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Organization and Polarity of Protoplasm

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Erste Sektion:

Cytologie und Protozoenkunde.

Einführender Vorsitzender: Herr Prof. Dr. R. Klemensiewicz (Graz).

Schriftführer: Herr Dr. L. Löhner (Graz).

Erste Sitzung.

Dienstag, den 16. August, Nachmittags $^1/_2$ 3 Uhr, im Hörsaale des Instituts für allgemeine und experimentelle Pathologie.

Den Vorsitz führt Herr Prof. Dr. A. Borgert (Bonn).

Herr Prof. Dr. J. P. Munson (Ellensburg, Wash.) hält seinen angezeigten Vortrag:

Organization and Polarity of Protoplasm.

By J. P. Munson, Contents Ph. D., (Ellensburg, Wash.).

Mit Tafel Ia, Ib und Ic.

Introduction — External Evidences of Polarity — fore and aft polarity in *Amoeba* — a walking cell — Internal Evidences of Polarity — caryolymph and metaplasm — centrosome and aster — evidences of persistence of centrosome and aster — nature of astral rays — nature of centrosome — the aster and cytoplasm — Polarity of the Egg — direction of egg-axis — general impression of specific characters — Theoretical Suggestions — development of structure and polarity.

Introduction.

Studies in embryology, cellineage, and cleavage often necessitate the assumption of a polarity of the fertilized egg, even where no positive evidence of such polarity has been demonstrated. But the existence of such polarity is often denied on the assumption that the egg, and protoplasm in general, is isotropic.

Polarity is supposed to be evidence of a permanent structure in the protoplasm. The existence of such a structure is often denied on theoretical grounds by those who look upon protoplasm as a chemical mixture of complex organic and inorganic compounds. Writers on protoplasm usually favour either the reticular theory, the alveolar theory, or the granular theory, often as a resultant of the method employed, and the viewpoint, whether morphological or physiological, from which the facts are observed.

The conclusion reached, in matters like these, where interpretation must so largely enter in, is necessarily a resultant of two factors: the observers habit of mind; and the evidence which the object of study presents. But the mental habit, or the habitual viewpoint, often seems to determine what evidences shall be attended to. It may as well be said, that the opinions of such mentally biased observers are of little or no value; and that sc ence would be benefitted if their dogmatizing were taken less seriously.

The physiologist is so apt to ignore the permanent aspect of things, and so eager to look for the changes which accompany functional phenomena. Such changes are facinating, not only because they are more readily seen, but because they stimulate more strongly the desire to discover an explanation of those changes. Most minds betray a disposition to reduce all phenomena to an unit. The habit of mind acquired in the study of chemistry and physics predisposes the observer to explain all life in terms of molecules and atoms. To account for life in terms of dead matter often seems more satisfactory than to explain life in terms of living units. In the latter case we are apt to feel that we assume what we are trying to explain. Properly considered, however, atoms and molecules are as purely theoretical as are combinations of these into living units of a higher order. A crystal is no less real because it is something more than a fortuitous concourse of atoms and molecules. If we say that molecules are alive, as some do, we do not simplify matters; for somehow chemical compounds have not yet been made to live. „Omne vivum ex vivo" seems to express a fact; and why not accept the fact? There may be an element of time entering in here, which we fail to take note of, in observing the more trancient aspects of our subject. The question then becomes: what are the morphological features which protoplasm presents; and what are the physiological processes by which structure is maintained in the midst of the multitude of changes which functional activity involves?

In higher forms of life, we usually concede the presence of an organ specialized for the performance of a function. In these higher forms coordination of organs, which entails a regular sequence in their functions, is readily made out; and it seems a mere platitude to say, of these, that organization determines the functions of the organism. To what extent can functions exist in the absence of organization; does the one precede the other; or have function and organization developed simultaneously, out of matter devoid of both structure and function?

In my studies on the cell, I have kept these problems in mind; and it is my purpose here to present some observations bearing on these questions.

I.

External Evidences of Polarity.

Fore and aft Polarity. — In *Amoeba verrucosa*, fig. 1, pl. I, there is a definite differentiation of an anterior and a posterior end. A line connecting these two ends constitutes a longitudinal axis, which divides this Amoeba into two approximately similar halves, — a right and a left side, — which constitutes bilateral symmetry.

The dorsal side is characterized by longitudinal folds, grooves, and ridges, usually four or five in number. They seem to be the outer expression of internal movements of the granules in the protoplasm. The ectosarc is of considerable thickness, and is differentiated from the granular endosarc. I have succeeded in removing the endosarc leaving the ectosarc as an empty shell, the longitudinal lines still showing.

The granules of the endosarc move constantly towards the anterior end; and the Amoeba moves, as a whole, in that direction. This movement is not always in the direction of light. It is often opposite to the force of gravity; and it cannot always be accounted for by assuming that the Amoeba is attracted by oxygen.

In *Amoeba villosa*, fig. 2, pl. Ia, a similar fore and aft polarity is evident. But the differentiation of the two ends is here still more marked. The external, longitudinal lines are not so evident as in *Amoeba verrucosa;* but the body, as a whole, is more elongated; and the posterior end is distinguished by a villous prolongation. The surface of this part of the Amoeba appears roughened, as if covered with short, thick ciliary processes or papillary villi. This form, too, has a longitudinal axis and bilateral symmetry. The granules of the endosarc move uniformly towards the anterior end, which shows a considerable development of ectosarc.

Dorso-ventral Differentiation. In *Arcella discoides*, fig. 3, pl. Ia, there is evidence of a radial symmetry and a dorso-ventral differentiation. This Amoeba lives in a shell, which is somewhat hemispherical in shape, being flat or concave underneath and convex above. In general outline, the shell is circular or nearly so. Seen from above, it is brown, with a clear round spot in the center. I take this to be, in part, an optical effect due to transmission of light through a round opening on the under side, through which the Amoeba projects, when its pseudopodia are extended. The pseudopodia are few in number; and come out radially from the sides of the Amoeba. They are large; and they are evidently extensions of the protoplasm, including part of the endosarc. In this form there is a vertical axis connecting a dorsal and a ventral pole.

A Walking Cell. The pseudopodia of *Arcella discoides* are somewhat changeable, both as regards thickness and length. But in another cell, (fig. 4, pl. Ib), sometimes found in the sediment, at the bottom of the aquarium in which the Amoebae live, the processes corresponding to the pseudopodia of Amoeba have become fixed; and assume the character of large cilia.

In this walking cell, which resembles a *Paramaecium* more than an *Amoeba*, there is a distinct fore and aft polarity. At the anterior end, and running along one side of the elongated body, there is a groove bordered by short, thick cilia, evidently the most primitive beginnings of a ciliated alimentary canal. Unicellular algae are swept into this canal by the motion of the cilia, and pass along the groove to the posterior end.

I call this a walking cell, because the few long cilia projecting from the body of the cell are used more for walking than for swimming. The cilia are about equal in length to the diameter of the body of the cell. At the posterior end, are two long cilia which are divided at the end into three long prongs. The long cilia do not move synchronously, as is usually the case in infusoria. They move alternately in such a way as to present, under the microscope, a good image of the movements of the limbs of a walking fly. This cell rarely swims; but crawls over and between the particles of sediment, somewhat as a fly would.

I take these cilia to be a further development of pseudopodia, such as *Arcella* presents. The forked feature of the posterior ones, suggests that such cilia may, in one sense, be considered as the ,,Anlage'' of the limbs of higher forms.

I am aware of the difficulties arising from such an assumption of homology between a unicellular and a multicellular organism. But the question of homology in this case is not so important as the fact that organs are developed in a cell, which perform the same functions as similar organs in higher forms; and which confirms the suspicion that functions in onecelled organisms may be performed by structures resembling those of higher forms.

I take these cilia to be connected at their base with the contractile protoplasm of the cell. They are probably constructed somewhat like the stalk of the *Vorticella*. The coordinated action of these cilia, causing a steady progression, must involve some definite protoplasmic areas within the cell, which cause the movements. A coordination by morphological continuity of protoplasmic fibers is clearly suggested.

If it be urged that this is an infusorian, and therefore a highly developed cell, it may be answered, that the mere fact of its being a single cell, instead of an aggregation of cells, ought not to justify us in denying, on „apriori" grounds, all organization, where so many evidences of organization exist. If we deny organization in the cell, why speak of one cell as being higher than another? What does this „high" or „low" mean if it does not refer to grades of organization?

II.

Internal Evidences of Polarity.

The animal ovum. A section of the ovarian egg of *Limulus* is represented in fig. 5, pl. Ic. The germinal vesicle, in this particular egg, is seen to be amoeboid, and contracted; but otherwise normal, showing perfect fixation. It contains a large, hollow nucleolus, open at one end, and filled with a granular substance, not unlike the caryolymph, in which the crowded nuclear reticulum lies imbedded. Surrounding the germinal vesicle, and occupying the invaginated wall of the nuclear membrane, is a granular substance, which, from its relation to the nucleus, seems to have been forced out from the nucleus. In this particular instance, it is more granular than the caryolymph and stains differently. But in many cases this is seen to be a clear substance resembling the caryolymph.

I have already expressed elsewhere[1]) that this substance in the neighbourhood of the nucleus is in fact extruded caryolymph, which on coming in contact with substances in the cytoplasm, unites with this forming a new substance which stains differently from both the nuclear chromatin and the caryolymph.

From the study of dividing cells, it is clear that the caryolymph is secreted in the chromatin substance, and in the case represented in fig. 5, pl. Ic, the large nucleolus is essentially an aggregation of chromatin. The case, therefore, seems to be this: the caryolymph is produced at the expense of the chromatin. It is periodically extruded from the nucleolus; and, at first, has the characteristics of caryolymph. But on contact with proteid substances in the cytoplasm, these are split up and new compounds are formed, which behave differently toward stains than either

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[1]) Munson: The Ovarion Egg of *Limulus*, a Contribution to the problem of the centrosome and yolk-nucleus. (Journal of Morphology, no. 2, Vol. XV, 1898).

nucleus or cytoplasm. Other students of protoplasm have made similar observations.

But it is maintained by some — on what grounds is not quite clear — that this extruded substance assumes the character of archoplasm which in turn is responsible for the appearance ,,de novo" of an attraction sphere, centrosome and aster.

According to this view, the centrosome and aster are temporary arrangements of the protoplasmic granules; or, in case of an alveolar protoplasmic structure, merely temporary, linear arrangements of the vesicles during caryokinesis; the centrosome and aster being considered temporary dynamic arrangements which disappear when the dynamic forces are again at rest.

That this is an erroneous interpretation will appear, if it can be shown that the aster and centrosome may exist independently of this extruded substance, and that the socalled Nebenkern, or Yolk-nucleus, are indeed different. That the latter is the case, can be seen from an inspection of figs. 6–12, pl. Ic, representing sections of the ovarian egg of *Clemmys*.

The centrosome and aster in these eggs can be traced back to the aster and centrosome in the dividing oogonia; and are clearly not new formations from the extruded nuclear chromatin as some observers have maintained of other eggs.

The centrosome and aster form, what (for want of a better term) I have to call a ,,receptacle" into which the extruded substance (metaplasm) most readily flows; and consequently it often happens that the metaplasm entirely obscures the aster. Fig. 6 represents a section of a very young egg, showing the centrosome and aster as a crescent-shaped archoplasm, partly surrounding the germinal vesicle. In fig. 7, the astral rays are spread out; and the meshes between are filled with the granular metaplasm. In many cases, this conceals the rays; and, in most cases, also the centrosome. I take the clear, round body, in the center of the large cytocenter (fig. 7) to be the centrosome, which appears more clearly in fig. 6. I do not see how such a thing could be a mere accident. My interpretation is sustained by the appearances in fig. 8. It is here seen, that the extruded metaplasm has pushed the centrosome and aster farther away from the germinal vessicle, and has accumulated between them instead of flowing all around them. That this metaplasm does change its position in the egg; that it actually flows is evident from its final distribution througout the cytoplasm. It appears first in surprisingly large quantities in the immediate neighbourhood of the germinal vesicle, gradually accumulating in that area which is occupied by the centrosome and aster; and then spreading out radially, as if flowing between the meshes of the astral rays. In fig. 9 can be seen the intimate connection of this yolk-nucleus, or metaplasm, with the germinal vesicle; and its gradual distribution, radially, to the distant parts of the cytoplasm. As it flows on, it may form large, conspicuous bodies, in the outside cytoplasm, which are entirely distinct from the true aster (fig. 10 and 11), and which never assume the characteristics of an aster, in as much as two asters are never seen in these ovarian eggs. But this metaplasm may so accumulate around the centrosome as to entirely obscure the aster as is shown in fig. 12.

When it first appears, it is very finely granular presenting a striking similarity to archoplasm. It is not surprising, therefore, that this substance, which manifestly is metaplasm, has been taken to be archoplasm out of which the aster is finally formed. But, as this substances flows away from the aster and centrosome, it undergoes a change; loses the appearance of archoplasm; becomes highly granular, and stains much more deeply. It is in this latter, transformed condition that it assumes the pronounced characteristics of the yolk-nucleus. Fig. 12 makes this clear.

This substance, which I have shown to be the effect of nucleoplasm combined with substances in the cytoplasm, evidently serves as food for the living substance of the cell; for there are numerous cases where the aster is not obscured by this substance, nor associated with it; doubtless because it has been absorbed.

Because of its continuity with the centrosome and aster of the dividing oogonia, and because of its presence in late stages of the growing egg, I take the aster to be a constant feature of cytoplasmic structure. The metaplasm, yolk-nucleus, and archoplasm are periodic accumulations of synthetic food substances which through the influence of the caryolymph (itself the product of chromatin) has been especially fitted to be built up into living substance, by the living substance already existing. In other words, growth by intussusception is made possible by this digestion and synthetic preparation of archoplasm, metaplasm, or yolk-nucleus; which, what-ever the name applied to it, seems to be essentially the same substance, having the same history, and the same ultimate uses in the cell.

I have shown, elsewhere[1]), that this centrosome and aster can be traced back to the centrosome and aster of the dividing oogonia; that both in *Clemmys* and in *Limulus*, the centrosome and aster persist, as such, during the growth period of the ovum, often, however, under highly disguised forms, owing primarily to the accumulation of those amorphous elements in these eggs, which serve as stored up foods.

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[1]) Munson: Researches on the Oogenesis of the Tortoise, *Clemmys marmorata*. (American Journal of Anatomy, Vol. III, No. 3, 1904.)

It seems highly important that we should learn to distinguish between metaplasm (archoplasm, yolk-nucleus and all such amorphous protoplasmic inclusions which serve as food), and the structurally formed living substance represented by the centrosome and aster.

That the centrosome and aster may persist in the growing ovarian egg is doubted by some, who seem not to have studied the matter very thoroughly. They seem to find no difficulty in admitting the persistence of the centrosome in the sperm cell, the middle piece of which is often said to form the centrosome of the male pronucleus.

I have shown[1]) that, in *Papilio rutulus*, the centrosome of the spermatogonia persists as the head piece of the spermatozoon fig. 13, 14, pl. Ic.

If it be admitted that this cytoplasmic structure may persist in the sperm cell throughout its many striking changes, and then after its maturity, valid reasons should be given for refusing assent to the suggestions, — which microscopic preparations afford so abundantly, — that the centrosome and aster found in the growing ovarian egg is a direct continuation of the centrosome of the dividing oogonia.

I have shown that in the butterfly the Nebenkern of the sperm cell has a nuclear origin, being a remnant of the spindle, and that it is a temporary protoplasmic inclusion like the yolk-nucleus in *Limulus* (not the vitelline body), and the similar but more striking bodies in the egg of the tortoise. I have shown that the centrosome is entirely distinct from the Nebenkern which is gradually absorbed as food material. As the Nebenkern of the sperm seems to consist of a substance very similar in appearance to the metaplasm or yolk-nucleus of ovarian eggs, they are probably much alike, the one being a remnant of the spindle in the maturation division, the other a product of the chromatin or extruded caryolymph united with proteid food substance. If the nucleus be forced into the cytoplasm by pressure, it presents the appearance of a Nebenkern. By such pressure, the chromatin in the form of spherical chromosomes or a nuclear reticulum assumes, under the microscope, the appearance of archoplasm. We thus have a basis for understanding the similarity in appearance of the yolk-nucleus, archoplasm, metaplasm and Nebenkern in sperm cells and in egg cells. Endless confusion is apt to arise when we fail to distinguish these amorphous bodies in the cell, from the formed structures in the cytoplasm represented most clearly in the centrosome and aster.

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[1]) Munson: Spermatogenesis of *Papilio rutulus*. (Proceedings Boston Society of Natural History, 33, No. 3.)

Evidences of Persistence of Centrosome and Aster. The discovery of the centrosome and aster in the fertilized egg of *Ascaris* aroused high hopes that we had finally found some positive evidence of protoplasmic structure, more particularly an orderly arrangement of parts of the cytoplasm, which seemed to offer some basis for a morphological explanation of heredity and natural selection. These high hopes, however, seem to have been needlessly shattered by the seeming disappearance of the aster and centrosome belonging either to the egg or to the sperm. As a result, too, of Weismann's theory, which locates hereditary qualities in the nucleus, cytoplasmic structure which might form a basis for heredity has been denied. The prominent aster and centrosome, so easily seen in leucocytes, have also been described as temporary, dynamic centers arising from the movements of these cells. These are all inferences based on negative evidences. Such evidences can easily be obtained by bad methods. One investigator of the egg of *Limulus* — to give one instance — has stated that there is nothing in the egg of *Limulus* corresponding to the yolk-nucleus (vitelline body) in the egg of spiders, a statement which I have shown to be untrue[1]).

