studied were those of aqueous solutions in contact with hydrocarbon oil or with mercury. The oil or mercury was in the form of droplets or spherules of uniform size, the surface area of each of which was calculated from data derived from the total quantity of oil or mercury used and the total number of droplets or spherules formed. The hydrocarbon oil and the mercury were employed because they do not absorb or dissolve in themselves a trace of the solute from the solutions bathing the surface of the droplets or spherules.

* For the development of this formula from the original values of Gibbs see S. R. Milner, *op. cit.*

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The hydrocarbon oil was employed in two ways: as an emulsion with the aqueous solution or as droplets, which, being lighter than the solution in which they were liberated, were allowed to ascend through a long vertical column of the solution. In the first instance a definite quantity of the oil was mixed with a known quantity of the solution and the mixture agitated for some hours in a shaking apparatus. The droplets of oil in the resulting emulsion were of approximately like diameter, which was in a large number of cases measured under the microscope. The average volume of each was determined, and from this and the total quantity of oil used the united surface areas of all the droplets present in the emulsion were calculated. The quantity of the solute in the emulsion, apart from that on the surfaces of the droplets, was ascertained by the application of the drop-pipette or stalagmometric method. As the concentration of the original solution was known, the difference between it and that ascertained stalagmometrically was the amount condensed on the united surface areas of all the droplets.

In the second method the hydrocarbon oil was allowed to ascend in droplets through a long column of the solution in a cylinder which terminated above in a broad cup-like receptacle. The apparatus was so arranged that the connection between cylinder and receptacle formed of rubber tubing could be cut off by a pressure clip. The oil droplets could freely ascend to the interior of the receptacle, but the flow of the contents of the latter back into the cylinder was reduced to a minimum. The quantity of oil used was known, and the total number of droplets which ascended through the column of liquid was determined from an average of counts made for several selected test periods. When the droplets had all ascended the connection with the cup-like vessel above was cut off by compression of the rubber tube below it, and the concentration of the fluid in the cylinder, whose volume was known, was determined. The difference between this concentration and that originally present, the total volume in the cylinder, and the united surface areas of all the droplets were the factors from which was deduced the concenttation of the solute on each cm.^{2} of surface area.

When mercury instead of hydrocarbon oil was used, the droplets, all uniform in size, fell through the fluid in a cylinder which ended below in a reservoir from which it could be cut off by the closure of a glass tap. As the volume of the mercury used was known and the number of droplets also ascertained through an average of the counts made for that purpose, the united areas of the surfaces of all the droplets were determined. The original concentration and volume of the solution being known and the final concentration ascertained, the amount of the solute condensed on each cm.^{2} of the surface area of the droplets was calculated.

Out of a large number of solutes used in these experiments only two, caffein and anilin, gave values which approximated the values postulated by the equation $S = -\frac{C}{iRT} \cdot \frac{d\sigma}{dC}$, while in the case of the others the experimentally ascertained values greatly exceeded the theoretic values. Thus, in a sodium glycocholate solution of 0·25-per-cent. concentration, the surface condensation on the droplets of hydrocarbon oil was 5×10^{-6} grm. per cm.^{2}, that is, the directly ascertained value was about 70 times the theoretic value. When mercury was used the ascertained value exceeded the theoretic about 25 times. In sodium oleate solutions the directly determined value was about 100 times the theoretic. With Congo red, methyl orange, and sodium hydrate the experimentally ascertained values were respectively 25, 43, and 20 times the values derived from the Gibbs equation. Even if errors in calculation were allowed for in every case, the excess of adsorbed solutes was still so great that Lewis suggested, as a possible explanation for the great discrepancy, that the adsorbed material is in a gelatinised or colloidal condition on the surfaces of the droplets of oil or mercury.

Lewis found also that in the case of inorganic salts the quantity adsorbed exceeded the theoretic amount, but it was chiefly the cation that was so affected. In silver nitrate the silver adsorbed was 5 times in excess; in cupric chloride the copper was 17; and in potassium chloride the potassium was more than 30 times the calculated amount. This excess of the cation is, in Lewis' opinion, probably due to electrical effects, since the oil used is negatively charged, and the potential difference between the oil and the water is approximately 0·05 volt.

The diameter of a water molecule is, according to Kundt and Warburg (5), $3{\cdot}39 \times 10^{-8}$, and, consequently, the diameter of its sphere of attraction at the oil–solution interface would be $6{\cdot}78 \times 10^{-8}$ cm., but Lewis, accepting the range as equivalent to that postulated by Parks (14), namely, $13{\cdot}4 \times 10^{-6}$ cm., and using also the value $5{\cdot}4 \times 10^{-6}$ grm. for the amount adsorbed per cm.^{2} from a 0·25-per-cent. sodium glycocholate solution as experimentally determined, calculated that the concentration of the bile salt in the superficial layer at the oil–solution interface was 40·3 per cent., or 160 times that obtaining in the rest of the solution. If, however, we assume that the range of molecular attraction is smaller than that postulated by Parks then the concentration of the bile salt at the interfacial surface must be extraordinarily high.

How such a concentration can obtain we do not know, but an explanation may be tentatively suggested. It is not certain that the solute adsorbed

is confined to a deposit within the range of attraction of the molecules on the interfacial surface. It is not improbable that there is no sharp break between the concentration at the interfacial surface and that in the solution generally—that, in effect, there is a shading off between the two. The amount adsorbed does, indeed, depend on the surface tension of the solution at the interfacial line, and the effects of this surface tension do not extend beyond the range of molecular attraction of the molecules on the interfacial line, but in the case of the solutes which are adsorbed in such extraordinary excess as to suggest the formation of a colloidal or gelatinous deposit, the latter must tend to produce successively superposed interfacial deposits until equilibrium is attained, in which case the deposit may extend with lessening concentration through a distance from the interfacial surface equivalent, it may be, to several, if not many, times the range of molecular attraction.

These facts make it evident that in solutions in which interfacial surfaces, numerous as in emulsions and consequently of very great areal value, exist, the concentration of the solutes may fall very considerably through condensation of the solutes on the interfacial surfaces. This must result in lowering the osmotic pressure. The dissolved molecules free in the solution are fewer, and their pressure is less than that of the molecules in the simple homogeneous solution unaffected by surface tension, except at its boundaries or limiting surfaces. The osmotic pressure of a solution of potassium chloride contained in a beaker is, therefore, different in value from that of the same solution and like concentration in which numerous interfacial areas obtain through the presence of very numerous foreign non-soluble systems.

The elemental unit of living matter, the cell, is constituted of unhomogeneous material in which the essential constituents are chiefly in colloidal condition, that is, the constituents are minute particles or dispersoids, separating which is a fluid containing in solution the salts and other compounds characteristic of living matter. Such dispersoids present interfacial surfaces of very great areal value, and if the surface tension of the fluid at the fluid–dispersoid interface is lowered by the solute or solutes there must be condensation of them on these interfacial surfaces. This would lower very considerably the concentration of the solutions in the fluid diffused through the living matter, and thus the osmotic pressure, otherwise due to the presence of such salts in living cells, would be nearly correspondingly reduced.

Surface tension, therefore, through the operation of the Gibbs-Thomson principle determines in a very considerable degree the distribution of salts

in the living cell. Further, between each cell of a tissue or organ and the lymph that bathes its surface there is an interface where the salts of the lymph may lower the surface tension, and in consequence undergo more or less of condensation there. This serves to lower the general concentration in the lymph, and the osmotic pressure is diminished accordingly.

That such surface and interfacial condensations of solutes in living tissues and organs can occur, and thus modify the distribution of their soluble constituents, though definitely indicated by the results of physical investigations, would largely remain of theoretical interest if there were not more direct evidence to this effect. Such direct evidence comes from investigation by microchemical methods of the distribution of salts in living cells. The microchemical localisation of salts in cells and tissues is as yet not advanced enough to enable us to determine the finer distribution in living structures of all its inorganic constituents, but along several lines it is complete enough to permit us to demonstrate a condensation of salts due to the action of the Gibbs-Thomson principle.

This localisation is most effective in the case of the potassium salts. Eight years ago the author found that the hexanitrite of cobalt and sodium represented by the formula $CoNa_3(NO_2)_6$, in an appropriately prepared solution, instantaneously precipitates potassium from its solutions as $Co(NO_2)_3,3(K/Na)NO_2+nH_2O$, in which the amount of the potassium ranges, according to K. Gilbert's (4) determinations, from 16·31 to 18·21 per cent. The sensitiveness of the reaction may be understood from the fact that, in a solution so dilute as 1 part of potassium in 275,000 parts of a solution formed chiefly of the reagent, the crystals of the triple salt, the hexanitrite of cobalt, sodium, and potassium, are formed.

In tissues the potassium is not always abundant enough to give with the reagent crystals of the triple salt, and in the vast majority of preparations the triple salt formed is evident only as a yellowish reaction, and, consequently, not sharply delimited in tissue preparations. If, however, the reagent is completely washed out of the preparation with ice-cold water, the application of ammonium sulphide, reacting as it does to form the black cobaltous sulphide, brings out, in an extraordinarily sensitive way, the distribution of the triple salt, and, therefore, of the potassium in it. This black reaction, as observed under the microscope, makes the distribution of the triple salt sufficiently sensitive to diagnose the presence of potassium when it is but 1 part in over 1,000,000 of tissue mass.

With this method of localising the distribution of potassium, the author succeeded in finding that, in a number of animal and vegetable cells, the potassium is condensed on surfaces in such a way as to make it very highly

probable that surface tension is the only immediate factor involved in this condensation. Of these he has given an account in recently published communications (10, 11, 12).

More recently, however, the author has found a unicellular animal form, in which the action of surface tension in influencing the distribution of salts in it is, to all appearance, placed beyond doubt.

This organism is *Acineta tuberosa*, a Suctorian Protozoan of marine habitat, a form which permits readily of technical manipulation and microscopic examination, especially for microchemical purposes. Some, at least, of the salts of sea water penetrate its cytoplasm, and amongst these certainly are those of potassium, of which the chloride is apparently the most abundant. The distribution of this element in the cytoplasm of this organism was carefully investigated, and the results were found to be of such significance as to justify the detailed description of them given in the following pages.

II. *Methods of Investigation.*

The specimens of *Acineta tuberosa* used in these observations were found in abundance attached to brown filamentous algæ growing on wooden wharves and floats, at and just below the surface of the water, near the Marine Biological Station of the Biological Board of Canada, in the Bay of St. Andrews, New Brunswick, in July and August of 1911. It was easy at any time during that period to get an abundant supply of these specimens by collecting a mass of the algæ, which was carried to the laboratory in a quantity of the sea water of the immediate locality. In nearly all cases the material so collected was used within a few minutes—20 at the most—after it was collected.

For the determination of the distribution of potassium in the *Acinetæ*, a mass of the algæ was lifted with a forceps from the sea water, and allowed to drop into a quantity of the solution of the hexanitrite of cobalt and sodium. For the method of preparing this reagent, the reader is referred to an earlier article of the author's (9). At the end of five minutes the mass of filaments was removed from the reagent and placed in ice-cold distilled water, which was renewed every three to five minutes, until, at the end of half-an-hour, all the uncombined reagent was completely extracted, and the only demonstrable cobalt compound present was that in the form of the triple salt, the hexanitrite of cobalt, potassium and sodium. The filamentous mass was now placed in a quantity of a mixture constituted of equal parts of glycerine and fresh or recently prepared ammonium sulphide, which gave the preparation a black reaction, due to the formation of the black cobaltous sulphide compound.

In the transference from one fluid to another, goose-quill points, glass needles, or platinum points were used, in order to avoid contamination of the preparations with iron or other metallic salts, which would tend to give a bluish-black, black or brown reaction with the ammonium sulphide.

The preparations were now made ready for examination under the microscope. For this purpose minute portions of the material subjected to the treatment described were teased out on a slide in a drop of a mixture of five parts of glycerine and one of ammonium sulphide, a cover-glass was added, and, after all the glycerine-sulphide mixture not included under the cover-glass was carefully removed, the edges of the cover-slip were luted to the slide with benzol balsam to prevent evaporation and to facilitate examination under high magnification with the microscope. Preparations so made have been found after 16 months to have retained all their original value and distinctness.

For revealing the structural and other characters of the *Acinetæ* fresh material was placed in 10-per-cent. formalin solution, in which also it was kept. This material was treated with a saturated solution of scarlet red in 70-per-cent. alcohol to show the distribution of fat in these organisms. Further fresh material was treated with Zenker's fluid, Flemming's chrom-osmio-acetic mixture, and with saturated aqueous solutions of mercuric chloride. The material so prepared was used to reveal the minute structure of the organisms.

III. *The Results.*

The structure of an *Acineta* can be seen from an inspection of a formol-scarlet-red preparation, such as is illustrated in Plate 14, fig. 1. The delicate lorica (*a*) is transparent and surrounds the organism, except at three points. Two of these latter are where the hillocks of cytoplasm, bearing the tentacles, project beyond the contour outline. The third is less distinct and is found at a point on the anterior border, midway between the two hillocks. It is a minute pore, where terminates the canaliculus which connects the central cavity of the cell with the exterior. Into this central cavity, which ordinarily is very minute, grows the bud of the cytoplasm by which the organism is reproduced. Such a young form is shown in fig. 1. The minute pore, or aperture, and the canaliculus may be seen most clearly in preparations made to show the distribution of potassium.

The cytoplasm is crowded with spherules of a proteid character which are best revealed by the scarlet red stain. These, through the action of the hardening reagent, shrink more or less, and thus the spherules appear to lie in cavities which they incompletely fill. Dissolved in the spherules is

a slight amount of lipoid material to which is due the faint stain given them by scarlet red. In some preparations the spherules appear to take with scarlet red a deeper stain. This is in large measure due to the fact that in the unstained condition they are impregnated with a reddish brown substance absorbed by the tentacles from the cytoplasm of brown Algae (fig. 8). With this brownish material there is associated lipoid material. Hence the deep brownish-red stain which these spherules have after treatment with scarlet red.

Here and there in the cytoplasm one can detect the presence of fat droplets, which, however, are very minute and few in number. The cytoplasm itself, apart from the spherules, is very finely granular. Especially is this the case in the hillocks from which the tentacles originate. In these hillocks there are no spherules or coarse granules of any kind, and even in the vicinity of, and immediately below, the hillocks, there are very few spherules and large granules.

The central cavity in which the germinal bud develops is ordinarily very small, but it is enlarged as the bud grows. The wall of the cavity and the surface of the bud are in close contact (fig. 1). The canaliculus which connects this cavity with the exterior is always patent, although in ordinary preparations it may be invisible.

The nucleus is placed below the germinal cavity and is invisible in the fresh preparation. When a germinal bud is developing the nucleus becomes irregular in shape, a lobate prolongation of it extends into the bud, and this lobate portion is separated by constriction at its narrow part and forms the nucleus of the cell of the developing bud. The process of division is thus amitotic.

The tentacles are 25 to 40, or even more in number, and they are usually disposed in a radiate direction in all planes from the convex surface of the hillocks. Their diameter is about 1·2–2μ, and their length, though varying from individual to individual, does not exceed, at the most, 35μ, but ordinarily is not more than 27μ. The outer end is capitate and its diameter is usually about 2·7μ, that is about half as much again as the average thickness of the tentacles.

The shaft of each tentacle consists of an axial portion constituted of very finely granular cytoplasm enclosed by an external sheath or layer of homogeneous material which, because of its slightly greater transparency and refringency, appears readily distinguishable from the axial substance. The thickness of this sheath is about 0·4μ in the capitate region of the tentacle. The sheath is an extension of the ectosarc, for, at the base of the tentacle, it passes directly into the clear homogeneous limiting layer of

the hillock, which layer, in other Protozoa, answers to what is known as the ectosarc.