In the very youngest eggs of *Limulus*, to be seen only in young Limuli a few inches long, when the eggs enter on the period of growth, after multiplication of the oogonia, the centrosome is to be found, in the midst of archoplasm, which is clearly a continuation of the centrosome and aster of the oogonia (fig. 19, pl. Ia). It forms a crescent-shaped body partly enclosing the nucleus, and with Lithium carmine and Lyons Blue can be differentiated as a blue body from all other parts of the egg. This same body can be traced in larger animals, after two or three years of growth, up to the adult *Limulus* with ovaries filled with mature eggs. In the smaller eggs, the body may still retain the crescent form, but more often, when the amorphous metaplasm is limited, it stands out clearly as a distinct aster (fig. 20, pl. Ib). In my work on *Limulus*, I have given a full account of this centrosome and aster. Space will not permit a description here of additional observations which I have made. I invite an inspection of figs 19, 20, 22, 23, 24, pl. Ib, 25, pl. Ib. The microscopic preparations from which my drawings are made are now on exhibition at this Congress.

Special attention may be called to figs. 20, 22 and 23. Who will venture to say that the astral formation in these ovarian eggs are not, in nature, similar to the aster in the fertilized egg of *Ascaris*, fig. 15, and in the first two cleavage cells of the same egg, fig. 16. And are they not also identical with aster and cen-

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1) Munson: The Ovarian Egg of *Limulus*, a contribution to the problem the of centrosome and yolk-nucleus. (Journal of Morphology, Vol. XV, No. 2, 1898.)

trosome of the resting leucocyte, represented in fig. 17, and with those of the dividing leucocyte represented in fig. 18?

In fig. 24, a section of the egg of *Limulus*, a distinct astral arrangement of the cytoreticulum is plainly visible. The absence of distinct astral rays, as in fig. 25, pl. Ic may be due to the obscuring effects of me meabolic products, which occur in all cells more or less; and which is especially apt to be present in variable amounts in egg cells, where stored food material is one of the chief causes of the exceptional size of eggs, as compared with ordinary cells.

The apparent disappearance of the aster in *Ascaris* after each cleavage may be due to the obscuring effect of those amorphous substances. As I have shown in plate Ic, the metaplasm moves from place to place in obedience to the varying condition of tension or contraction of different cytoplasmic areas, possibly in the same way as the granules in the cytoplasm of *Amoeba* move or flow. Hence the aster may be entirely obscured or may stand out very clearly, owing in part to the union of several fibrils, and also in part to the radial arrangement of the amorphous substances.

If other ovarian eggs be examined, we shall find corresponding bodies in the cytoplasm with more or less ease and certainty according to our skill in preserving and preparing our material for sectioning. The importance of such skill need not be emphasized; for clearly, these delicate structures require the most perfect fixation.

In the resting cell, as the ovum in its long period of growth, the astral rays separate apparently into ultimate fibrils that are almost too delicate to be seen individually, with even the best microscope. Even less perfect technique reveals this body in the ovarian egg of the spider, fig. 26, pl. Ib; of the frog, fig. 27; in the fish, fig. 28; in crustacea, fig. 30; and in the bird (dove) fig. 31.

A body similar to these has been seen by, Balbiani, v. Wittich, Carus, Schütz, Julin, Mertens, Henneguy, and by many other reliable observers. In many cases, as in the crayfish, fig. 30, the body is usually found in a notch of the nucleus where it is easily overlooked.

I find a similar body in the cytoplasm of nerve cells of the brain, fig. 21. It occupies a notch in the nucleus, and is apparently the point of convergence of the many fibrils, permeating the cytoplasm of the multipolar nerve cell, and extending out into the axons and dendrons of these cells.

I have no more reason to suspect that these are temporary arrangements of the fibrils, than that the fibrils of the cytoplasm which extend into the neuraxon and spread out to form the dendrons are so many temporary aggregations of amorphous granules.

The nature of astral rays. In the striated eye-muscle of the horned toad, fig. 32, can be seen, still more clearly, perhaps, the persistence of cytoplasmic fibrils; as well as the character of the material of which the fibrils are composed. There is a beautiful serial arrangement of the cytomicrosomes, embedded in an unstainable matrix, probably more liquid than the microsomes themselves.

From what I have been able to see of the fibrils of the nerve cells, I consider them similar to the fibrils of the muscle cells, with the exception that the cytomicrosomes in the nerve fibrils are far more minute, and not so regularly arranged with reference to the other fibrils. In the muscle fibres, the elements are so arranged as to act in unison; while that seems not to be the case in nerve cells.

As the muscle cell and the nerve cell are specialized, each for the important general physiological properties of contractility and irritability which belong to protoplasm in general, it seems reasonable that we should encounter a fibrillar structure in the unspecialized cytoplasm: For clearly these fibrillar structures in the muscle cell and in the nerve cell belong to the cytoplasm, not to the nucleus primarily, as those would like to maintain who claim that structure is to be looked for in the nucleus only.

The reticular nature of cytoplasm, as opposed to the purely granular or the alveolar, becomes apparent in the formation of the aster and spindle, when cells divide, fig. 16, 18, pl. Ib. But it is equally apparent in all of those resting cells, like the ovarian egg of *Limulus*, fig. 20, and the ovarian egg of *Clemmys*, fig. 11, pl. Ic, where the aster is most distinct.

There seems to be no valid reason for assuming that the aster is a separate archoplasmic formation which grows out into the cytoreticulum or between the alveoli. On the contrary, the aster in these cells, fig. 20, as well as in the spermatocytes of *Papilio*, fig. 13, is obviously continuous with the fibrils of the general cytoplasm. The characteristic appearance of archoplasm is due to the fact that the cytomicrosomes are exceptionally small, the fibrils also being too fine to be seen distinctly. Both fibrils and microsomes seem capable of uniting with others into larger strands, which, of course, become more evidently fibrous as this union advances. The distinctness of the aster and also of cytoreticulum, is therefore subject to variation.

There are good reasons for assuming that the fibrils possess the contractility of the muscle fibre and the irritability of the nerve fibre; for as I have shown, even in *Amoeba*, the cell may contract in one region and expand in another; these, however, being so coordinated as to lead to a definite forward progression of the movable elements or granules within the cell. It is especially when the astral rays are in a state of contraction that several fibres are apt to unite. Consequently the aster becomes more conspicuous during caryokinesis than during the resting period, when the component fibrils again separate.

The presence of amorphous granules in the cytoplasm cannot of course be denied; but they are metabolic products which appear and disappear according to the physiological condition of the cell; and, in my opinion, cannot be identified as formed living substance. When those amorphous granules are abundant, they obscure the reticular features of the cytoplasm, especially in poorly prepared material, where even the fibrous network, itself, may crumble into disconnected granules.

The coalescence, as well as the separation of the cytoplasmic fibrils, may be entire or partial, in which case vacuoles appear, which give the impression of an alveolar structure. That many of the unformed metabolic substances within the cytoplasm may form emulsions, enclosing air bubbles, and other gases, as well as fatty particles surrounded by albuminous membranes like a foam, may be readily admitted; especially as observation confirms the statement. Very large bubbles of this kind are seen in beautifully preserved ova of *Clemmys*, figs. 6—10, 12. But the presence of these bubbles, emulsified fats, yolk substance, or whatever else it may be, does not necessarily make the actual, living substance a mere emulsion, having an alveolar structure.

From the appearance of the astral rays, both in the dividing cell and in the less typically arranged corresponding body, in resting cells, known as the vitelline body, archoplasm and attraction sphere, as well as from the general appearance of well preserved protoplasm, like that shown in fig. 33, d, pl. Ia, it is difficult to adopt any other view of protoplasm than that of the reticular. It seems probable that the peculiar appearance of archoplasm is due, at least in part, to the fact that, in sections, we look at the cut ends of very fine fibers.

Nature of Fibrils and Centrosome. Like the fibrils, the centrosome appears to be capable of subdivision into parts, and also of increasing in size by the agglomeration of granules. It can be resolved into a delicate network of interlacing fibrils. When the fibrils contract, the minute granules coalesce and form a larger, more conspicuous body. The occasional absence of a central granule, usually called the centrosome, is, therefore, not of much consequence, since it is the arrangement of the fibrils, which is the visible expression of cytoplasmic structure. Many recorded observations regarding the disappearance of the centrosome, as for instance, in the fertilized egg of *Ascaris*, may be due to this separation of the component particles when the fibrils are relaxed, as they are apt to be, after each mitosis.

The included granules of amorphous substances then obscure the far smaller component particles of the centrosome.

The Aster and Cytoplasm. In the spermatogonia and spermatocytes of *Papilio rutulus*, the entire cytoplasm seems to be converted into a huge aster during caryokinesis. The same is also true of the cleavage cells of *Ascaris*, fig. 16, and also in caryokinesis of leucocytes of *Necturus*, fig. 18. In this cell, the size of the aster remains undiminished during the resting or reconstruction stage, fig. 17. In *Ascaris*, the aster seems to fade away after division; and this is the case, also, in spermatocytes of *Papilio*. May it not be true, that this disappearance of the aster is more apparent than real? It is possible to trace the aster during the long period of growth of the animal ovum, even when this growth continues for several years, as in the case of *Clemmys* fig. 6—11, pl. Ic, and also in the case of *Limulus*, figs. 19, 20, 22, 23, 24, pl. Ib.

While the aster becomes less and less distinct, as the yolk granules increase, there is no excuse for inferring that it is dissolved. Exceptionally successful preparations reveal a very distinct aster in the various growth stages of the egg, figs. 20, 22, 23. These asters show distinctly the continuity of the astral rays with the general network of the cytoplasm, fig. 20; and can often be seen to occupy practically the entire cytoplasm. To suggest that these are artifacts is manifestly absurd. Compare the aster in the ovarian egg of *Limulus*, fig. 20 with the aster in the fertilized egg of *Ascaris*, fig. 15. Compare also the aster in the ovarian egg of *Limulus*, fig. 22, with the aster in the leucocytes of *Necturus*, fig. 17. They are evidently similar bodies.

The framework, so to speak, of the entire cytoplasm is an aster with rays extending from a common center in the cytoplasm (often but not always the geometrical center of the sphere), to the periphery; where, by the hardening of the interfibrillar substance, the rays form the striation of the chorion as in the egg of *Limulus*.

When projecting beyond the limits of the cell proper, these radial fibers form cilia, after secreting a thin outer covering which hardens more or less. Several such fibers may combine to form larger cilia. Where part of the reticulum enters in, a pseudopodium results, fig. 33, b, c, pl. Ia.

In typical cases, the system of astral rays present the appearance of a geometrical spider's web, but the system is the same in whatever plane the spherical egg is cut, provided the section passes through the center.

The concentric arrangement of larger granules, so clearly visible in the aster of leucocytes, fig. 17, and also evident in the segmenting eggs of *Ascaris*, is prominent, also, in ovarian eggs of

Limulus, figs. 22, 23, and also in that of *Clemmys*, fig. 11. This appearance seems to be due to a regular system of concentric fibers connecting the radial fibers at regular intervals. Occasionally this system of concentric zones is so marked, the limits between the zones so sharply defined, as to suggest the presence of concentric membranes, as in fig. 23. However that may be, it seems clear that this concentric arrangement is the same as that which appears so conspicuously in the concentric arrangement of the yellow and white yolk in the hen's egg. The latebra of the birds egg seems to be the original center of the astral system of rays, and its position is evidently determined by the original aster and centrosome, as in the pigeon's egg, fig. 31. It may be reasonable to expect that some day we shall find the egg-centrosome of the hen's egg in the latebra.

The concentric arrangement is evident also in the reptilian ovum, even after yolk bodies have formed, fig. 33, e, pl. Ia. which I have shown more fully in my work on *Clemmys*.

The arrangement of the radial and concentric fibers is illustrated in the diagram, fig. 33, a, pl. Ia. The number of radial and concentric fibers is, of course, vastly more numerous than those shown in the diagram, which is drawn solely with a view to clearness.

It is conceivable that this system of radial and concentric fibers may be compacted into a very small body; and that the whole system, when the granular inclusions are absent might occupy a space like the vitelline body, fig. 25, or even be reduced to the size of a centrosome. Would it be permissible, then, to suggest that the structural elements of the cytoplasm might be contained in the body called the centrosome; and that this may grow, when food is supplied, by a process of expansion, as is apparent in the growth of the sperm center, on entering the egg? In that case, we might say that the centrosome is the cytoplasm in miniature. The history of the vitelline body in the egg of *Limulus* sustains such a hypothesis.

III.

Polarity of the Egg.

In the typical cell, the center of the astral system, the centrosome, occupies the geometrical center of the cytoplasm. But owing, doubtless, to difference in tension of the astral rays and the accumulation of amorphous substances, flowing to different parts of the cell between the fibers, the center is often eccentric. It nevertheless determines a fixed point in the cytoplasm, which together with the nucleus, or germinal vessicle, fixes the egg axis. A line drawn through the latebra and the germinal vesicle of the hen's egg forms an axis at one end of which the vegetative pole, at the other end the animal pole, is fixed. The position of the two primary germ lapers may therefore be said to be predetermined in the ovarian egg, even from the beginning of growth, by the position of the nucleus and centrosome of the oocyte.

The position of this axis with reference to surrounding cells and tissues is difficult to determine in most eggs. But the ovarian egg of *Limulus* is especially favorable in this regard, since its connection with the ovarian tube is a point easily seen. An ovarian tube of *Limulus* is represented in the diagram fig. 34, pl. Ic.

Originally the ovary of *Limulus* consists of strings of germinal cells arranged in the form of a network. As the cells multiply the strings form hollow tubes, the cells forming a lining epithelium of the tube, and being surrounded by a network of muscle fibers. As the original oogonia multiply a differentiation occurs, some of the descendents becoming secreting cells, forming the permanent lining epithelium of the tube. These lining epithelial cells correspond to the follicle cells of other eggs; and they seem to have the same nutritive function as ordinary follicle cells have. I have shown that in *Clemmys marmorata*, the follicle cells are originally the sister cells of the egg; and that is probably the case also in *Limulus*. But, in this case, as the egg grows, it sinks down beneath the epithelium and between the muscle fibers, so that it finally develops outside the tube being connected only at one point with the epithelial cells, through which it doubtless is nourished. In my work on *Limulus*, I have shown how the ovarian egg is nourished; namely, the secretion of the lining epithelial cells is often supplied so abundantly that it accumulates outside the egg, where it resembles entirely the amorphous food substances of the egg itself.

The ovarian tube thus formed is organically united with the alveolar tissue of the ovary only along one side fig. 34, x, pl. Ic. At this point, new eggs fig. 34, a, are formed as the tube increases in diameter. Thus we find larger and larger eggs b, c, d, e, f, g, h, i and so on till we reach a point opposite x, at o, where the first and consequently the largest egg is found. These eggs are, however, discharged into the ovarian tube while still comparatively small, the true yolk bodies being formed after the egg has left the follicle and entered the tube.

If the ovarian tube be slit open, and laid out flat, the surrounding muscle fibers are seen to be so arranged as to leave oval openings large enough for the eggs to pass through into the tube. The contraction of these muscles may possibly have something to do with the expulsion of the egg into the tube. After this, the egg is apparently nourished by the secretions of the lining epithelium, which probably enters the egg through the chorion, formed by those protoplasmic processes which are responsible for the distinct radial striation of the chorion.

Direction of Egg-Axis. Examination of the diagram, fig. 34, pl. Ic, will give some idea of the different forms of centers actually observed in microscopic sections. It will be seen that some are typical centrosomes and asters. The point of attachment of these eggs (actual cases selected at random), bears no constant relation to the axis of the egg, indicated by a line drawn through the center and the germinal vesicle. It is noticable, also, that the axis of one egg bears no constant relation to neighbouring eggs. Evidently gravity does not determine the egg axis.

That this egg axis is a real, not merely an imaginary axis, is shown by the fact that when the egg matures, the germinal vessicle moves toward the periphery, away from the center. The largest accumulation of yolk is found around the center which thus locates the vegetative pole.

In the case of the epithelial cells lining the ovarian tube, the polarity might be accounted for as a result of contact or pressure from neighbouring cells, perhaps. But the eggs seem to be a law unto themselves in this regard, the axis being determined by internal structures which, aside from the actual, visible evidences of such structure, clearly point to an organization which does not in all matters and immediately adjust itself to external influences; but offers such resistance to these, as to give the egg an individuality and life of its own.

General Impressions of Specific Characters. Besides such positive evidences of structure as I have attempted to present, there are others which can neither be represented in drawings, nor expressed in words. It is the general effect or impression which one acquires gradually in a prolonged study of this kind.

It is sometimes affirmed in support of the view that chromosomes are the bearers of heredity, that it is possible to know one species of organism from another merely by the appearance of the chromosomes. It is safe to say that, besides the distinctive features of the nucleus in each case, the general appearance of the cytoplasm is different in different animals, notwithstanding the general similarity in thé cytoplasmic fibrils. The cytoplasm of *Amoeba* differs as much from the cytoplasm of the egg of *Limulus* as the Amoeba itself differs from the egg. It is possible to identify *Clemmys marmorata* or *Astacus fluviatilis* by an microscopic examination of the cytoplasm of their eggs. But just what the distinguishing features are, can better be felt than described, after a prolonged study of the subject.

IV.

Theoretical Suggestions.

It seems evident that, in dealing with protoplasm, we are not studying matter in its elementary form. When we speak of the functions of the cell, we assume that it is an organism. The history of an individual cell, like that of the sperm cell of *Papilio rutulus*, forces upon as the conviction that it is an organism, which by a long evolutionary process, has acquired an autonomy sufficient for its own needs. The ectosarc of *Amoeba*, the chorion of eggs, are, in part, secretion products, which tend to isolate the living protoplasm from other bodies; and to give a definite limit to the area within which a balance between waste and repair is maintained. There results a restricted area in which a delicate balance between analytic and synthetic chemical processes is maintained, and in which the transformation of physical forces is nicely adjusted. Antibodies, antitoxins and ferments, which many, if not all cells, produce, are also well adapted to preserve the autonomy of the cell. In watching the growth of the ovarian egg, which may extend through many years, one cannot fail to observe how food material, metaplasm, and yolk accumulate, aided by the secreting follicle, and epithelial cells. Much more of this is elaborated than is needed for the immediate use of the cell. It is probably the same with other needed chemicals and oxygen. That this gives the cell a certain independence or staying power, so to speak, is evident every where; but especially so in the egg of *Limulus*, which is able to maintain its vitality under the most adverse conditions, for many months, without food and without even the usual supply of salt water.