The axial portion of the tentacles is constituted of the ordinary cytoplasm of the organism. It is almost homogeneous in composition, or hyaline in appearance. The material composing it does not appear to be derived from the cytoplasm of the hillock, but from that at a considerable distance below the hillock. This is clearly shown in fig. 2, in which are represented the channels in the cytoplasm, along which flows the more fluid and very finely granular material constituting the axial portions of the tentacles.

The currently accepted view as to the action of these tentacles in the absorption of food is that they are hollow and are provided at their ends with a cup-like sucking organ through which the food enters and is carried by suction action into the cytoplasm. The existence of a cup-like terminal for each tentacle I am unable to establish. There is nothing to suggest the occurrence of such a structure. The structure of the capitate point is that represented in fig. 7. As is not infrequently the case, the surface film of the point may be deeply impregnated with fatty substance which is sharply demonstrated in formol-scarlet-red preparations, but lipoid material is found in droplets irregularly at other points in the film along the course of the tentacles.

The tentacles are not always straight. They may exhibit a slight or a marked curve, but, as a rule, only one, or at most several, in a group, are so affected. When the tentacles are being retracted they become straight and the capitate end of each is reduced in diameter until the latter equals the transverse diameter of the tentacle itself, which does not decrease, however much the longitudinal diameter may diminish. The retraction affects all the tentacles in the two groups on a form equally, and it may proceed until, finally, they are but slight prominences on the external surface of the hillocks. It is, indeed, very rare to find them completely withdrawn into the hillocks.

From all the phenomena involved in the extension and retraction of these tentacles it is to be inferred that alterations in surface tension are involved. These alterations may affect different portions of the surface of the organism. The protrusion of the tentacles may be due to an increase of the tension of the general surface while the tension of the film at the points where the tentacles originate may remain as before, or the tension of the general surface may be constant while the tension of the film at the points where the tentacles develop may diminish. There is the possibility also that, while the tension of the general surface may increase, that of the film at the points of origin of the tentacles may decrease.

Whether, however, the tension of the surface film over the general surface

of the organism is raised or not is a question on which there is no evidence, direct or indirect. It is, nevertheless, tacitly assumed that in other organisms, which exhibit at points on their surface extensions of the protoplasm in the form of pseudopodia or flagella, the negative answer to this question is the correct one. It may turn out eventually to be the right answer, but, on *a priori* grounds, it is not improbable that in an organism like an Amœba the distribution of energy may be so adjusted that the tension over the general surface may be raised. In a specimen of Amœba which has been moving continuously in one direction for more than four or five times its diameter such an elevation of the tension must be continuously occurring, as otherwise the film at the point where the pseudopodium develops would spread over the whole surface and movement would quickly cease.

However much uncertainty there is on this point there is none on the question of relatively low tension in the films of the tentacles. The surface tension in these may be the same or lower than it was before they were protruded, but when they develop the tension is in all cases less than that of the general surface as otherwise there would be no development of tentacles.

There should, in consequence, be in the surface films of these tentacles the condition which promotes surface condensation as the result of the action of the Gibbs-Thomson principle. The solutes which lower the surface tension of the tentacles should be found in greater concentrations in their surface films than elsewhere in the cytoplasm of these organisms.

This is the case with the potassium salts. When these organisms, in the very active condition, are treated, after the manner described above, with the hexanitrite reagent, followed by washing in ice-cold water and by application of ammonium sulphide, the distribution of potassium salts thus revealed is, in the vast majority of preparations, like that represented in fig. 3. In such the potassium is seen to be localised in the surface films of the tentacles, at the interface formed by the maternal and germinal cytoplasms and at the interface formed by the cytoplasm and each of the included spherules. Elsewhere in the cytoplasm the potassium salt is so minute in quantity as to be undemonstrable by the reagent employed for that purpose.

The occurrence of potassium salts at the interface formed by the maternal and germinal cytoplasms is in part at least due to surface condensation. The film covering the germinal cytoplasm must have a tension less than that of the film of the adjacent maternal cytoplasm, as otherwise the germinal bud would not develop in the central cavity of the organism. The condition at the interface would then be more or less like that at the interface formed by a drop of oil suspended in water, in which salts, *e.g.* those of potassium, are

dissolved. In both cases there would be a condensation of potassium salts from the enclosing or surrounding medium on, but not in, the surface of the enclosed object or system.

Some portion, however, of the potassium salts found to occur at the interface between the maternal and germinal cytoplasms must be explained as involved in the process of excretion. In the canaliculus connecting the germinal cavity and the exterior potassium salts are frequently found (figs. 3, 4) and these may extend in the form of a plug or mass through the pore in the lorica. Sometimes the salts so excreted do not reach the exterior, for if the external end of the canaliculus is not in line with the pore in the lorica the salts pass to the right and left in the grooves above the cytoplasm formed by the two parallel folds of the lorica. The position of these folds and the presence of potassium salts in them is shown in fig. 9, which represents a specimen as seen when its anterior surface is turned towards the observer. Even when the canaliculus is flush with the pore in the lorica potassium salts may be found in the groove for some distance to the right and the left of the pore (fig. 3, *a*).

The occurrence of potassium salts at the interface formed by the cytoplasm and each of the included spherules can be demonstrated only in a few specimens of *Acineta* and even when the demonstration is clearest it is nevertheless of such a character as to escape observation unless it is specially examined with that end in view. That there should be such a surface condensation of potassium salts in the cytoplasm on the surface of the spherules would follow from the fact that the spherules must have a different surface tension from that of the cytoplasm. Why this condensation is not evident in every specimen of *Acineta* cannot be explained unless it be that the surface tension of the cytoplasm at the cytoplasm–spherule interfaces is not, in the majority of *Acinetæ*, diminished as it is in the cytoplasm immediately adjacent to the germinal bud or in the surface film of the tentacles, in which case there would be little condensation of potassium at the cytoplasm-spherule interfaces.

The condensation in the tentacles is sharply confined to their surface films. This is shown particularly in fig. 6, representing the terminal portions of two tentacles greatly magnified. The coarse granules there revealed represent the crystals of the hexanitrite of cobalt, sodium, and potassium, blackened by ammonium sulphide. The crystalline deposit appears to be very voluminous but this is due to the fact that potassium forms only about 16 per cent. of the crystals themselves. Were, however, the potassium much less abundant than this the precipitate would not be crystalline but one finely diffused throughout the surface film. Such a finely diffused precipitate

is sometimes observable between the crystals in the surface film of the head of the tentacle.

The potassium salt is more abundant in the capitate portion of the tentacle than elsewhere in the latter.

Contraction of the tentacles is rarely observed in specimens very recently taken from their habitat, but when it is developing the potassium salt diffuses from the surface films of the tentacles into the axial portions, and in consequence in such, when treated to reveal the potassium, the tentacles are black throughout (fig. 4). In certain of such preparations the cytoplasm of each hillock and of the immediately underlying part has also a dark shade which indicates that the potassium salt, as a result of the retraction, has begun to diffuse from the tentacles downward into the cytoplasm. When the tentacles are completely retracted the potassium is then diffused throughout the hillocks into which they are withdrawn and also downward into the underlying cytoplasm. In some specimens even the hillocks may be inverted and then one finds their outline marked out by the deep black reaction they give. In fig. 5, which represents this occurrence, a more marked diffusion downward into the cytoplasm is shown by the dark shade which becomes less and less marked the further downwards this diffusion proceeds. As this diffusion develops the potassium salts tend to condense more on the surface of the spherules than was the case when the tentacles were still extended, especially on those spherules which are found in the anterior half of the organism. This may be explained as due to the greater concentration of potassium salts in the surrounding cytoplasm in this region.

Occasionally in specimens one observes crystalline bodies of unknown composition in the cytoplasm, on the surface of which potassium salts may be condensed. The condensation on their surfaces has been observed to be more pronounced when the tentacles are partially or wholly retracted (fig. 5).

The salt of potassium most abundant in these condensations is probably the chloride. This was shown by the silver reaction. When living *Acinetæ* were placed for 30 minutes in N/10 solution of silver nitrate, to which some nitric acid was added, and afterwards exposed to bright sunlight for 10 minutes, a brown-black deposit of the reduced silver chloride was found in them only in the superficial films of the tentacles and at the germ-bud–cytoplasm interface, where the potassium reaction was obtained. This would seem to indicate that the potassium is present as chloride only, but as the reaction for the SO_4 of sulphates was, unfortunately, not applied one must admit the possibility of some of the potassium being present as sulphate. If, further, sodium, calcium, and magnesium, as chlorides, are present in these organisms they must undergo condensation on the same surfaces and

interfaces, though possibly to an extent different from that obtaining in the case of the potassium salts, and, consequently, some of the haloid chlorine demonstrated as present may be united with them.

IV. *General Observations.*

The foregoing observations make it evident that surface tension controls the distribution of potassium salts in the cytoplasm of *Acineta*, and that, whenever the surface tension at a point changes, there results a redistribution of the salts, which conforms to the altered conditions of surface tension. The quantity of potassium so affected appears to be very great as compared with the amount which is diffused throughout the cytoplasm. In fig. 3 the surface films of the tentacles, and the interfacial surfaces between the maternal and germinal cytoplasms, seem to hold by far the vast part of the potassium in the organism. What is the exact proportion so condensed, as compared with that in the cytoplasm generally, cannot be determined, but the very marked concentration in the surface films of the tentacles, and the almost entire absence of a reaction for potassium in the cytoplasm elsewhere, suggests that the degree of concentration greatly exceeds the value $S = -\frac{C}{iRT} \cdot \frac{d\sigma}{dC}$. As already pointed out, Lewis found that the condensation of potassium on the surface of the droplets of hydrocarbon oil in a M/20 (0·373 per cent.) solution of potassium chloride was 5×10^{-8} grm./cm.^{2}, or thirty times the value $1{\cdot}7 \times 10^{-9}$ grm./cm.^{2}, calculated from the equation. In a solution of the concentration M/20, the hexanitrite reagent would give a dense precipitate, and a precipitate is given* when the concentration of potassium is as dilute as 0·00039 per cent., or M/10000 KCl. It is obvious, then, that the concentration of potassium chloride in the cytoplasm is below this value, while the concentration of the condensation in the surface films of the tentacles must be much above it.

The thickness of the layer of condensation in the films is probably not as great as appears indicated in the preparations. The thickness depends in part only on the diameter of the molecules forming the surface films. If the molecules were those of water, they should have, according to Kundt and Warburg, a diameter of $3{\cdot}39 \times 10^{-8}$ cm., and a range of molecular attraction equal to $6{\cdot}78 \times 10^{-8}$ cm. If, further, the surface tension in the films were as

* The precipitate is found at the bottom of the test-tube containing the mixture of the solution and reagent, after it has been allowed to stand for some hours. The precipitate is also similarly given when the potassium chloride is of the concentration M/20000 in a mixture of the solution and reagent. Crystals of the precipitate may, however, be found in a drop of the mixture examined under the microscope a few minutes after it is made. (The mixture in these cases should consist almost wholly of the reagent.)

low as that of the solution in contact with the oil droplets in Lewis' determinations, and the concentration in the cytoplasm were M/20, then the concentration in the condensation would be $(5 \times 10^{-8} \times 100) \div (6{\cdot}78 \times 10^{-8})$, or 73·7 per cent. of potassium; 73·7 per cent. of potassium is equivalent to 140·4 per cent. KCl, which is an impossibility. With a solution concentration less than M/20, on the other hand, the concentration of the condensation in the surface films would still be high, probably higher than if it corresponded to the value of the Gibbs equation for M/20, that is, $(1{\cdot}7 \times 10^{-9} \times 100) \div (6{\cdot}78 \times 10^{-8})$, or 2·5 per cent. of potassium, which is equivalent to 4·76 per cent. KCl, or 0·638 M.

If the surface film were constituted of molecules of protein in which water was absorbed, the thickness of the zone would be very much greater than if it were formed of water alone, and this would provide for a concentration of potassium chloride much less than the impossible 140·4 per cent., yet greater, perhaps very much greater, than the lower limit, 4·76 per cent.

With such a concentration in each superficial film, the precipitate would be marked, but this would not be confined to the surface film, for the crystals formed would project into the underlying zone. This, perhaps, explains why the deposit seen under the high powers appears to have such a thickness as indicated in fig. 6.

What is the source of the potassium found in these condensations?

The amount of potassium found in the sea water around the wharf of the Biological Station at St. Andrews, as ascertained from analyses made by the author, ranges from 0·0272 per cent. to 0·0353 per cent. according to the tide. As chloride the potassium of the higher concentration would correspond to 0·0673 per cent. or slightly less than M/110. The presence of potassium salts in sea water suggests that the potassium salt diffuses into the organism from without, but the author found that when a mass of the filamentous Algæ to which a very large number of *Acinetæ* were attached, was placed for 24 hours in a large quantity of filtered sea water the majority of the *Acinetæ* examined contained very little potassium, although the tentacles were protruded. Further, in a few of the *Acinetæ* in every preparation there was little or no potassium present. It may also be noted that sea water with a concentration of 0·035 per cent. of potassium will not give with hexanitrite reagent a precipitate of the potassium salt which will at all compare in density with the precipitate of the same salt in the superficial films of the tentacles, and it is consequently improbable that the much higher concentration of potassium salt in the tentacles is derived by diffusion from the sea water.

The only other source of the potassium in the condensations in the tentacles is the potassium of the material absorbed as food through the tentacles. The organisms which are the prey of marine *Acinetæ* are chiefly vegetable and the cytoplasm of these is charged with potassium which, with their other constituents, is absorbed through the tentacles. Sometimes, indeed, such organisms heavily impregnated with potassium may be found attached to the tentacles of an *Acineta*.

The potassium absorbed seems to play no part in digestion or metabolism and though its condensation in the superficial films of the tentacles would indicate that it lowers their surface tension this diminution is not dependent wholly on its presence. The fresh-water form *Podophrya* (*Tocophrya*) *quadripartita*, which grows attached to *Cladophora*, *Vaucheria*, and other Confervoid forms, does not contain any potassium even when it is absorbing material from its prey. It does not become impregnated, even in the slightest degree, with potassium salts when it is placed in sea water for 24 hours. It is manifest, therefore, that the tentacles and the general surface of these organisms are ordinarily impermeable to the salts of their medium.

The low surface tension on the tentacles of *Acineta*, and at the points on the hillocks where the tentacles arise, is doubtless due to formation in these structures of a substance derived from the metabolism of the proteins and other constituents of the cytoplasm. When potassium salts are present they may co-operate with it in its action on the surface tension. What this substance is cannot definitely be indicated and conjecture is our only resource. On first consideration a lipoid is suggested. To determine whether such a body is present in sufficient quantity to permit it to be distinctly shown under the microscope, preparations of active *Acinetæ* hardened in 4-per-cent. formol were treated with a saturated solution of scarlet red in 70-per-cent. alcohol for 2 hours and after being washed for a few minutes in 60-per-cent. alcohol and then in distilled water, were mounted in 50-per-cent. glycerine. A careful examination of such preparations under the microscope revealed minute spherules of fat in the membranes of the tentacles. In some examples of *Acineta* these spherules were very numerous, in others they were few. Occasionally a distribution of fat like that shown in fig. 7 was observed. In such the superficial membrane of the terminal capitate end of the tentacle was deeply impregnated with fat or it presented the appearance of a mosaic formed of closely placed very minute spherules of fat. The superficial membrane, however, in the vast majority of the tentacles gave no indication of fat uniformly diffused throughout it.

Scarlet red is not a staining reagent to demonstrate the occurrence of

soaps, or certain lecithins, which may be present in the membrane of each tentacle. The application, however, of a solution of osmic acid of 0·5-per-cent. strength to living examples of *Acineta* has failed to give any indication of the presence of these lipoids, while it demonstrated by a black reaction the minute spherules of fat revealed by the scarlet red.