While we attempt to explain vital phenomena by the various tropisms, the fact seems to be that protoplasm, as it develops, becomes more and more self determined, more and more independent of external influences; and indeed resists those changes which external influences normally produce in lifeless matter. The cell seems to possess the power to regulate its responses to external influences by a process of inhibition. There is a disproportion between the stimulus applied and the results which follow. This distinguishes living protoplasm from ordinary chemical substances. Hence the difficulties always experienced in attempting to explain life in terms of chemistry and physics.

What is there in living protoplasm which is responsible for this resistance to physical and chemical influences? I take it to be the structure which has been gradually developed through a long process of natural selection, by trial and failure. The various elements of the protoplasm have finally become so adjusted to one another as to supply, for a while at least, each others physical and chemical needs; and thus giving the cell a power of resistance to new factors introduced or new forces impinding upon it. In its final analysis, is not this resistance that which maintains the species; and, in fact, that which we call heredity?

This resistance seems to remain so long as protoplasm remains uncoagulated. It ceases when coagulation sets in. The application of an acid, for instance, may so violently disturb the existing adjustment of various currents of forces, as to forever prevent their resuming the original balance, in which case the protoplasm is killed.

Development of Structure and Polarity. If we compare *Amoeba proteus* with *Amoeba verrucosa*, fig. 1, pl. Ia, *Amoeba villosa*, fig. 2, *Arcella discoides*, fig. 3, and the walking cell, fig. 4, pl. Ib, we may observe an evolution of structure of a more or less permanent type. As compared with *Amoeba proteus*, for instance, the granules in *Amoeba verrucosa* flow more regularly in restricted paths. If we attempt to explain the pseudopodia in *Amoeba proteus*, by the assumption of variation in surface tension, then we are confronted with the question why those variations do not occur also in *Amoeba verrucosa* and in *Amoeba villosa*, living as they do in the same water, under precisely similar conditions. We avoid this difficulty if we assume that the formation of pseudopodia is due to unequal contraction of the cytoplasm in different parts of the cell. With an uncoordinated protoplasmic contraction, pseudopodia will occur on any point of the body; while with a definite coordination of protoplasmic contractions, a definite direction must also be given to the flow of the protoplasmic granules.

It is conceivable that this definite flow of the protoplasmic granules leads finally to a definite linear arrangement of the particles of which the protoplasm consists.

Accompanied by a corresponding thikening of the ectosarc, which such repeated lateral contraction may in some way promote, the cell gradually acquires a definite external form, which reveals the polar differentiation already described. External evidences of polarity become marked in proportion to the development, within, of a perfect sequence between those primordial activities called irritability and contractility. The one property makes the cell aware, so to speak, of external influences; the other enables it to seek or avoid the influence and consequently to preserve its own integrity.

Both irritability and contractility are probably due to a regular sequence in the transformation and transmission of energy; and it is conceivable that out of an uncoordinated transformation and transmission of energy, a regular sequence may be established in proportion as the limited sphere in which this transformation and transmission takes place becomes isolated from direct disturbing influences from without. The all important problem, it seems to me, is the question as to the origin of the first stages in this isolation. For, when once initiated, it tends to increase as a permanent structure is developed, till the cell, like living things, instead of being at the mercy of every external influence, becomes at last able to modify those external influences, by contractility and motion, and to create to a certain extent, an environment favorable to its own well being.

In this respect and in its staying power, a cell differs from a flame of the candle to which it has been compared, and with which, indeed, it has many points of resemblance. If we could observe the structure of a burning flame, we should probably discover a regularity in the flow and sequence of the ignited particles. Similarly, it may be that regular currents of the particles composing protoplasm may acquire, from mere inertin, a permanency which resists change, and which consequently give rise to permanent lines of force, which become visible in serial arrangement of particles be they atoms, molecules, or vital units. These definite paths and permanent lines of force, like those shown by iron filings at the poles of a magnet may be responsible for the visible structure which in the foregoing pages has been described as the basis of polarity and organization. When once this regular sequence in the discharges of energy is established, it tends to perpetuate itself; and the structure becomes more definite, the external form more fixed, and polarity more pronounced.

Analogies may be of no scientific value, but they help us to make clear the ideas we wish to convey. As in the social world, so in the cell, order is a gradual development out of chaos. The processes which we see at work in the world at large gradually producing those exquisite harmonies, which we see everywhere, are probably active also in that minute world which a cell, like *Amoeba*, or an egg, represents. As potential and kinetic energy are states of one energy, so structure and function the static and the dynamic are counterparts of each other.

As there are different forms of energy, so we may assume that there are different forms of protoplasm, which indeed the microscope reveals. Protoplasm is too complex a thing to be reduced to a single chemical formula. Its activities are too varied to be explained by natural selection alone. In ignoring morphology, physiology disclaims the very reason for its existence. But on ignoring physiology, morphology becomes a mere metaphysical system of negations. Structure and function must be comprehended together if comprehended at all. The duality which otherwise arises, is probably due to the limitations of the human mind itself. Being only a part of a larger whole, it strives in vain to explain the whole in terms of a part, such as a molecule, an atom, or a biophore.

Explanation of Plates.

Plate Ia.

Fig. 1. *Amoeba verrucosa.*

Fig. 2. *Amoeba villosa.*

Fig. 3. *Arcella discoides.*

Fig. 19. Ovarian Egg of very young *Limulus*, showing vitelline body, archoplasm and centrosome.

Fig. 33. Diagram showing similarity in radial and concentric striations of different cells. a) Ideal representation of what appears in b) the egg of *Limulus*, in c) leucocyti, d) *Ascaris*, e) *Clemmys.*

Plate Ib.

Fig. 4. A walking cell with two posterior forked cilia, a ciliated groove where food is taken in and several long cilia used more for walking than for swimming.

Fig. 15. Fertilized egg of *Ascaris*, showing polar body and aster and centrosome.

Fig. 16. First two cleavage cells of *Ascaris*, showing asters and centrosome.

Fig. 17. Testis cells of *Necturus* showing aster and centrosome in resting stage.

Fig. 18. Testis cells of *Necturus* showing asters and nucleus in Karyokinesis.

Fig. 20. Ovarian egg of *Limulus* showing aster and centrosome, similar to that of the fertilized egg of *Ascaris.*

Fig. 21. Multipolar nerves cells showing fibrils, and their convergence in a body resembling centrosome being in a notch of the nucleus.

Fig. 22. Ovarian egg of *Limulus* showing vitelline body as a centrosome and aster with concentric zones resembling those seen in the diving testis cells ef *Necturus*, Figs. 17 and 18.

Fig. 23. Ovarian egg of *Limulus*, gold chloride preparation, showing a more distinct limitation of the concentric zones.

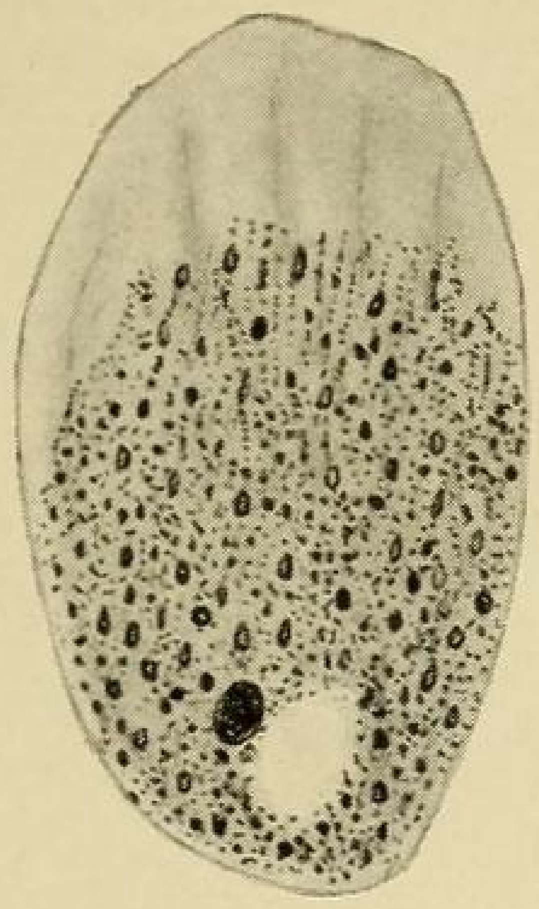
Fig. 24. Ovarian egg of *Limulus*, showing vitelline body with astral rays.

Fig. 26. Ovarian egg of the spider, showing yolk nucleus resembling that of *Limulus*, Fig. 25.

Fig. 27. Small ovarian egg of the frog, showing a body in the cytoplasm resembling a yolk nucleus or vitelline body.

Fig. 28. Ovarian egg of the goose fish, showing at one pole of the nucleus, a sphere with central granules and archoplasm extending part way around the nucleus.

Fig. 30. Ovarian egg of the crayfish, showing an indented germinal vesicle, with modified protoplasm at the indented pole, in which a round vesicle appears, the whole resembling archoplasm.



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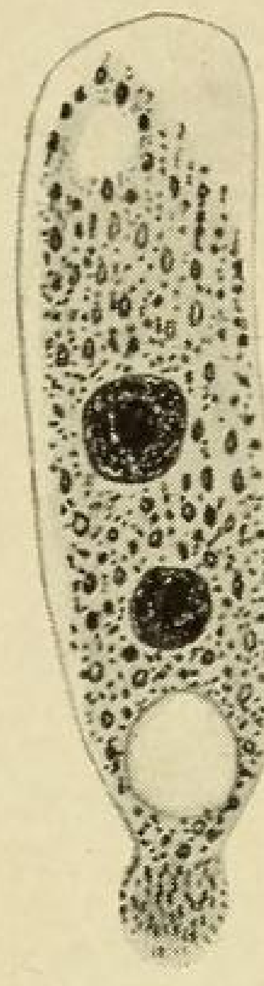

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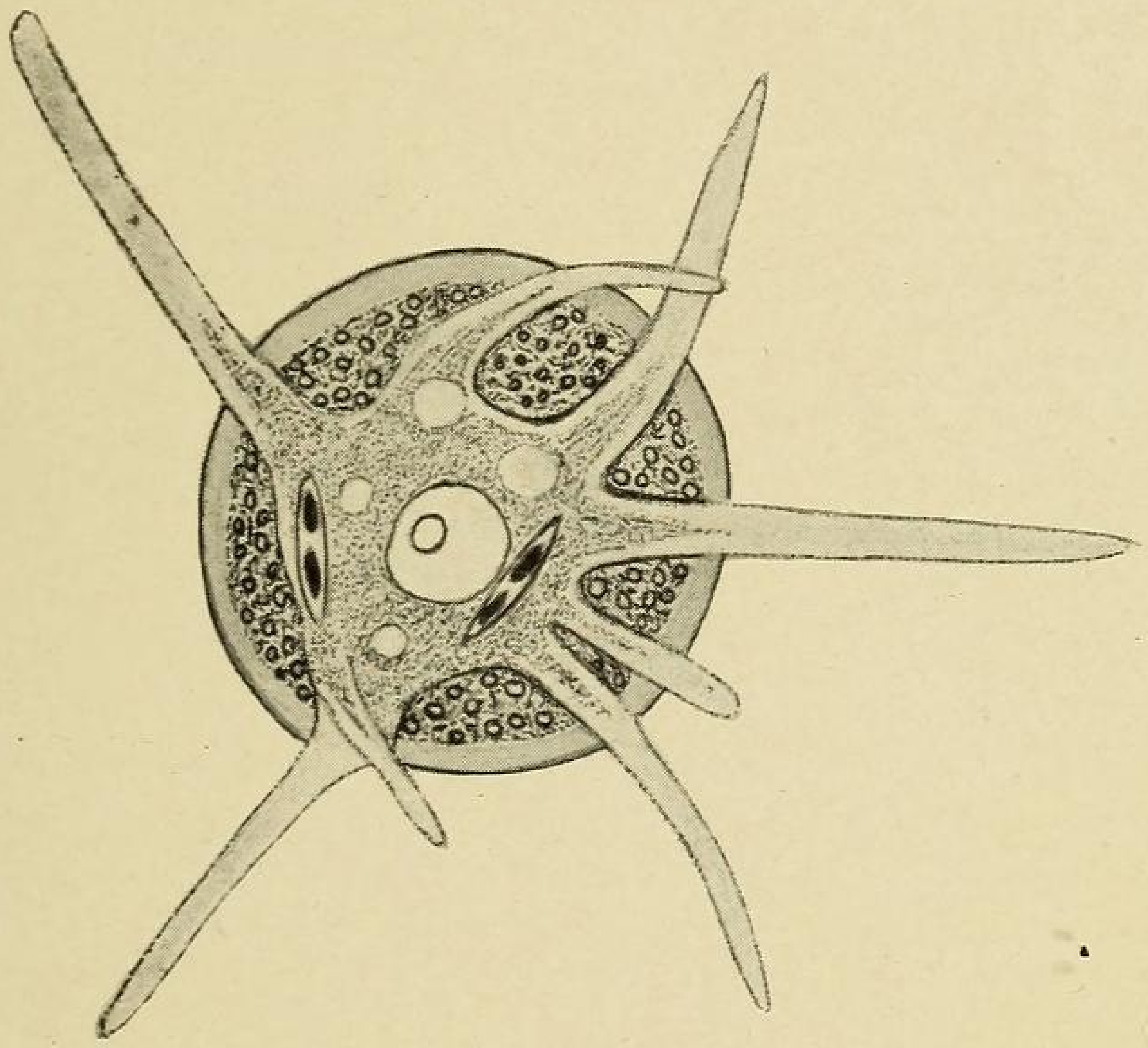

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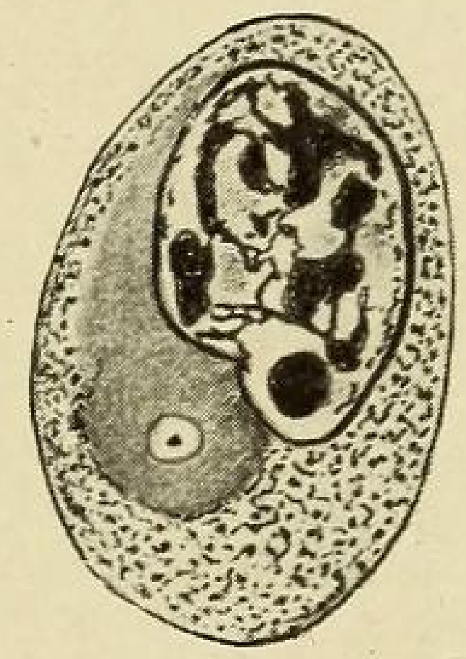

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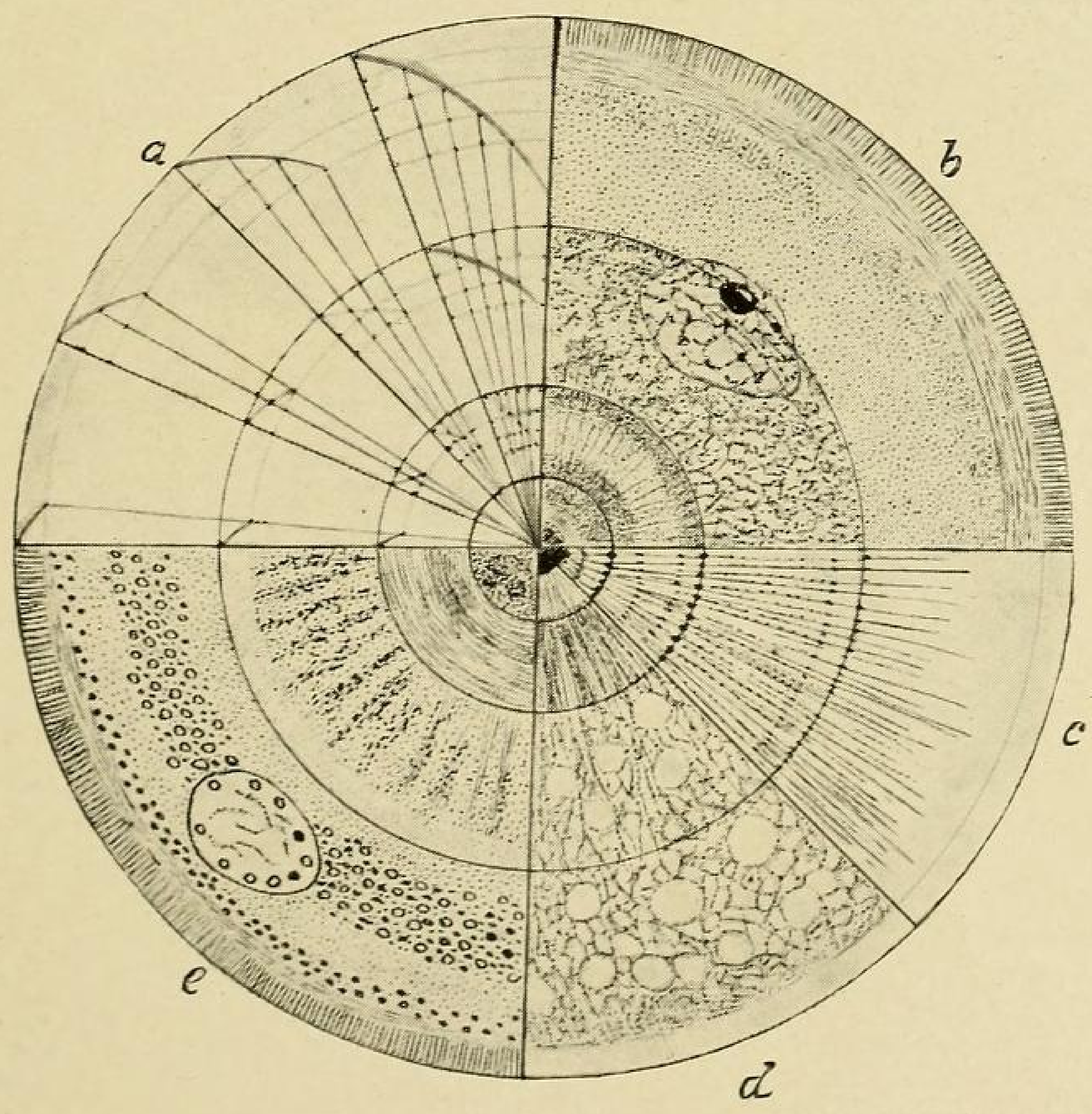

Fig. 33.

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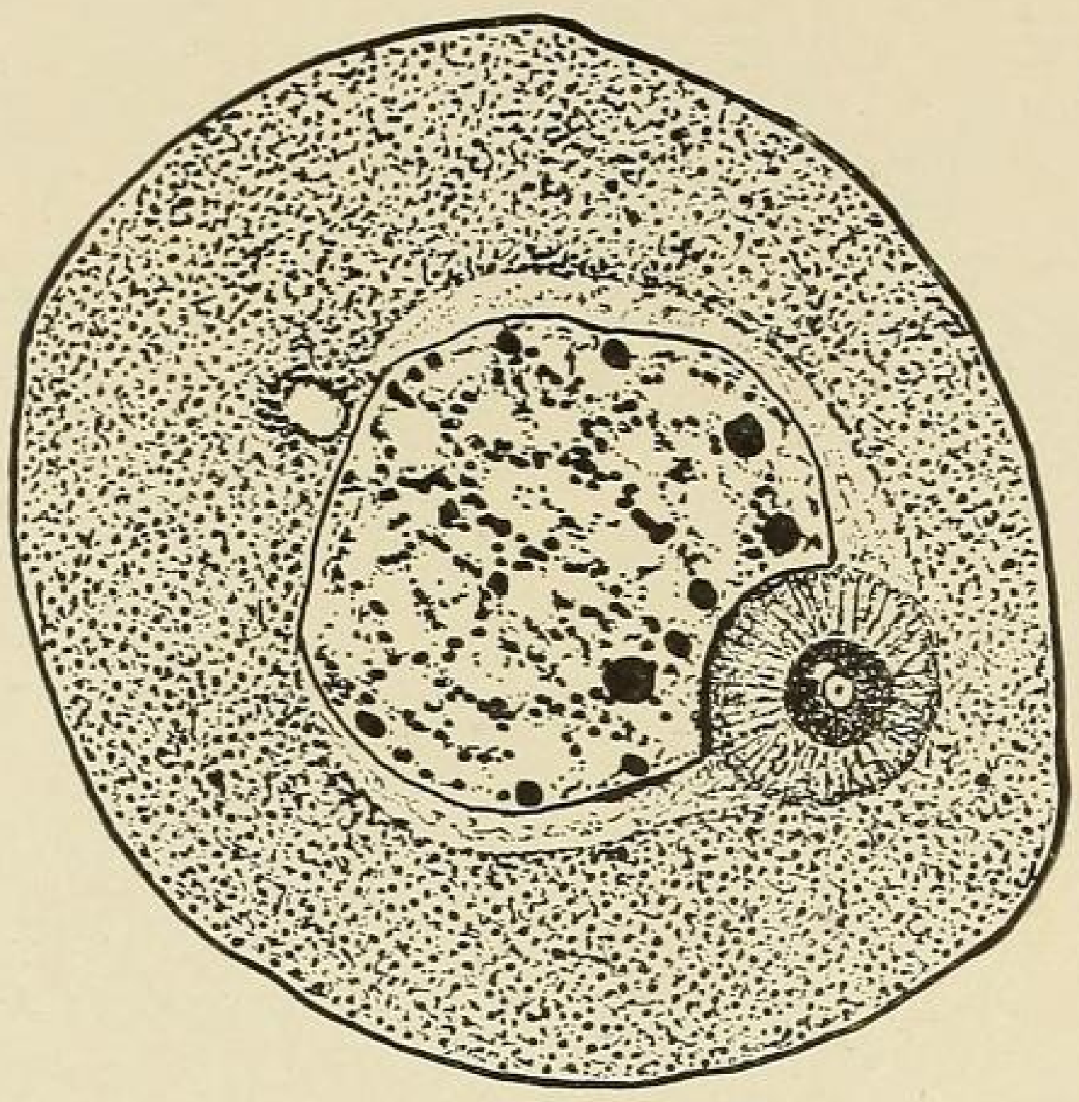

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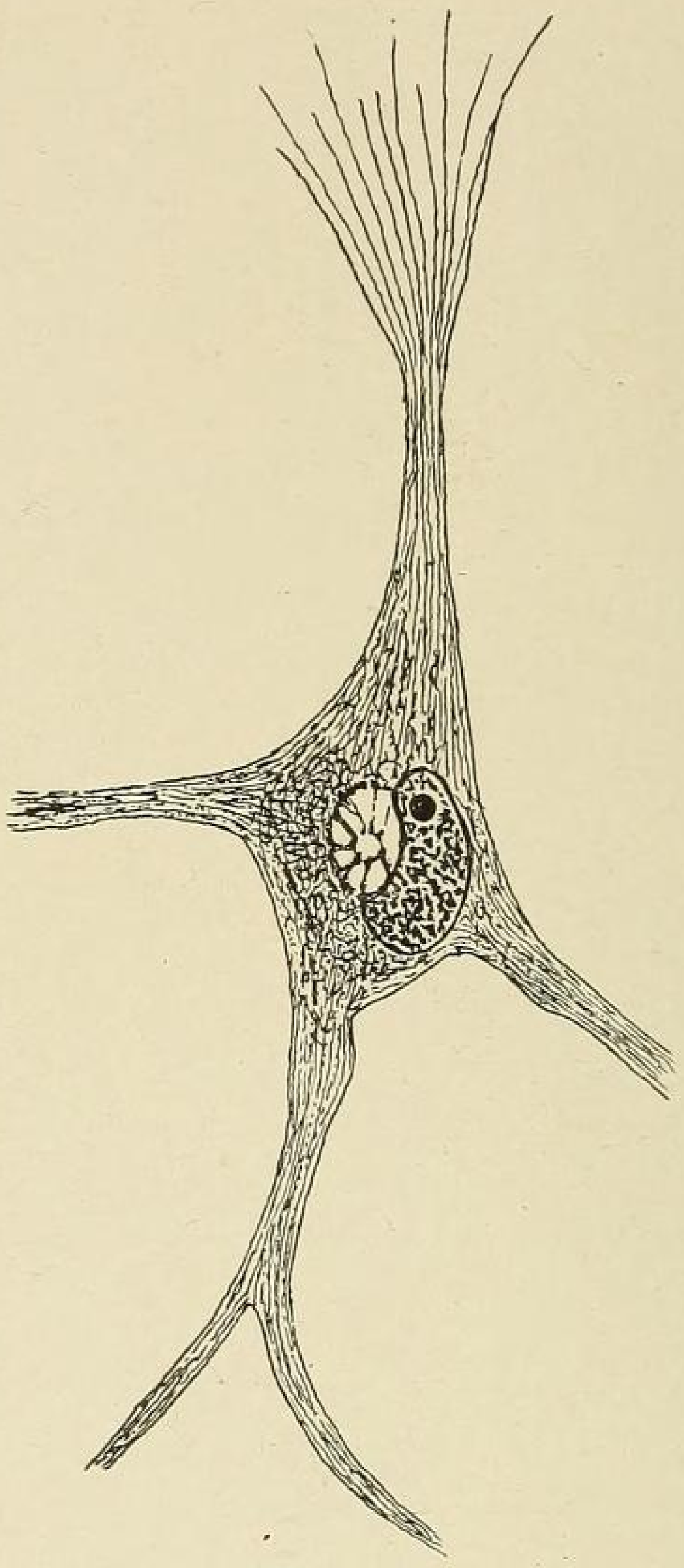

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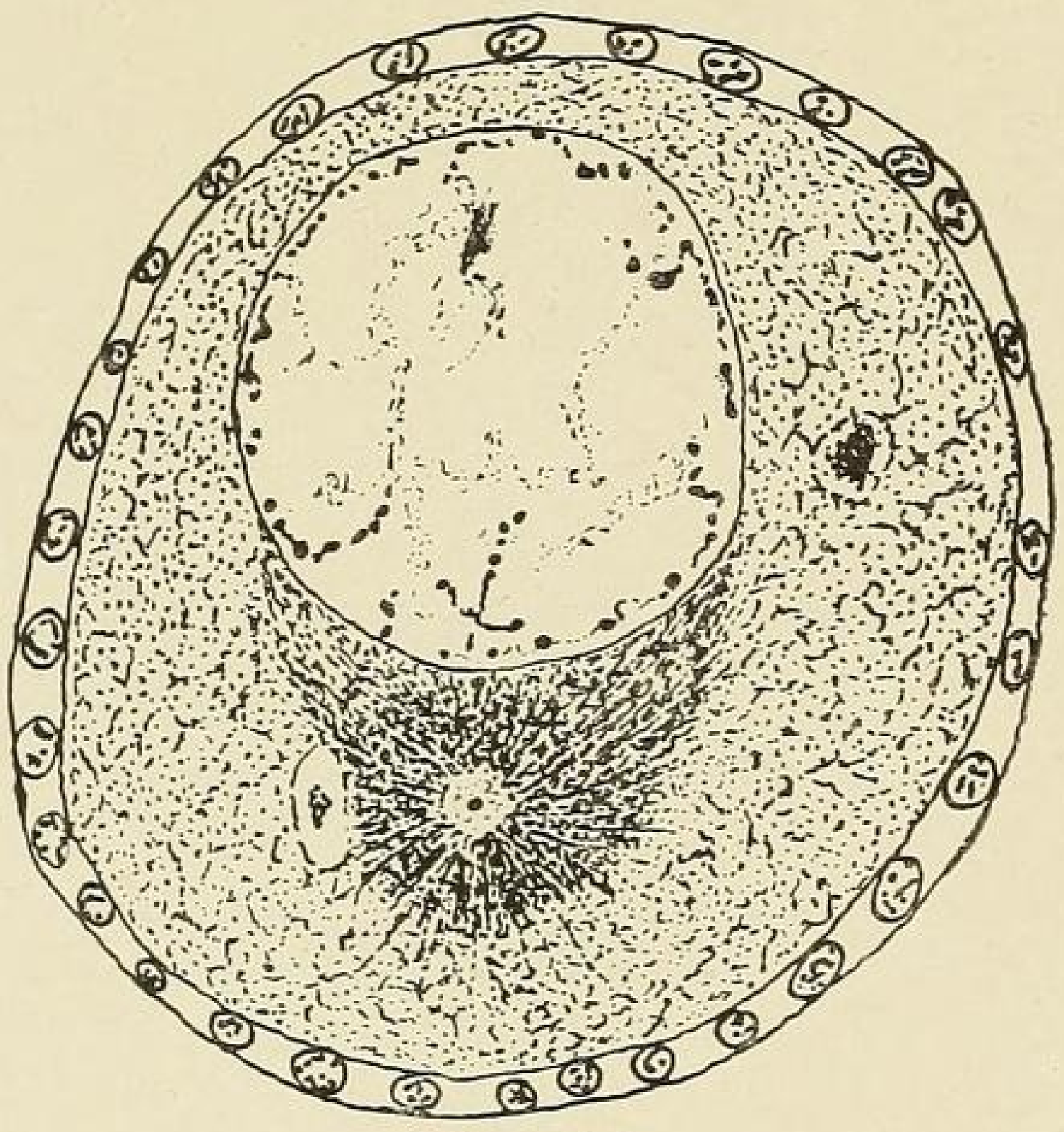

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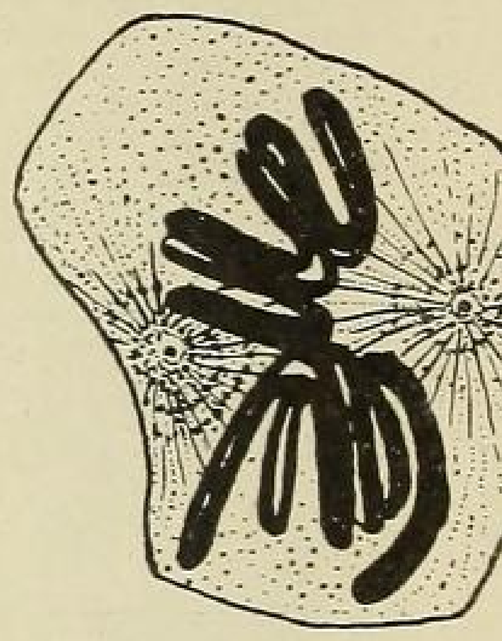

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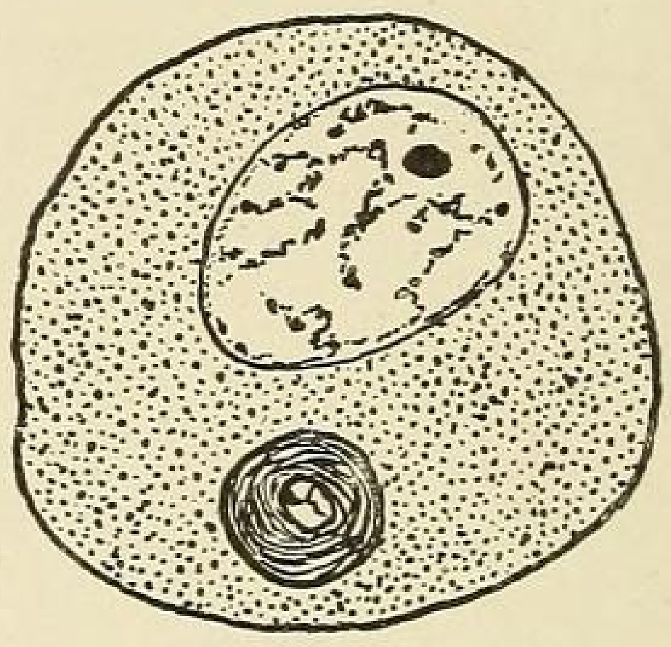

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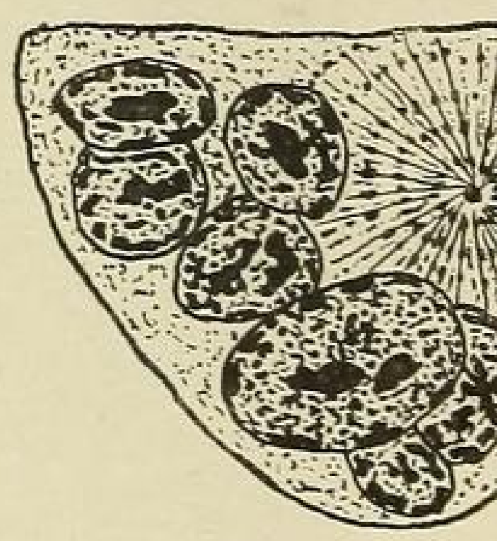

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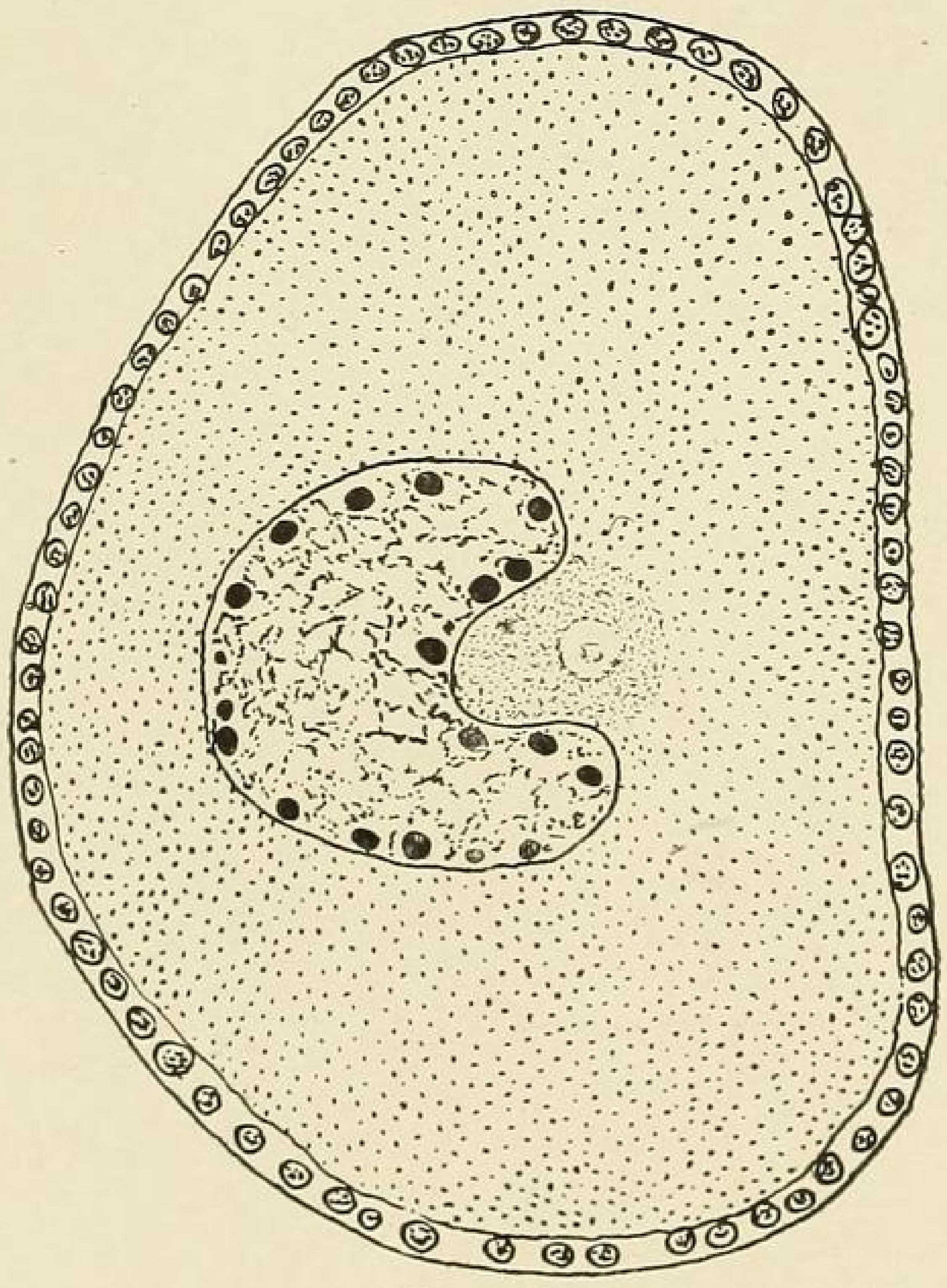