There, consequently, appears to be little direct evidence that the low surface tension of the tentacles is due to the presence of fat. It may, indeed, be held that the presence of minute spherules of fat in their membranes predicates a saturation of the latter with fat, which, however, is so scanty that scarlet red and osmic acid fail to demonstrate it. If that were the case it would be difficult to explain why the saturation did not prevail over the whole surface of each hillock instead of being localised as it is.

The presence of minute spherules and other deposits of fat in the tentacles may be the result and not the cause of the low surface tension in the tentacles. Fats lower surface tension, it is true, but if the surface tension should, through the action of some non-lipoid substance, be diminished, the lipoid material, it is presumed, may condense where the tension is low and give appearances in the tentacles like those described.

The view that the tentacles of *Acineta* are hollow tubes open at their capitate ends finds no support in the observations of the author, and it follows that the food matter absorbed from the prey of these forms is not taken into the cytoplasm of the tentacles unchanged or absorbed indiscriminately by them. The homogeneous appearance of the axial portion of each tentacle while it is attached to the prey suggests that the material which is being taken into each tentacle is in a digested condition and that the digestion so effected occurs outside the tentacles or in the interior of the capitate ends. This involves the assumption that the tentacles secrete one or more ferments. Now, the existence of proteolytic ferments in the tentacles, even in minute quantities, would render the presence of amino-acids possible and these latter would bring about a diminution of surface tension which would maintain, if not originate, the extension of the tentacles. These ferments would be more abundant, in the state of hunger, and specially at the points where they would come most into service, and their activity, however slight, directed upon the proteins at points in the hillocks, would cause the protrusion of the tentacles there.

The presence of free amino-acids in living protoplasm is not unknown and especially in structures in which cellular activity is marked. In the growing points of vegetable structures free amino-acids have been found in quantities sufficient to render the demonstration of their presence certain. It is possible

that in such growing structures they may play, amongst other parts, that one of lowering the surface tension at points on the cells where extensions of the latter occur. As there is not a great gulf fixed between the metabolism of an active vegetable cell and that of a vigorous animal cell the existence in the latter of free amino-acids on occasions is not improbable.

The observations just now advanced are, of course, largely of the order of speculation. They have been put forward because they are in a measure concerned with another problem for which the lipoid theory of membrane action has been proposed as a solution.

With a concentration of potassium in the superficial molecular layer of each tentacle giving a precipitate much denser than sea water gives with the hexanitrite reagent, the question arises why the potassium salt in the surface film of each tentacle does not diffuse into the sea water and equalise the concentration on both sides of the tentacle–sea-water interface. The presence of a lipoid in the superficial film would perhaps account for this inequality, for the lipoid would make the membrane more or less impermeable, not only to water but also to salts in the latter. This, however, does not aid much in the way of explanation, for if the superficial film owes its impermeability to a lipoid constituent there should be no penetration of the superficial film by a solution of a potassium salt from the cytoplasm underneath and no condensation of potassium would occur in the film. It is obvious, therefore, that the impermeability of the superficial layer of protoplasm of the tentacles cannot be due to a lipoid and that some other factor plays the important part. That force is surface tension, in all probability, for the force that condenses the potassium salt in the superficial film of each tentacle would hold it there, especially as the surface tension of the sea water is higher than that of cytoplasm and a diffusion of potassium salt into the sea water would tend to raise the tension of the latter, thus increasing, instead of decreasing, the inequality of the forces on both sides of the membrane–sea-water interface. If the surface tension of the external medium were equal to, or lower than, that of the superficial film of the cytoplasm of the tentacles, diffusion outward would take place in order to equalise the tensions on the two sides of the dividing interface. In support of this it may be pointed out that Czapek (2) has found that it is only when the external medium of vegetable cells has its surface tension lowered to 0·68 times that of pure water that the tannin diffuses from them into the external fluid.

Besides the influence that surface tension has upon the distribution of salts in the interior of living cells it has, it would appear, a very important effect upon the diffusion from them and, therefore, into them. The factors operating in cellular osmosis are, consequently, not so simple as those postulated in the

van 't Hoff-Arrhenius theory of osmosis based on the results of the experiments of de Vries and Pfeffer. This compels a revision not only of the doctrine of the semi-permeable membrane as applied in physiology but also of not a few of the conclusions that were based on it.

V. *Summary of Results and General Observations.*

1. In *Acineta tuberosa*, a marine Suctorian Protozoan, the potassium salts are localised: (1) On the interface between the cytoplasm and each of the spherules strewn throughout the cytoplasm; (2) on the cytoplasm–germ-bud interface; (3) in the superficial film of each extended tentacle.

2. The quantity of potassium found at each cytoplasm–spherule interface is generally very minute, and may be observed only after careful examination in some preparations; the quantity on the cytoplasm–germ-bud interface is usually richer and more readily demonstrable; while the potassium is most abundant in the surface film of each extended tentacle.

3. The potassium in the remainder of the cytoplasm does not give a reaction with the reagent used, the hexanitrite of cobalt and sodium. With this reagent crystals of the triple salt, the hexanitrite of cobalt, sodium and potassium, may be formed in solutions as dilute as 1 of potassium in 275,000, but in microscopical preparations of cells appropriately treated with the reagent and subsequently with ammonium sulphide the sensitiveness of the reaction exceeds that limit, perhaps to the extent of demonstrating 1 part of potassium in 1,000,000. In any case the absence of a reaction in the cytoplasm generally is an indication that the potassium salt or salts present are in exceedingly attenuated dilution.

4. When the tentacles begin to retract, the potassium salt or salts in the film of each begins to diffuse into the cytoplasm of the main body of the organism. This diffusion results in a greater concentration at first in the cytoplasm near the base of the hillocks from which the tentacles take their origin, but, as the retraction proceeds, the diffusion progresses downward into the cytoplasm, which is now more or less deeply impregnated with potassium salts and the deposit on each cytoplasm–spherule interface becomes, as a rule, more distinct.

5. Complete retraction of the tentacles alone is rarely seen, but more often, though never frequently, one finds a complete retraction of hillocks into which the tentacles have been completely withdrawn. In the masses derived by retraction of such hillocks the potassium salt is still present, but a distinct reaction for potassium in the underlying cytoplasm shows that diffusion of potassium into the cytoplasm, as a result of the retraction, has proceeded.

6. The condensation of potassium in the superficial layer of the extended tentacles, and the diffusion downwards into the cytoplasm when the tentacles are being retracted, indicates that the Gibbs-Thomson principle of condensation, due to the action of surface tension, is the factor in bringing about the concentration of potassium salts in the superficial layer or film of each tentacle, and that the deposit on the cytoplasm–germ interface, as well as those on the cytoplasm–spherule interface, would appear to be due to the operation of the same principle.

7. Surface tension thus makes the concentration of potassium in the cytoplasm of the cell body of *Acineta* less than 1 in 275,000, and condenses it to an extraordinary degree in the surface film of each tentacle, and at other interfaces where the tension is low. Surface tension is, therefore, an all important factor in determining the distribution of potassium salts and, inferentially, of other solutes in active *Acinetæ*.

8. How the low surface tension is brought about which leads to the formation of the tentacles is not known. With microchemical methods for demonstrating fat, very minute spherules of fat are found in the superficial layer or film of each tentacle, and the superficial film of the capitate end of a tentacle may be, now and again, deeply impregnated with fat. Fat or lipoid substance may, consequently, be the cause of the low tension. It is, however, suggested that amino-acids are the substances which lower the surface tension.

9. The quantity of potassium salt condensed in the surface film of each tentacle appears to be of greater concentration than obtains in the sea water of the habitat of the organism. This inequality of concentration on the two sides of the surface-film–seawater interface is, it is explained, due to the action of surface tension in maintaining the condensation on that side of the interface where the surface tension is less. Lipoids, it would appear, are not concerned in preventing the exchange of potassium salts, for they should also prevent the penetration of the surface film by potassium salts derived from the underlying cytoplasm.

10. The current conceptions regarding cellular osmosis and the distribution of salts in living cells, based on the van 't Hoff-Arrhenius theories of solutions, must be revised.

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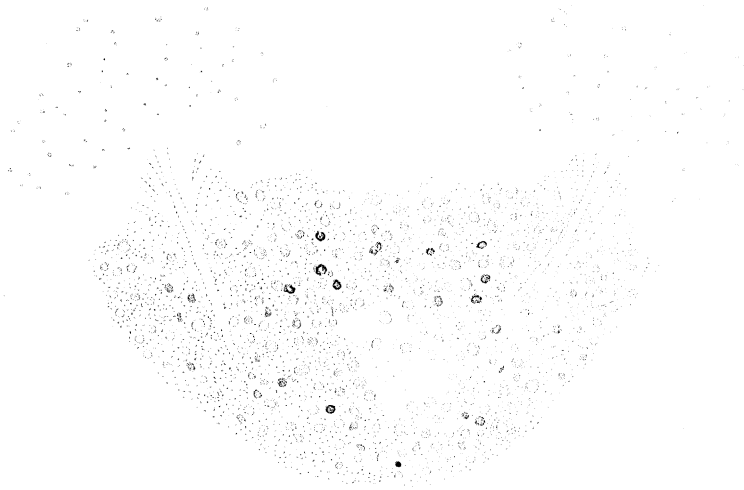

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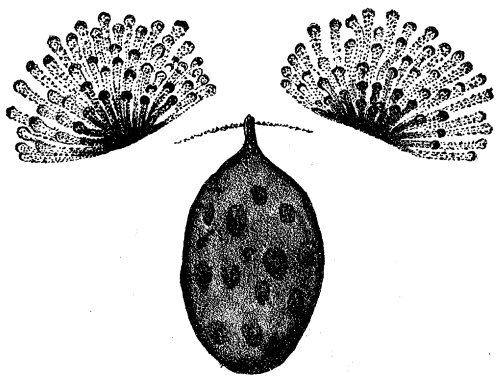

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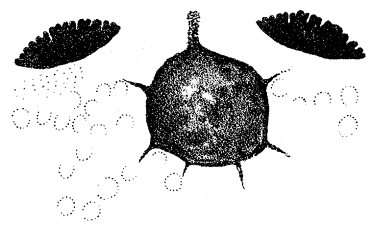

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VII. EXPLANATION OF PLATES.

Note.—The dark shading in the drawings indicates the cobaltous sulphide reaction developed from the treatment of the cobalt, sodium and potassium hexanitrite with ammonium sulphide. The dark shading thus represents the distribution of potassium throughout the organism.

Plate 14.

Fig. 1.—Mature specimen of *Acineta tuberosa*, treated to reveal the distribution of fat in its cytoplasm. Formol, scarlet red, glycerine. × 700.

Fig. 2.—Young adult specimen of *A. tuberosa*, showing the channels in the cytoplasm along which the axial contents of the extending tentacles flow. Formol, scarlet red, glycerine. × 1200.

Fig. 3.—Fully adult specimen of *A. tuberosa*, tentacles extended, treated to reveal the distribution of potassium in it. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 750.

Fig. 4.—Fully adult *Acineta*, with tentacles partly retracted and the potassium salt or salts already to a certain extent diffused from the tentacles into the underlying cytoplasm. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 750.

PLATE 15.

Fig. 5.—Adult specimen of *A. tuberosa*, with the tentacles and hillocks wholly retracted and the potassium salt or salts diffused in the underlying cytoplasm more than was the case in the form indicated in Fig. 4. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 750.

Fig. 6.—The terminal portions, greatly magnified, of two of the tentacles of the specimen from which fig. 3 was drawn. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 3200.

Fig. 7.—The terminal portions of two tentacles of a specimen of *A. tuberosa*, stained to show the distribution of fat in them. Formol, scarlet red, glycerine. × 1680.

Fig. 8.—Specimen of *A. tuberosa* unstained, to show the distribution in it of the pigment which it absorbs from the vegetable forms on which it preys. The hillocks and tentacles are free from it. Formol, glycerine. × 500.

Fig. 9.—Specimen of *A. tuberosa* seen with its anterior border tilted forward, showing the distribution of potassium salts in the grooves formed by the two parallel folds of the lorica. Cobalt sodium hexanitrite, glycerine-ammonium sulphide. × 450.

Carbohydrate Metabolism in its Relation to the Thyroid Gland.—The Effect of Thyroid Feeding on the Glycogen-content of the Liver and on the Nitrogen Distribution in the Urine.

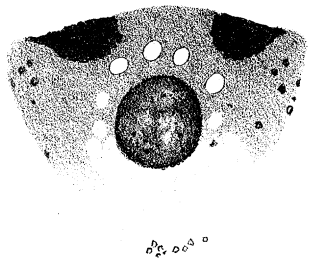
By W. CRAMER and R. A. KRAUSE.

(Communicated by Sir E. A. Schäfer, F.R.S. Received June 10,—Read June 26, 1913.)

(From the Chemical Laboratory of the Physiology Department, Edinburgh University.

Introduction.

Our present knowledge of the relation of the thyroid gland to metabolism is based almost entirely on observations of the disturbances of metabolism produced by the diseases of the thyroid gland. By the application of physiological methods to patients suffering from Graves' disease or from myxœdema an increase in the total metabolism and in the nitrogen metabolism in Graves' disease on the one hand, a decrease in the total metabolism in myxœdema on the other, have been definitely established. From these facts the conclusion has been drawn that the secretion of the thyroid gland increases the oxidative processes, so that an inadequate functioning of the gland brings about the condition of obesity and depressed nitrogen metabolism, characteristic of myxœdema. As regards the carbohydrate metabolism it has been observed clinically that in Graves' disease there is sometimes a tendency to alimentary glycosuria; the opposite condition—an increased



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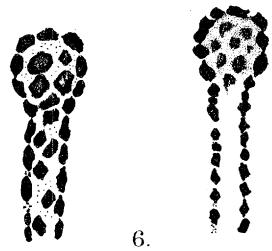

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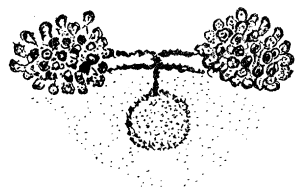

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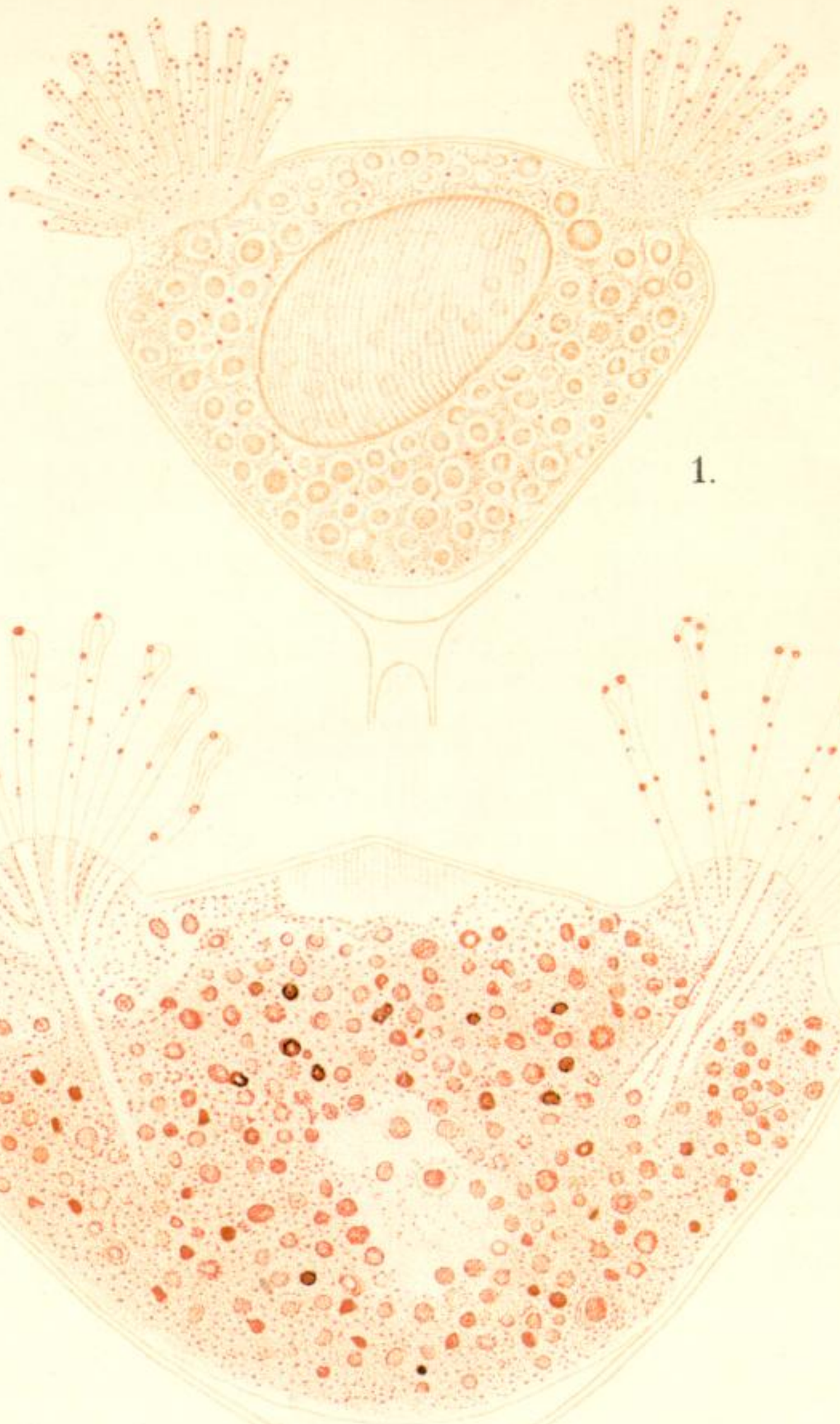