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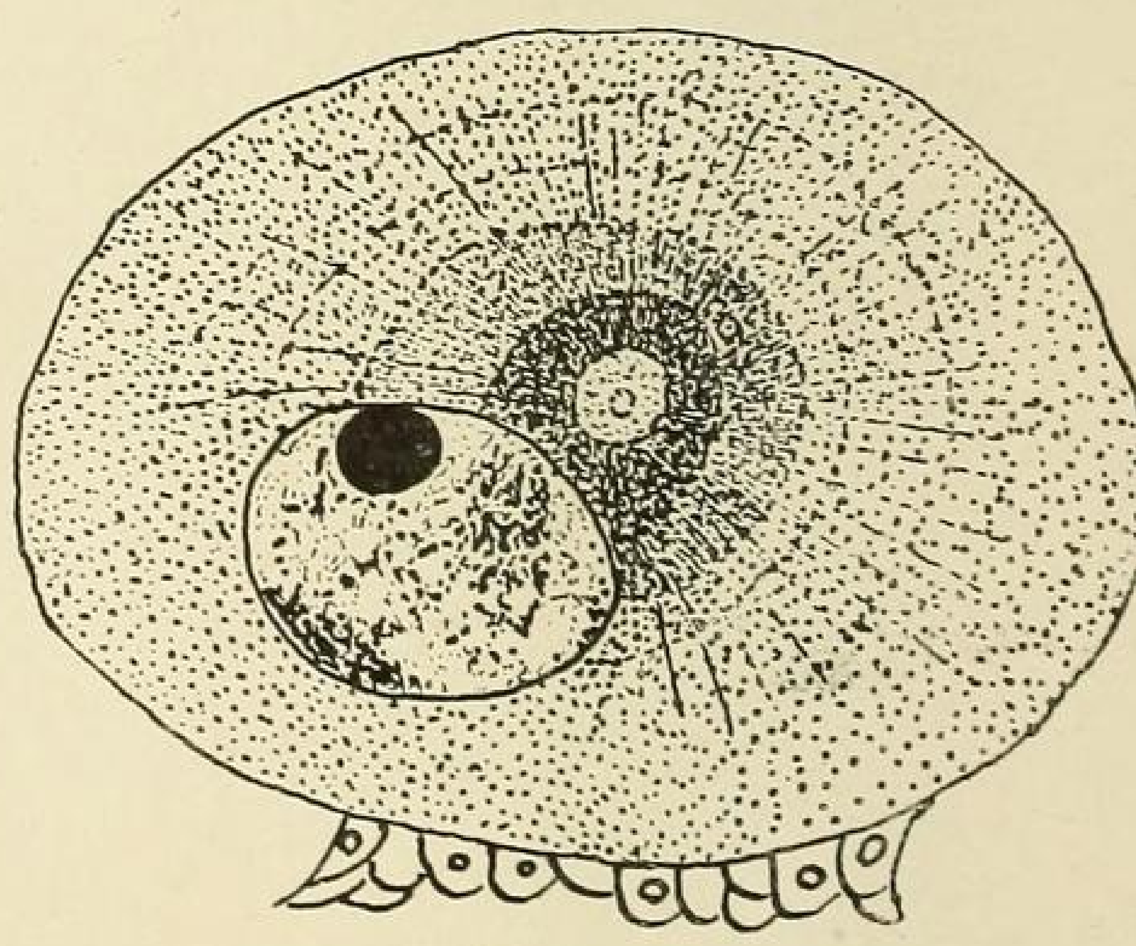

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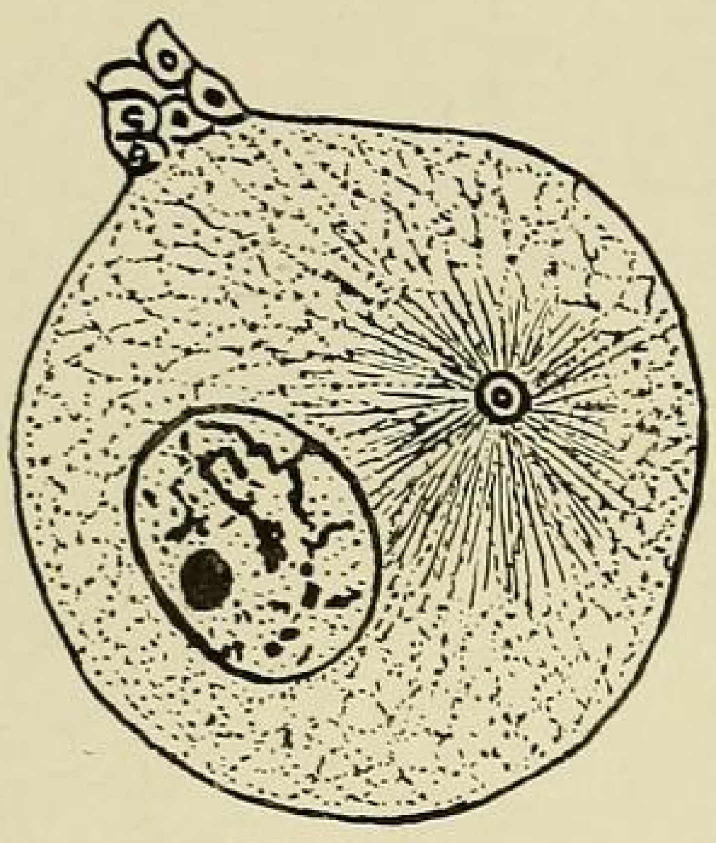

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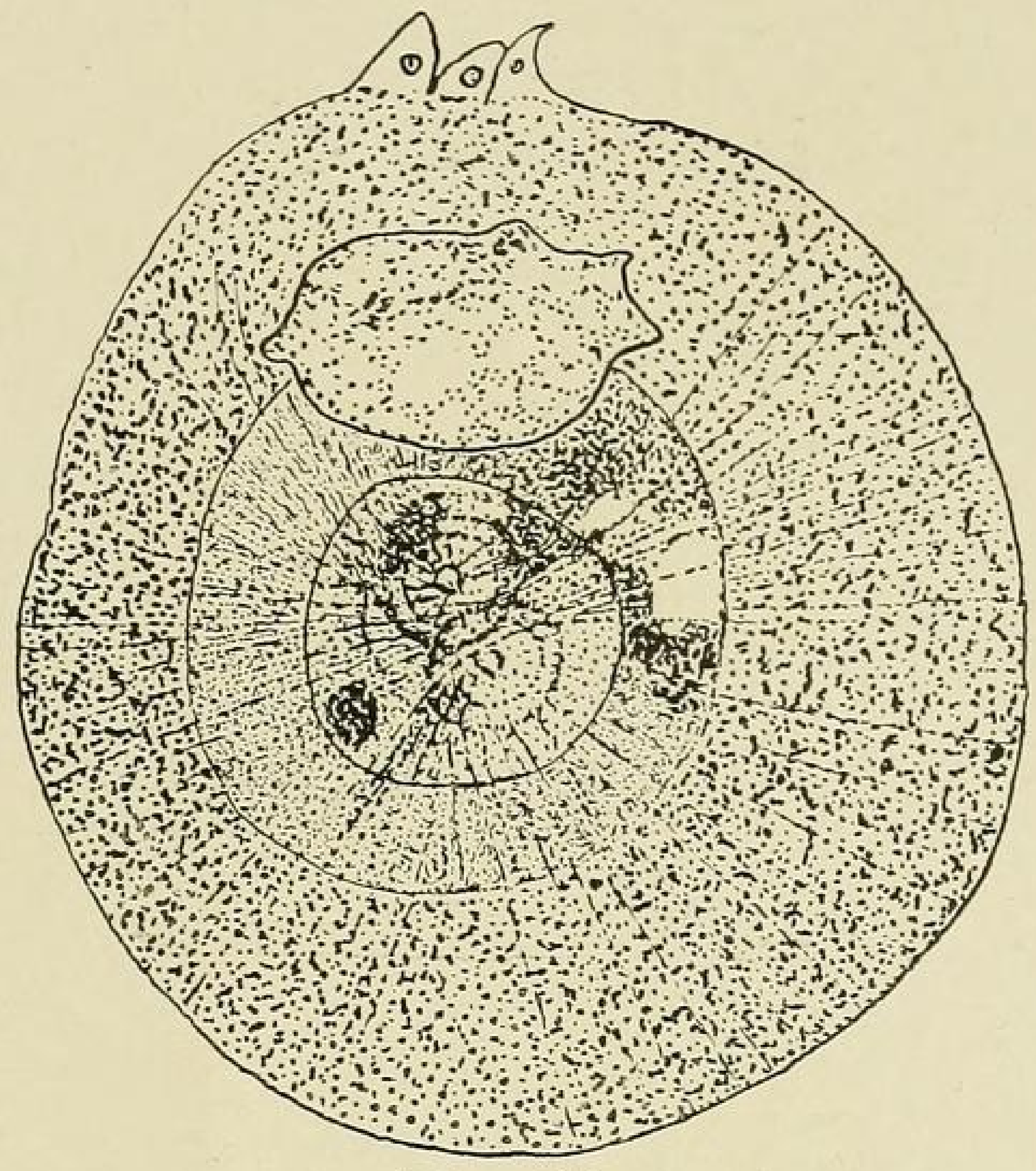

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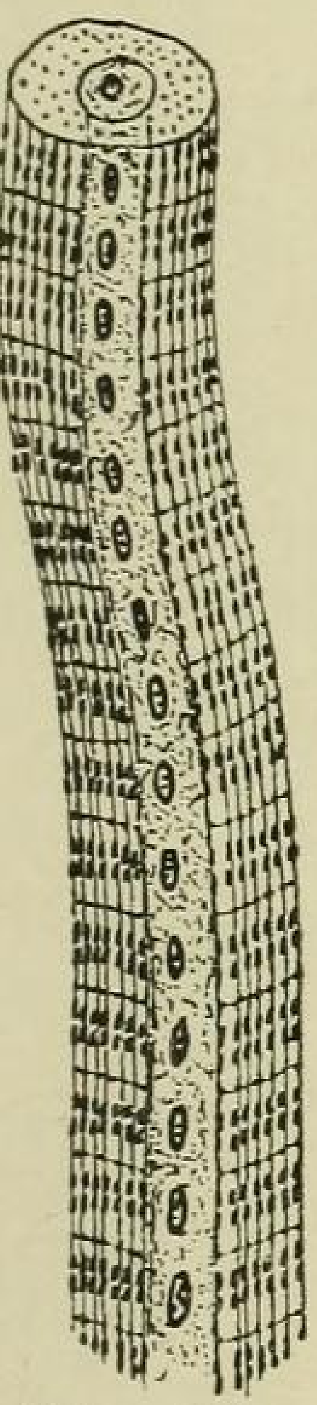

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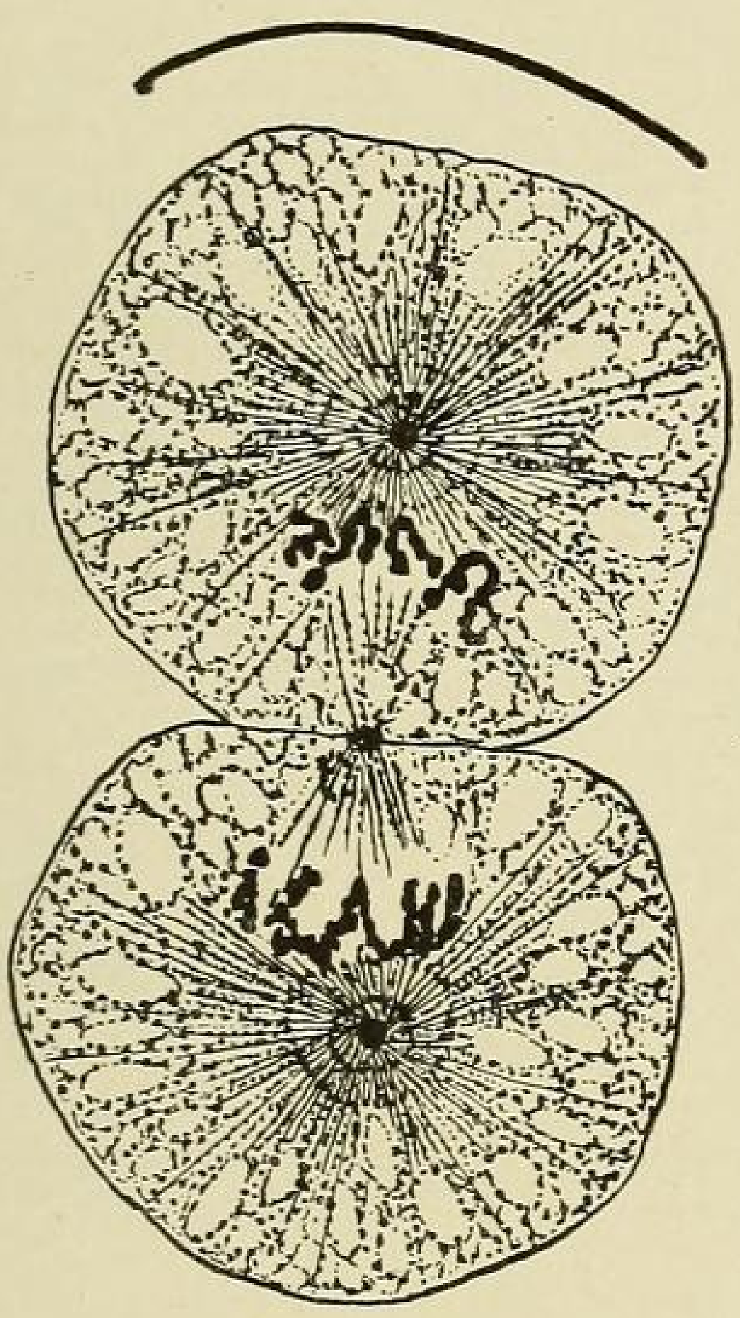

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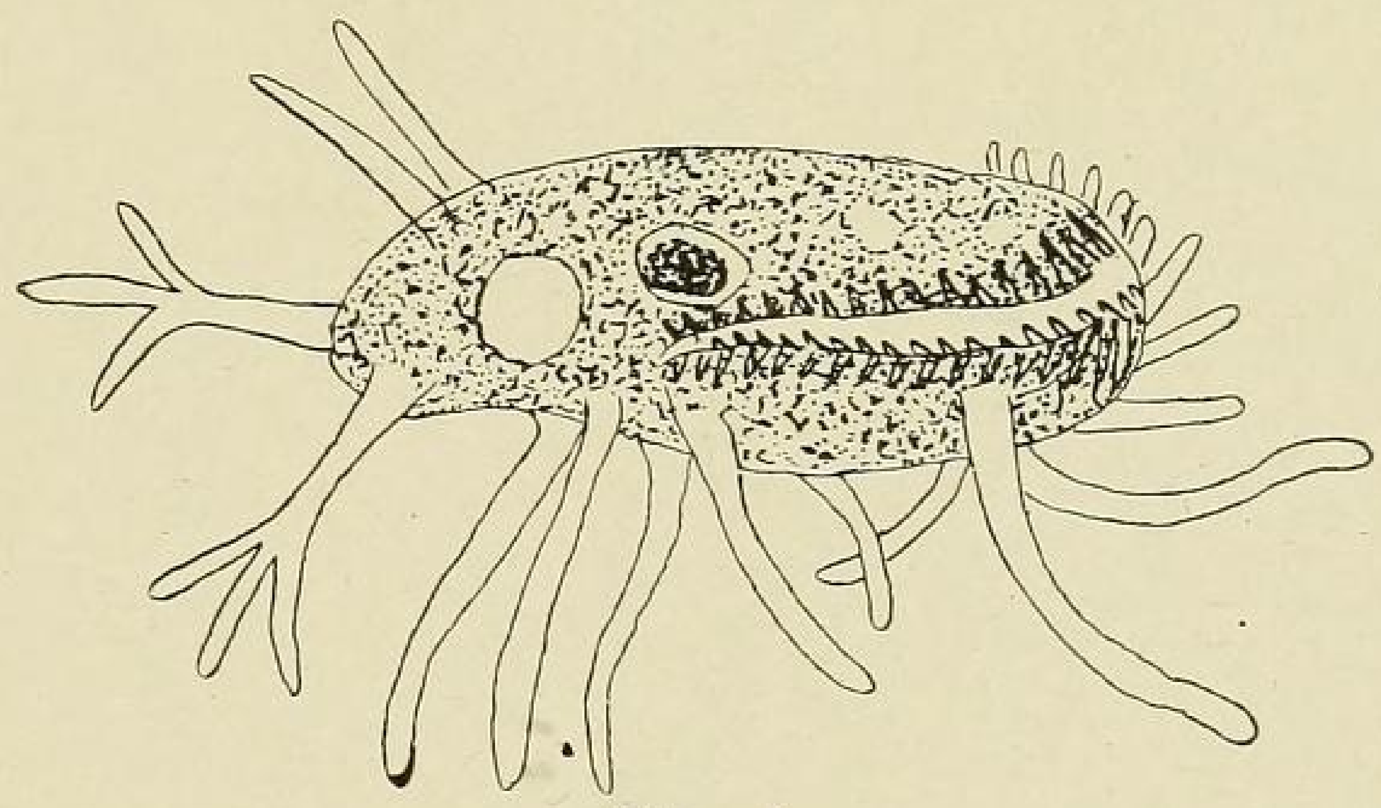