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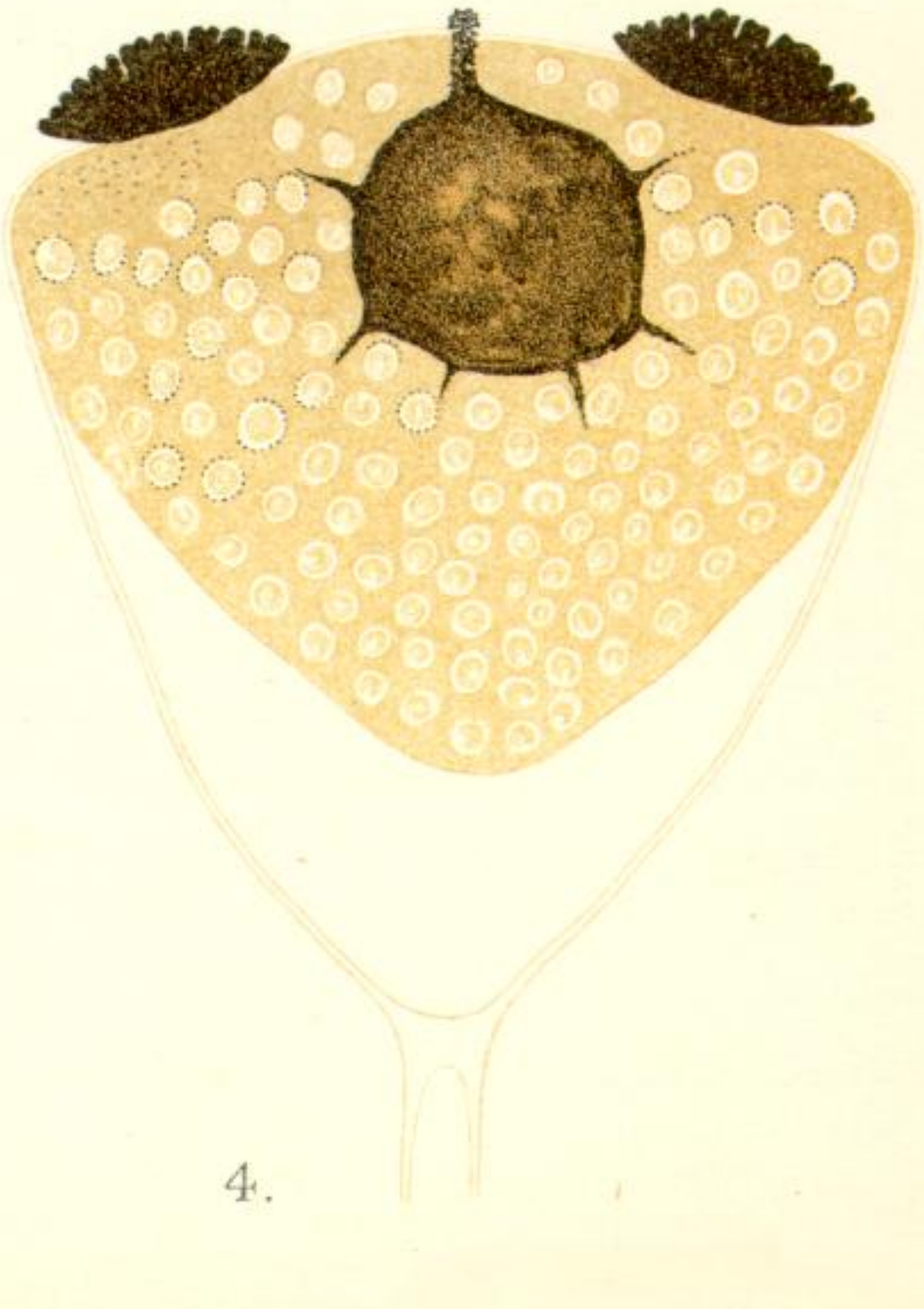

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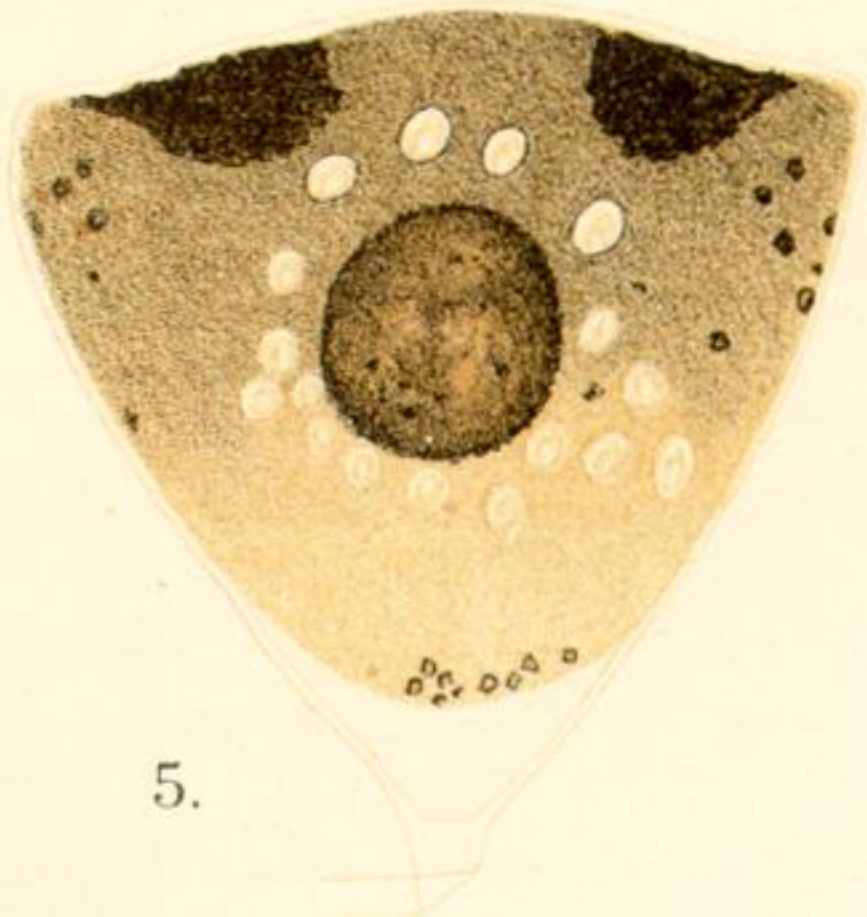