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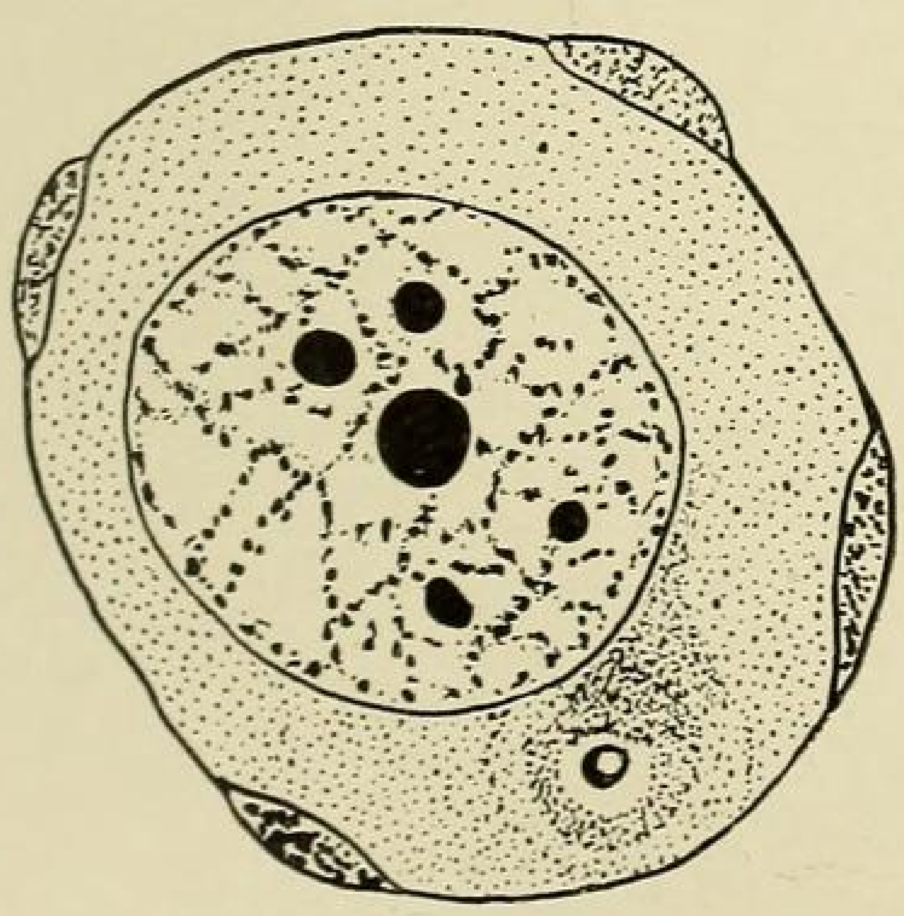

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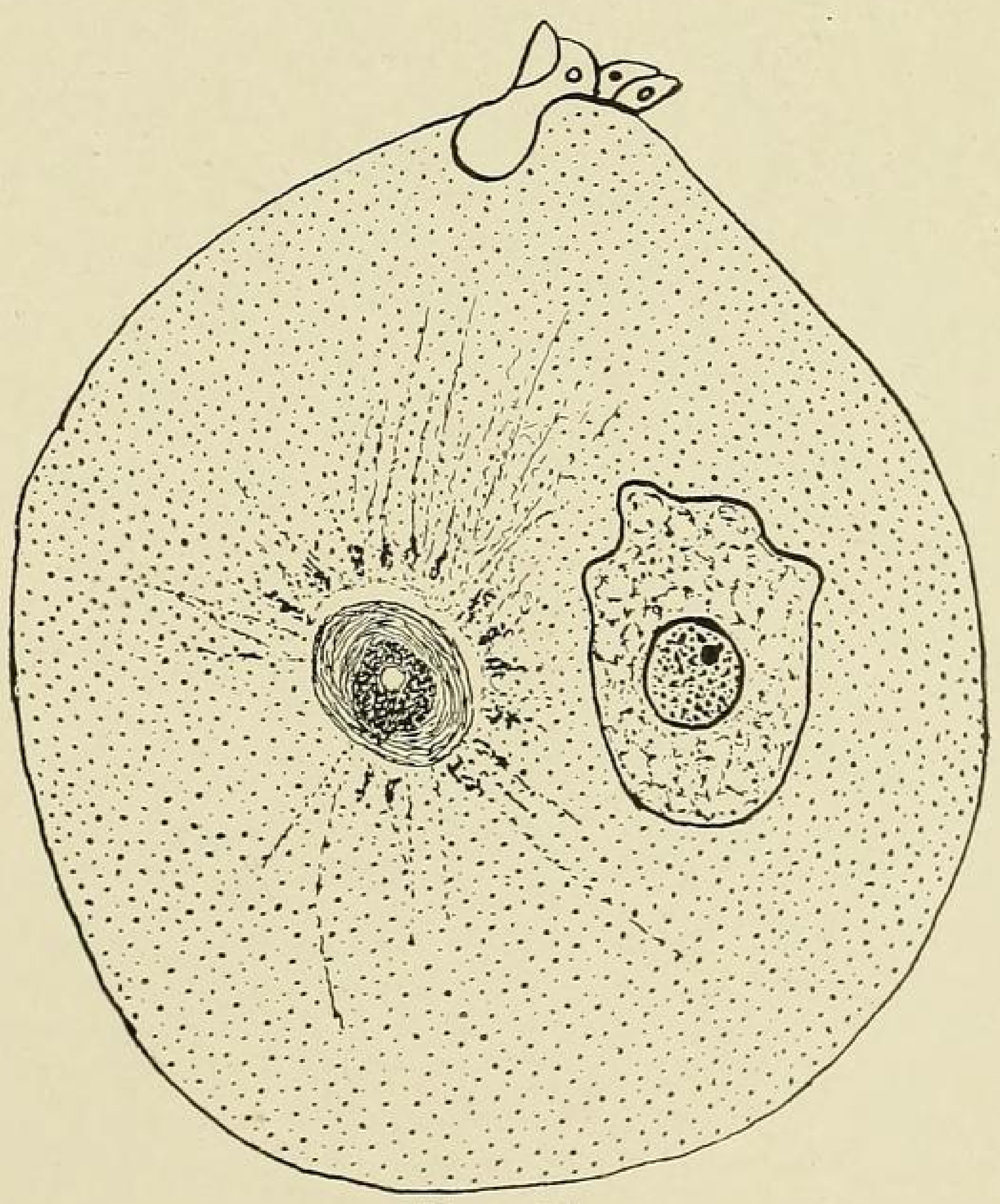

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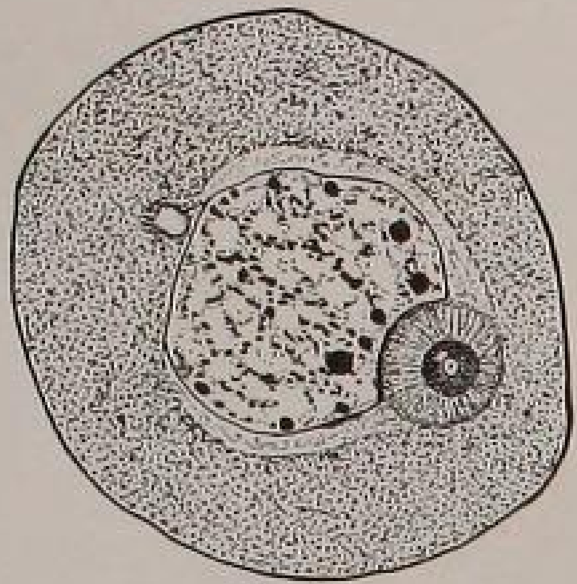
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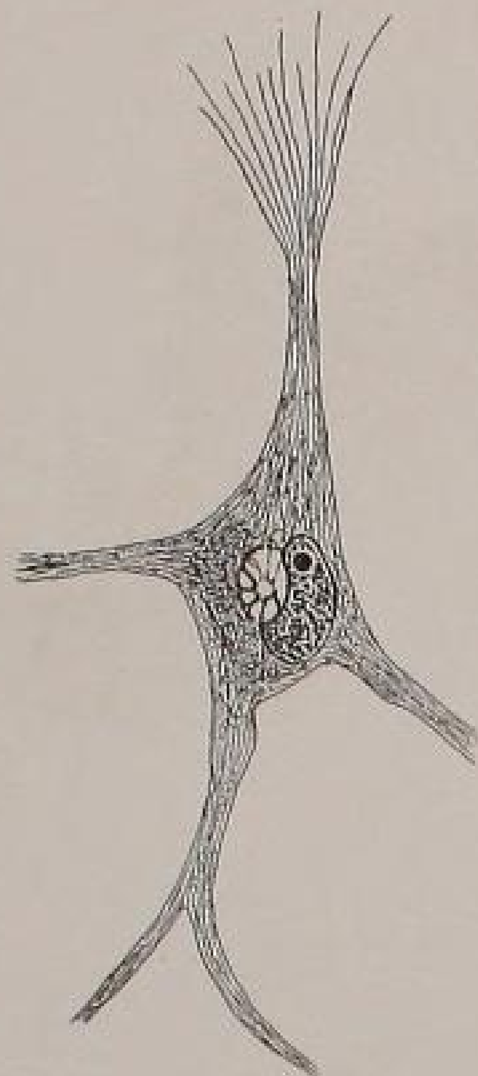
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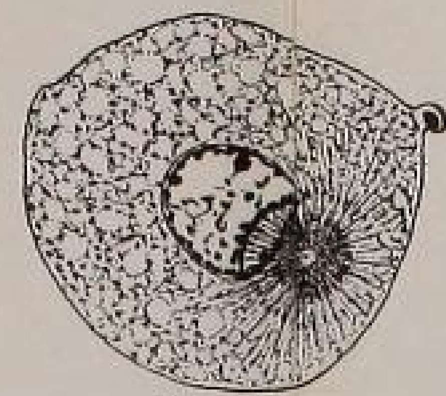
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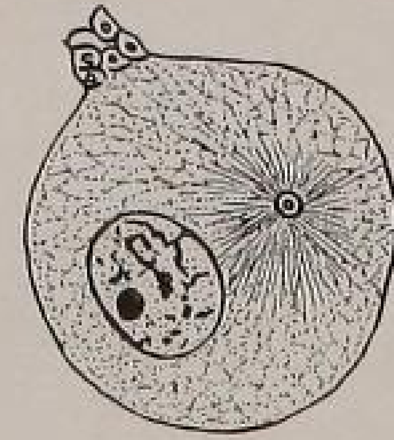
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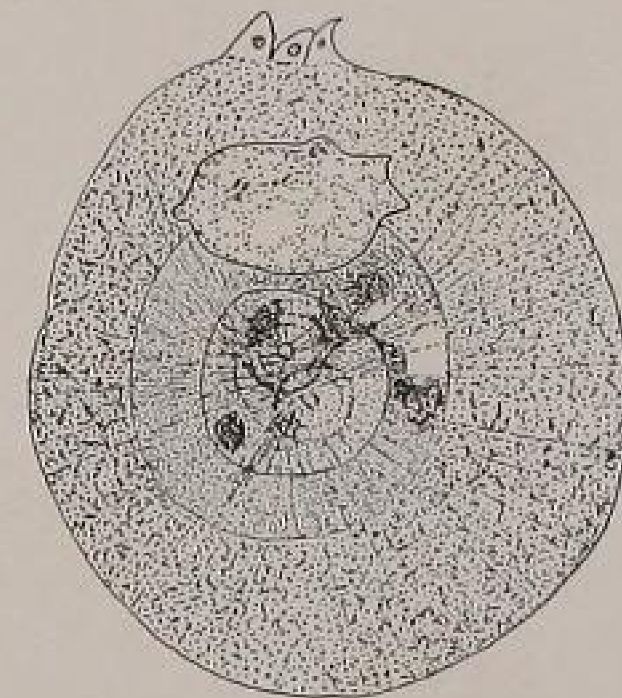
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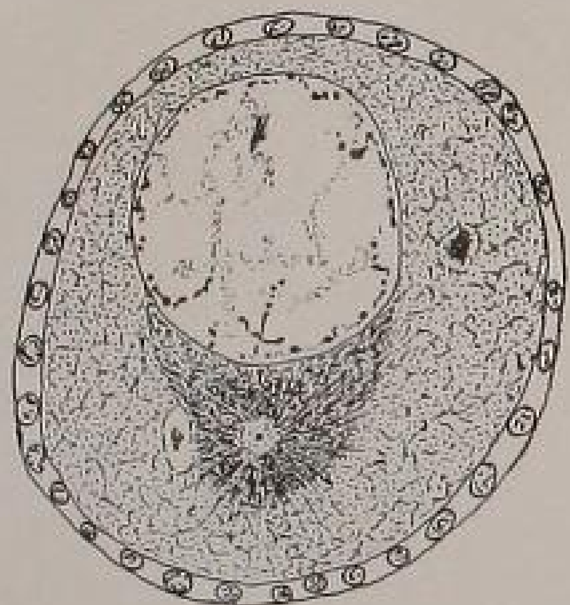
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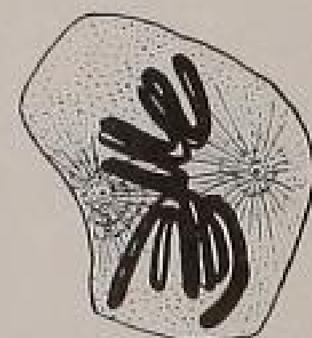
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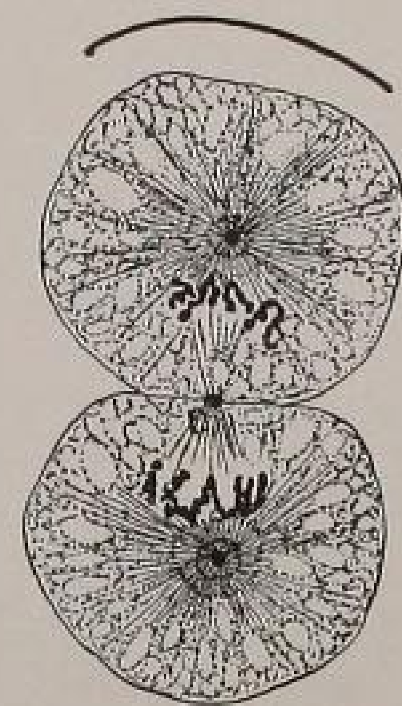
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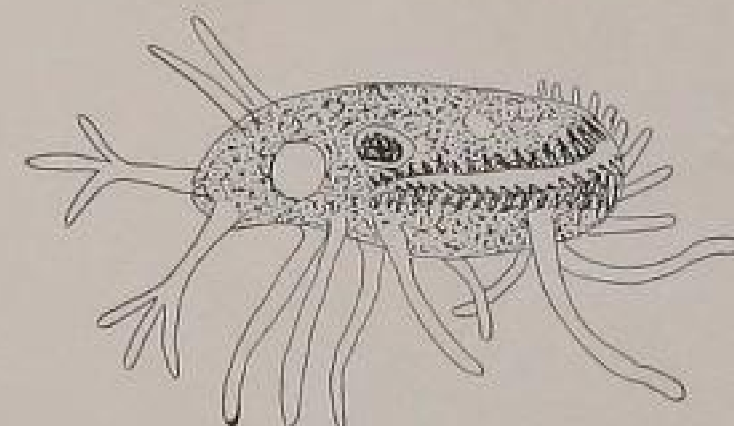
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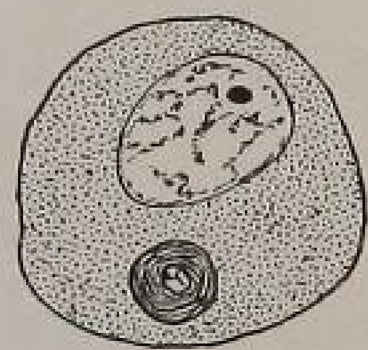
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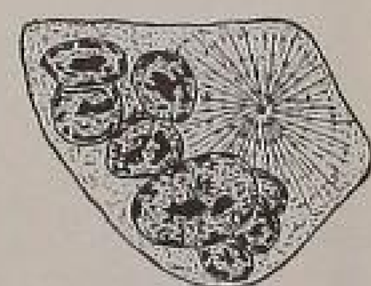
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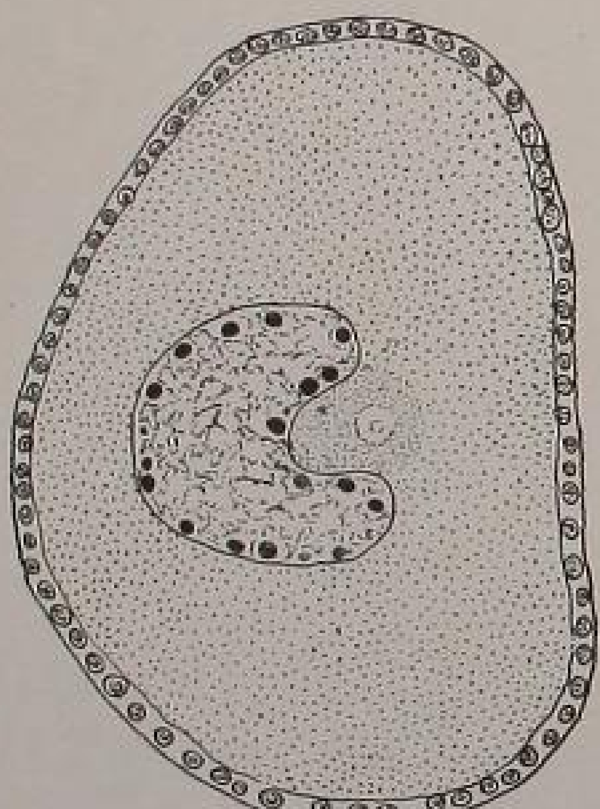
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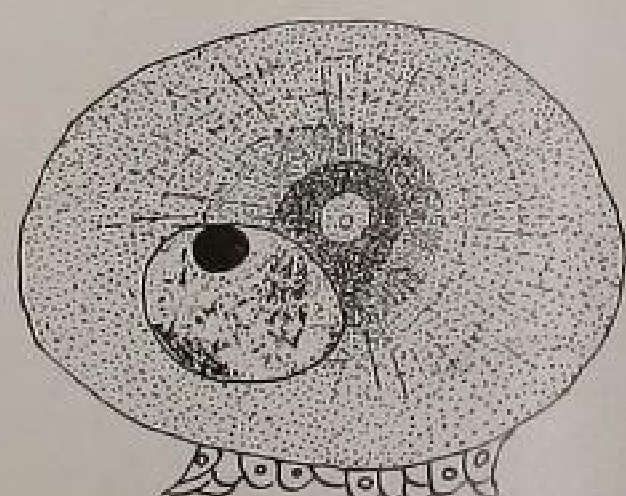
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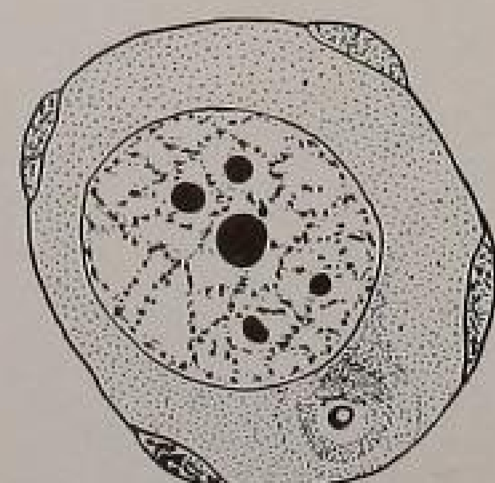
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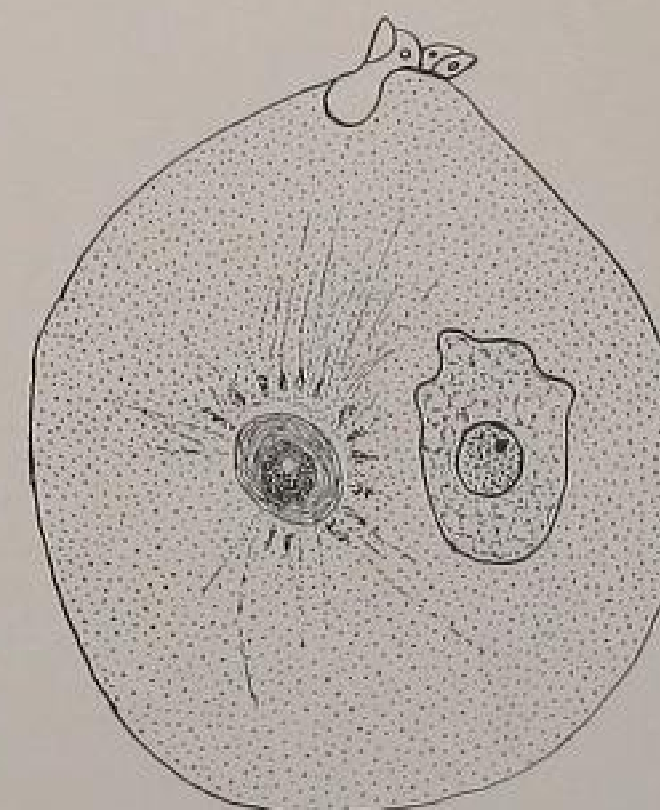
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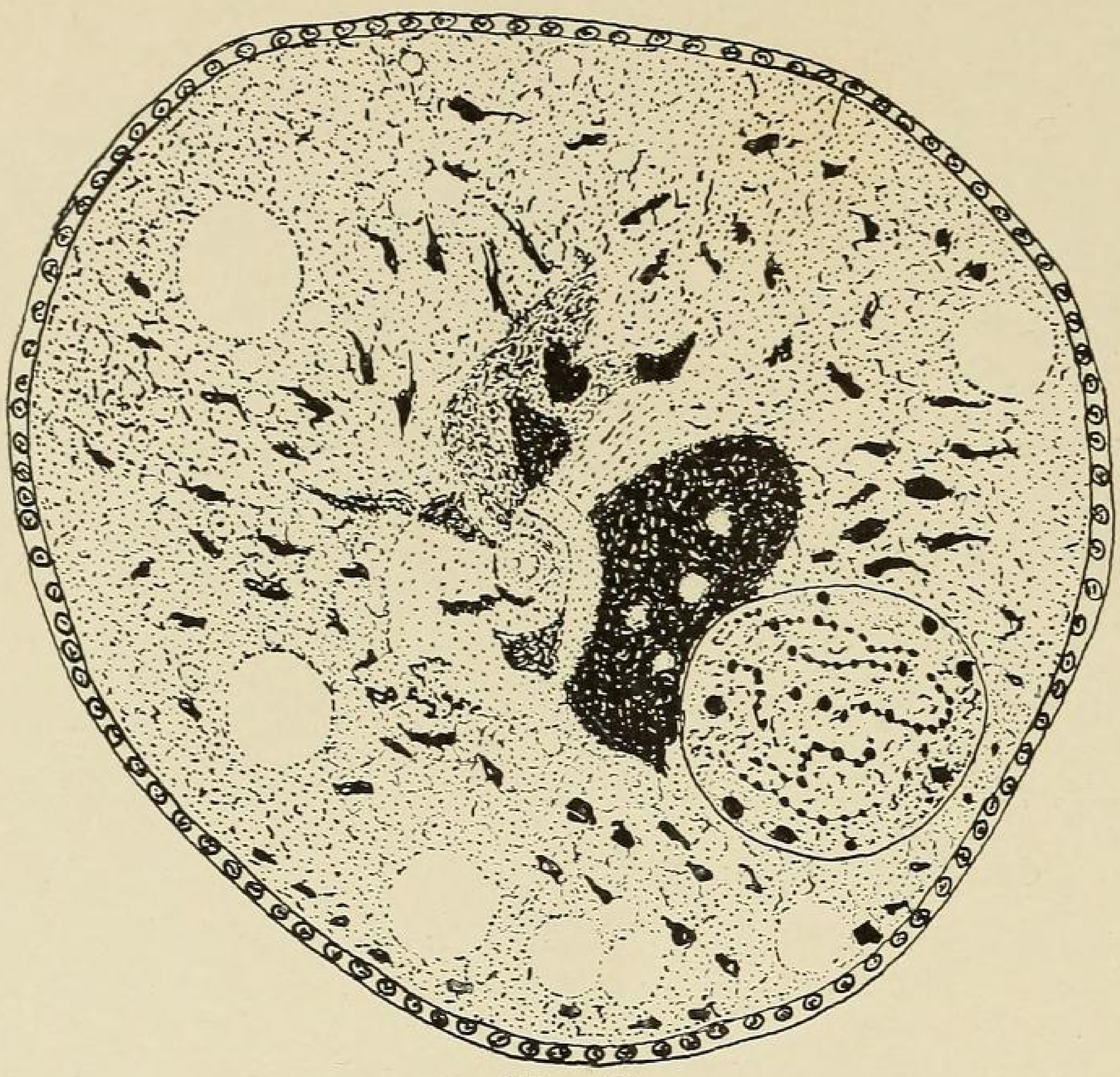
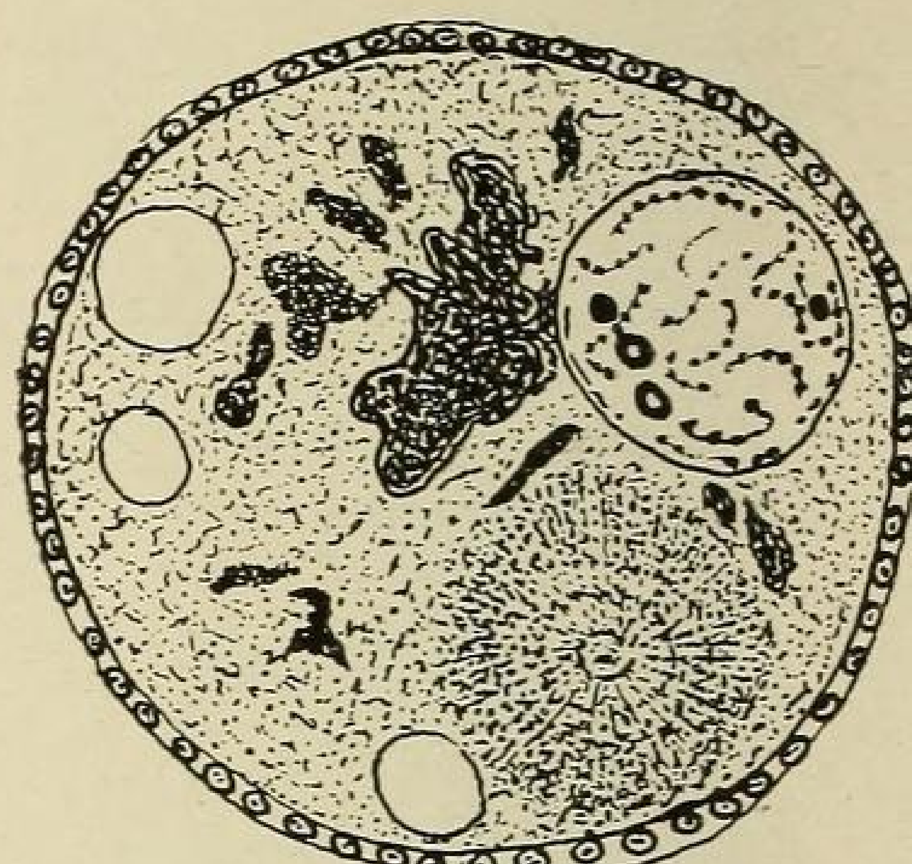


Fig. 8.



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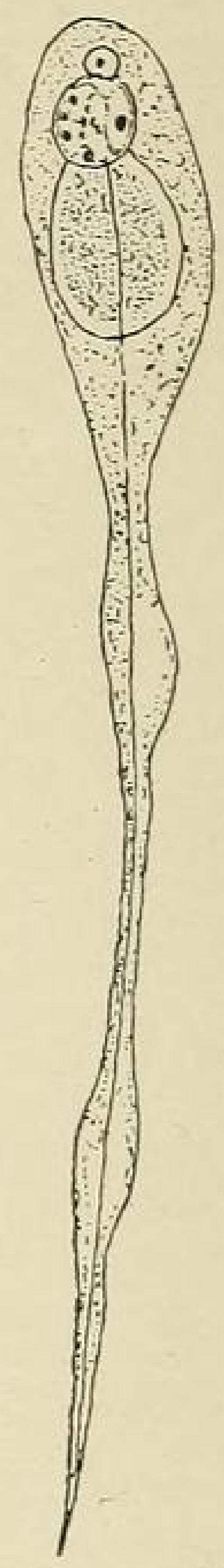

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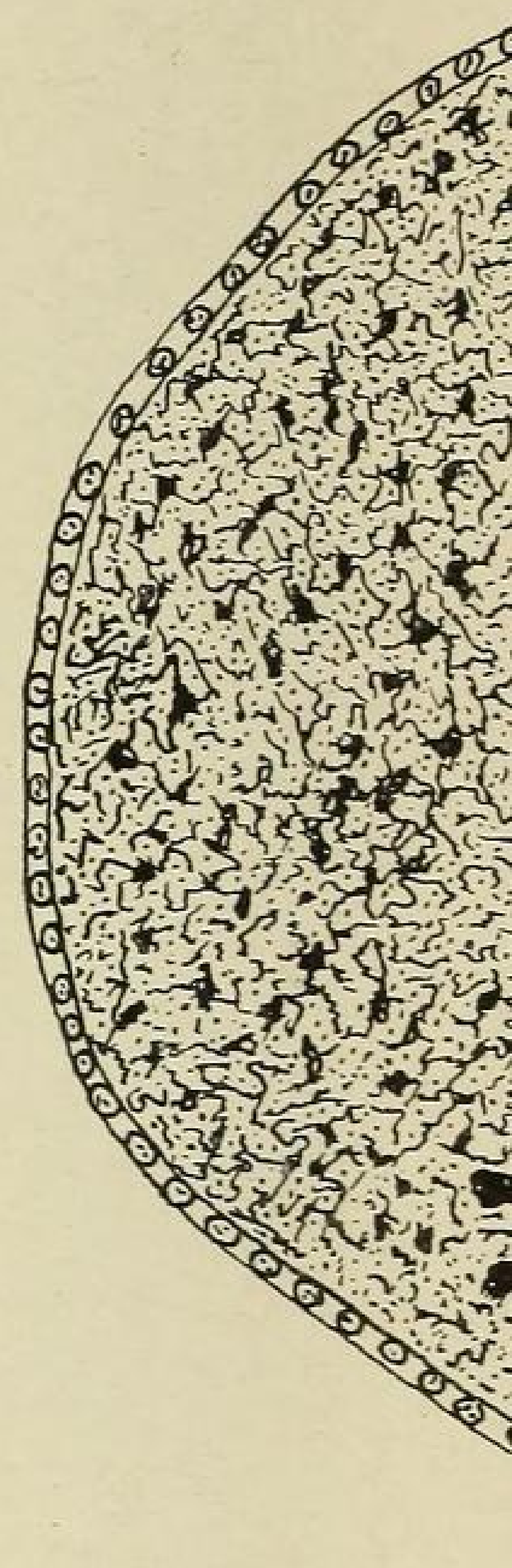