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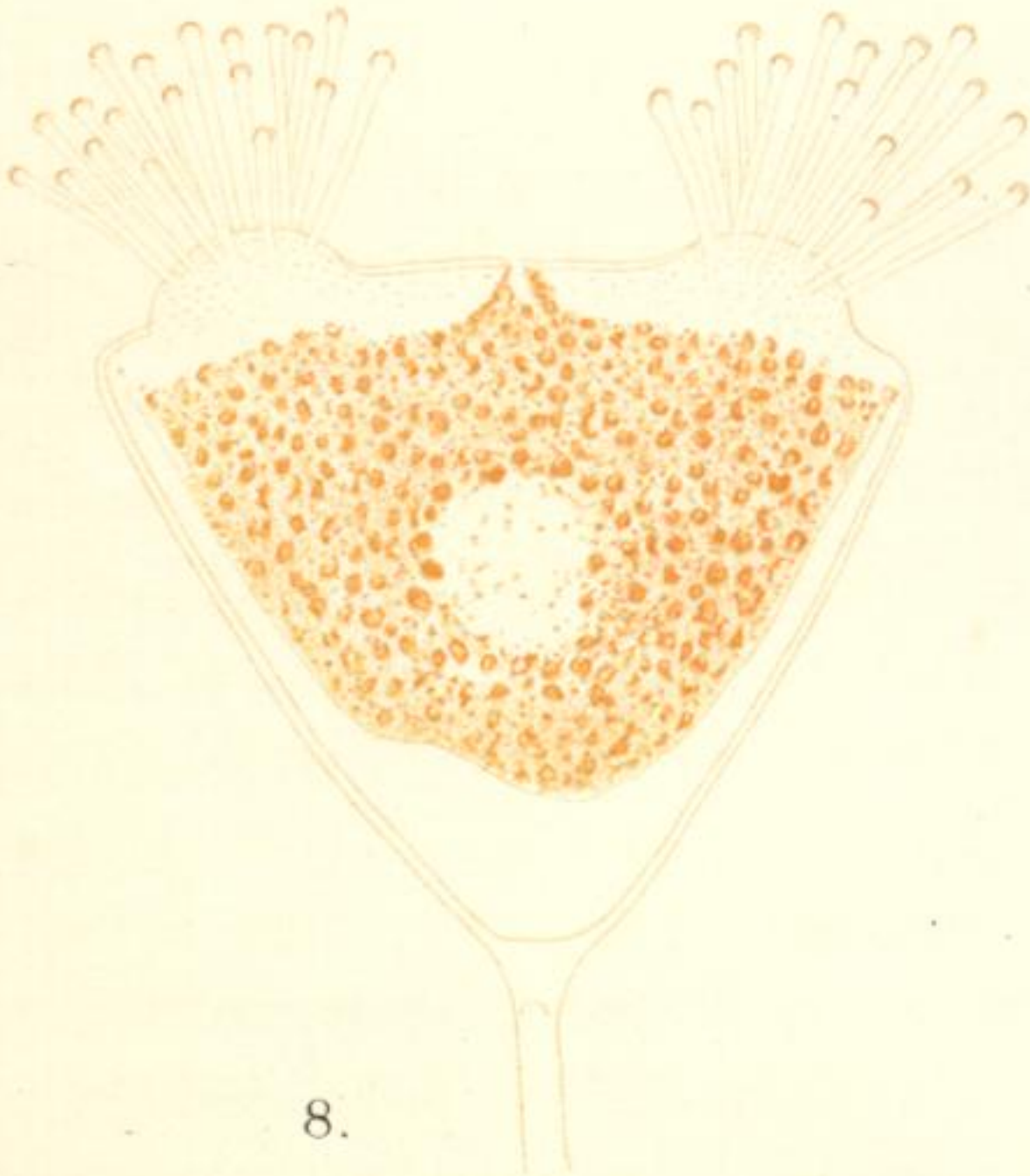

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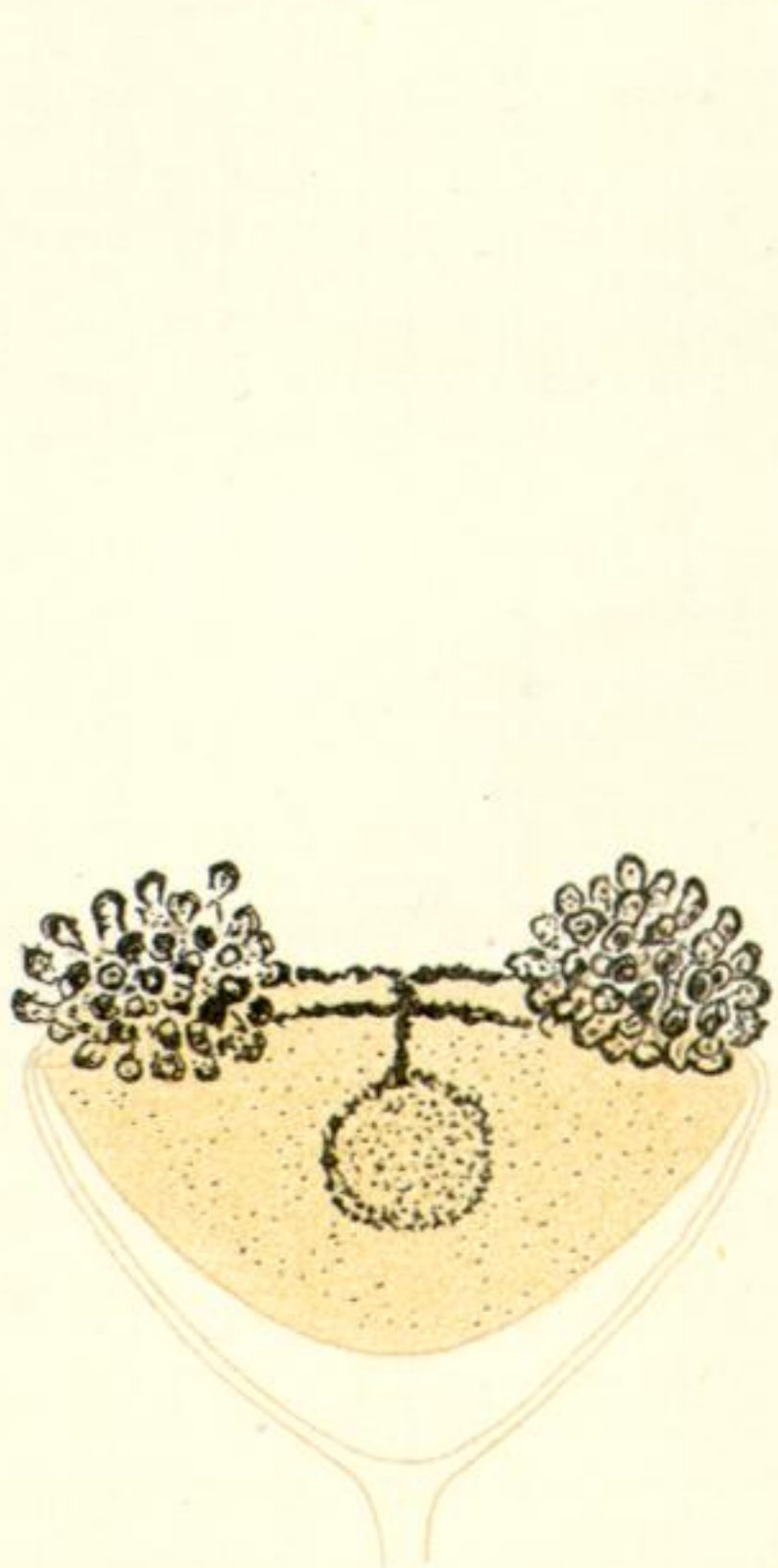

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