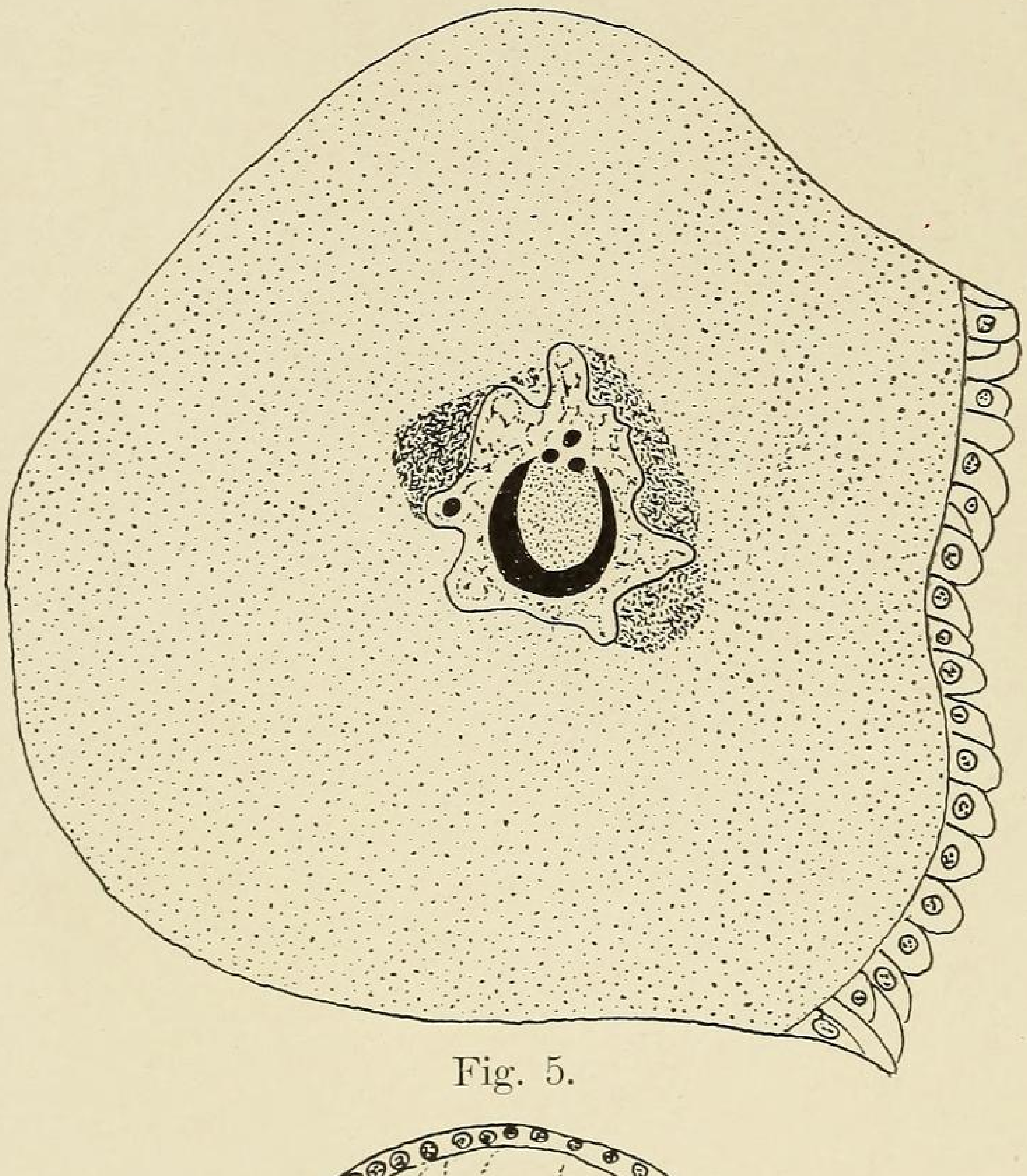

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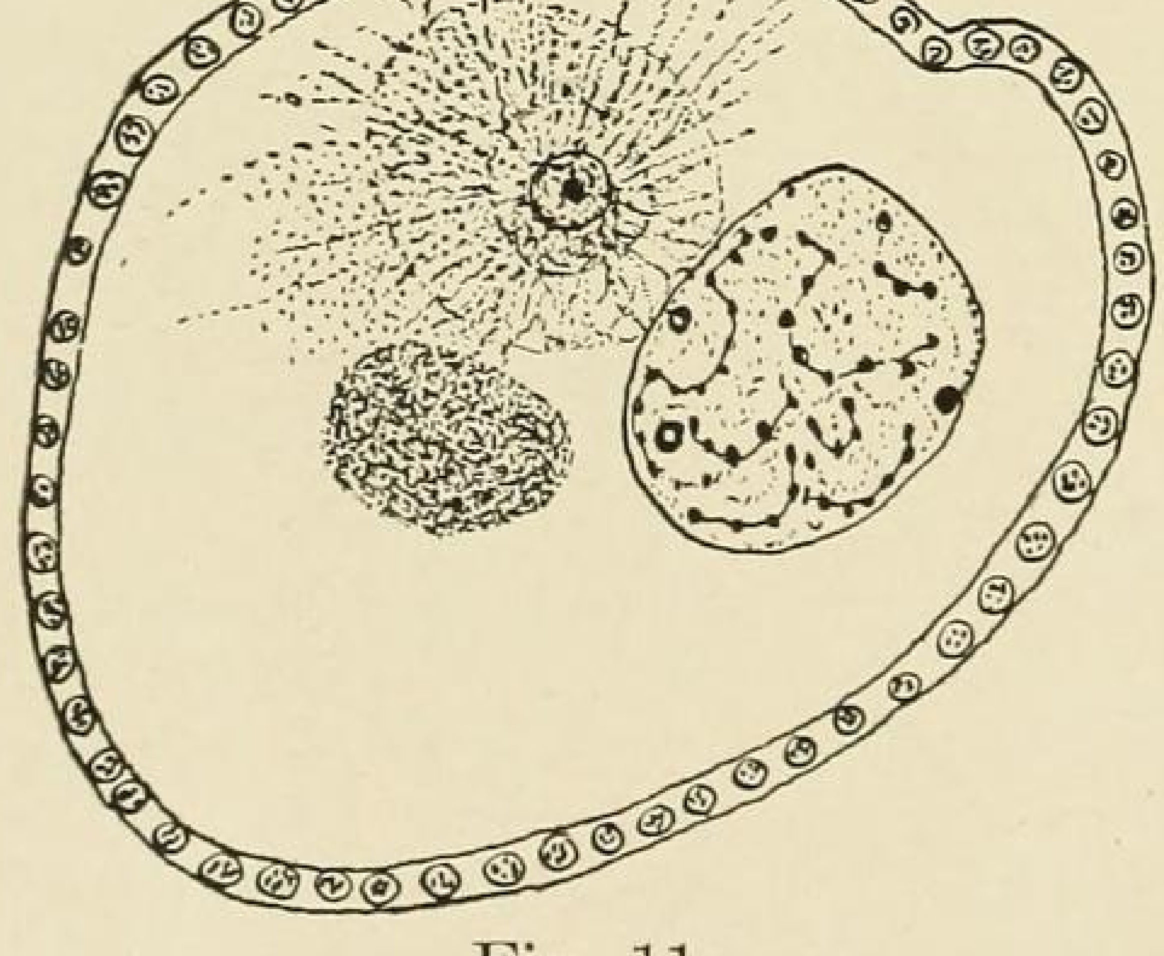

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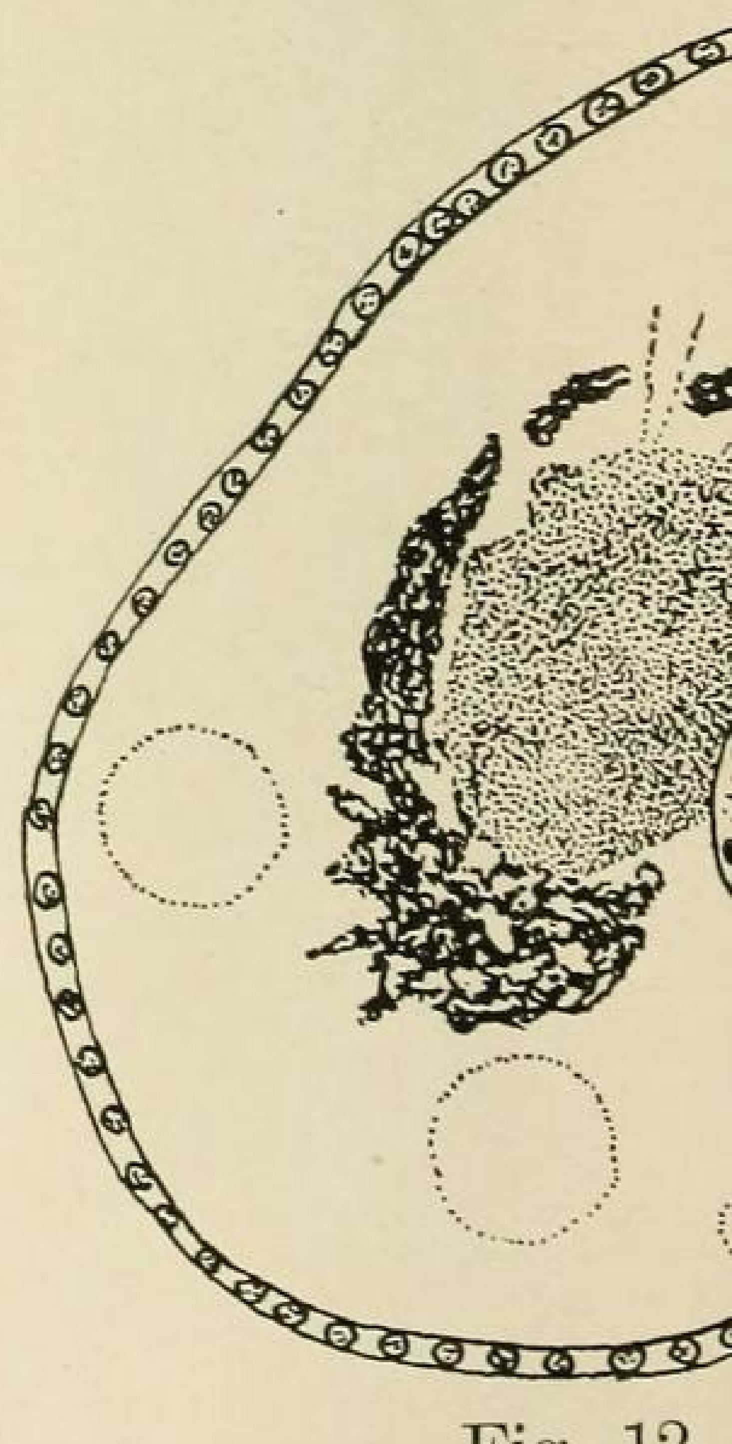

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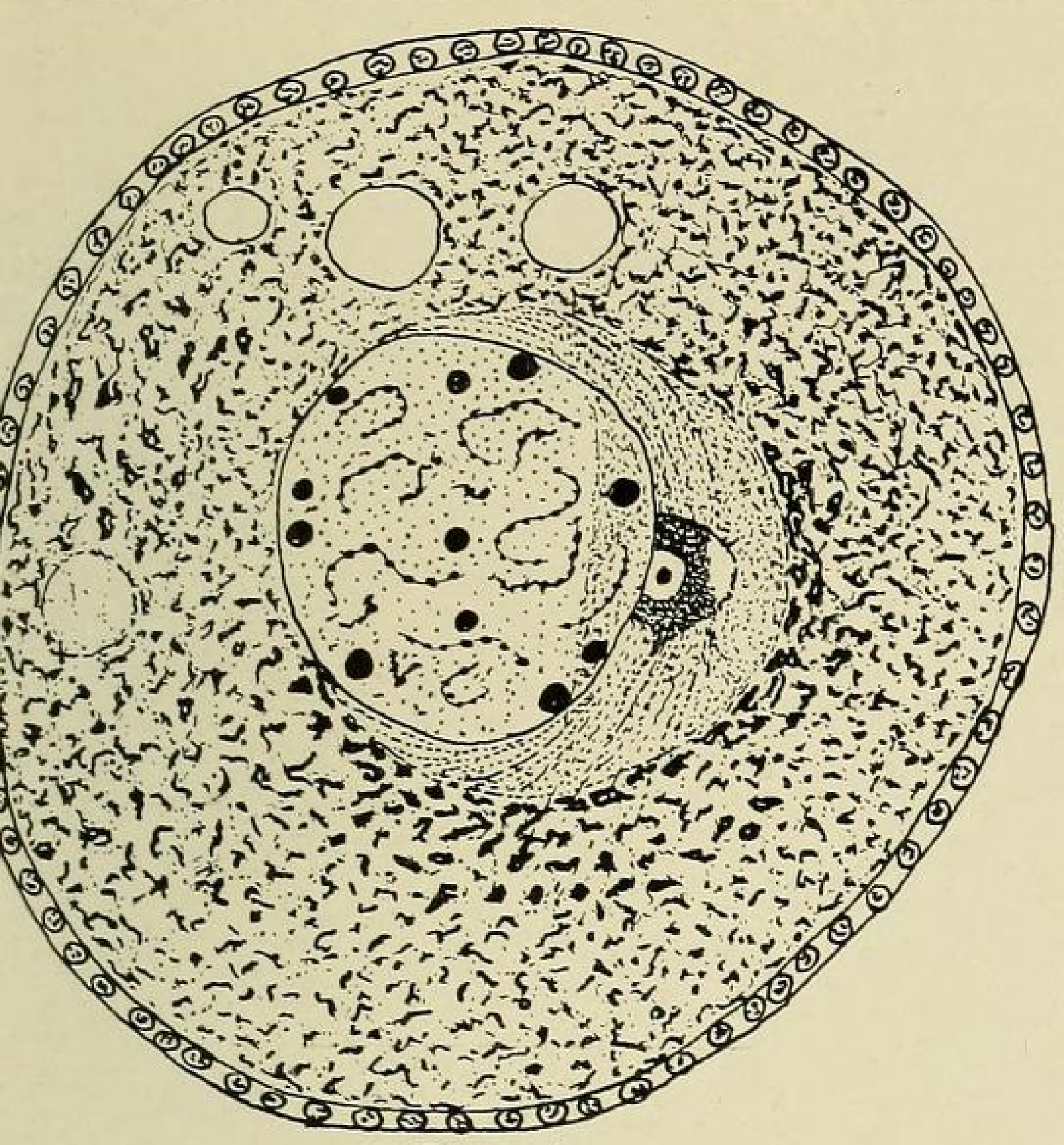

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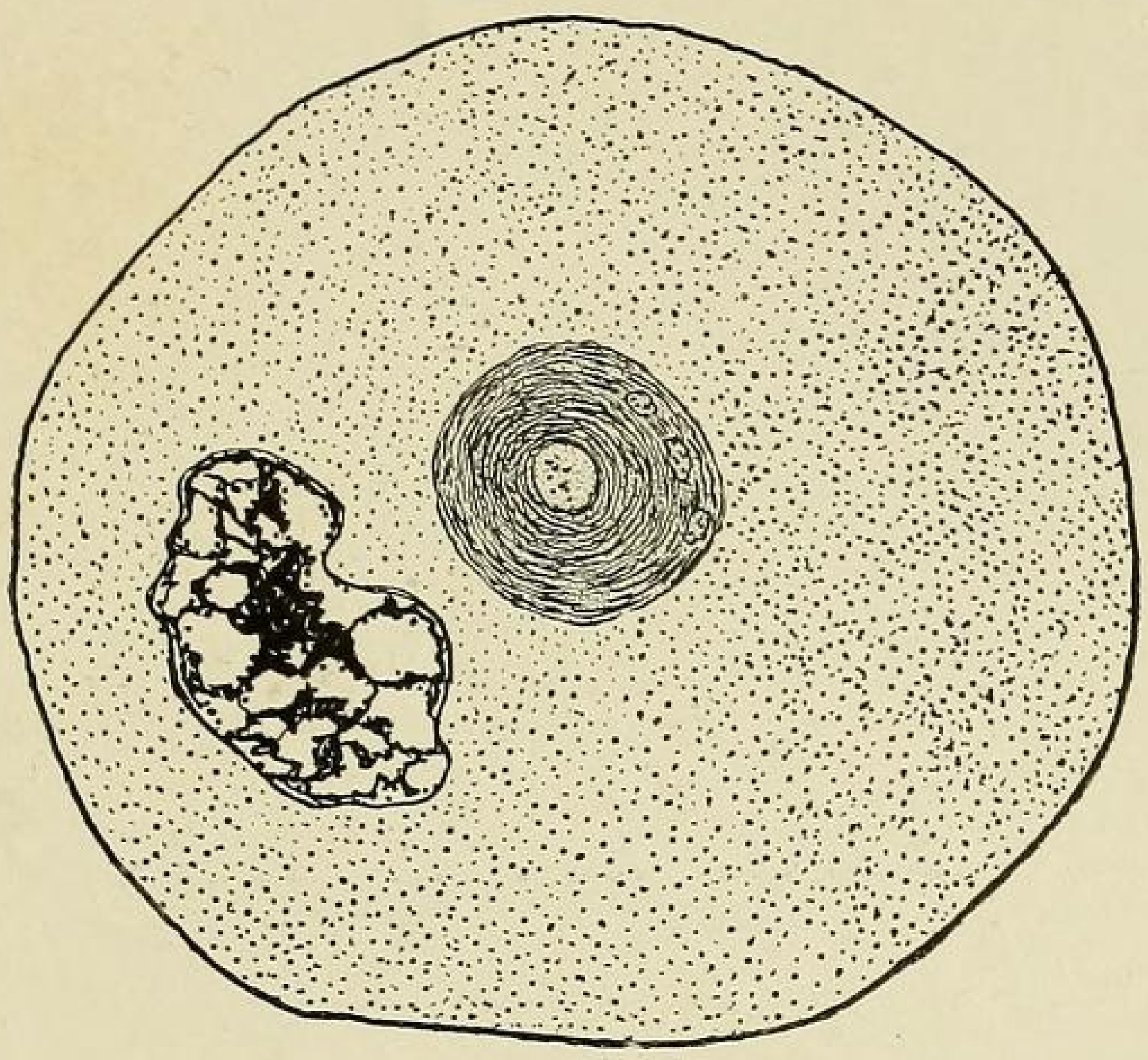

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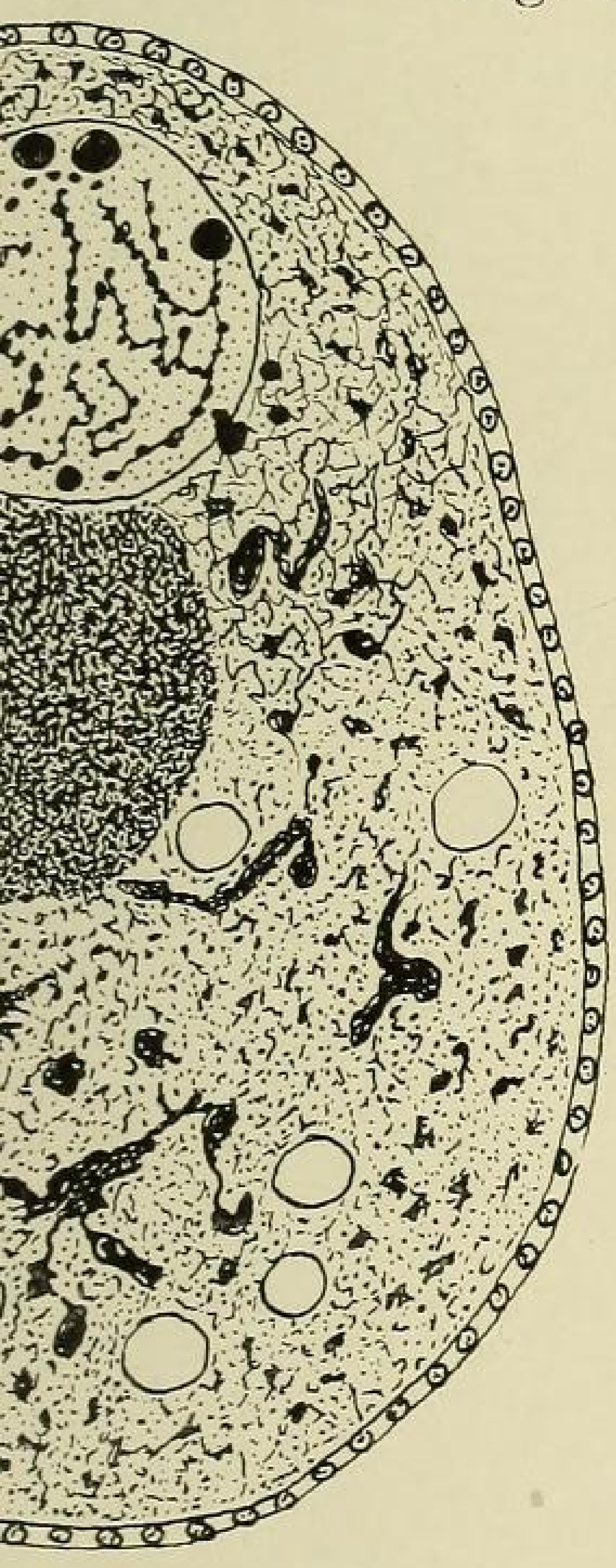

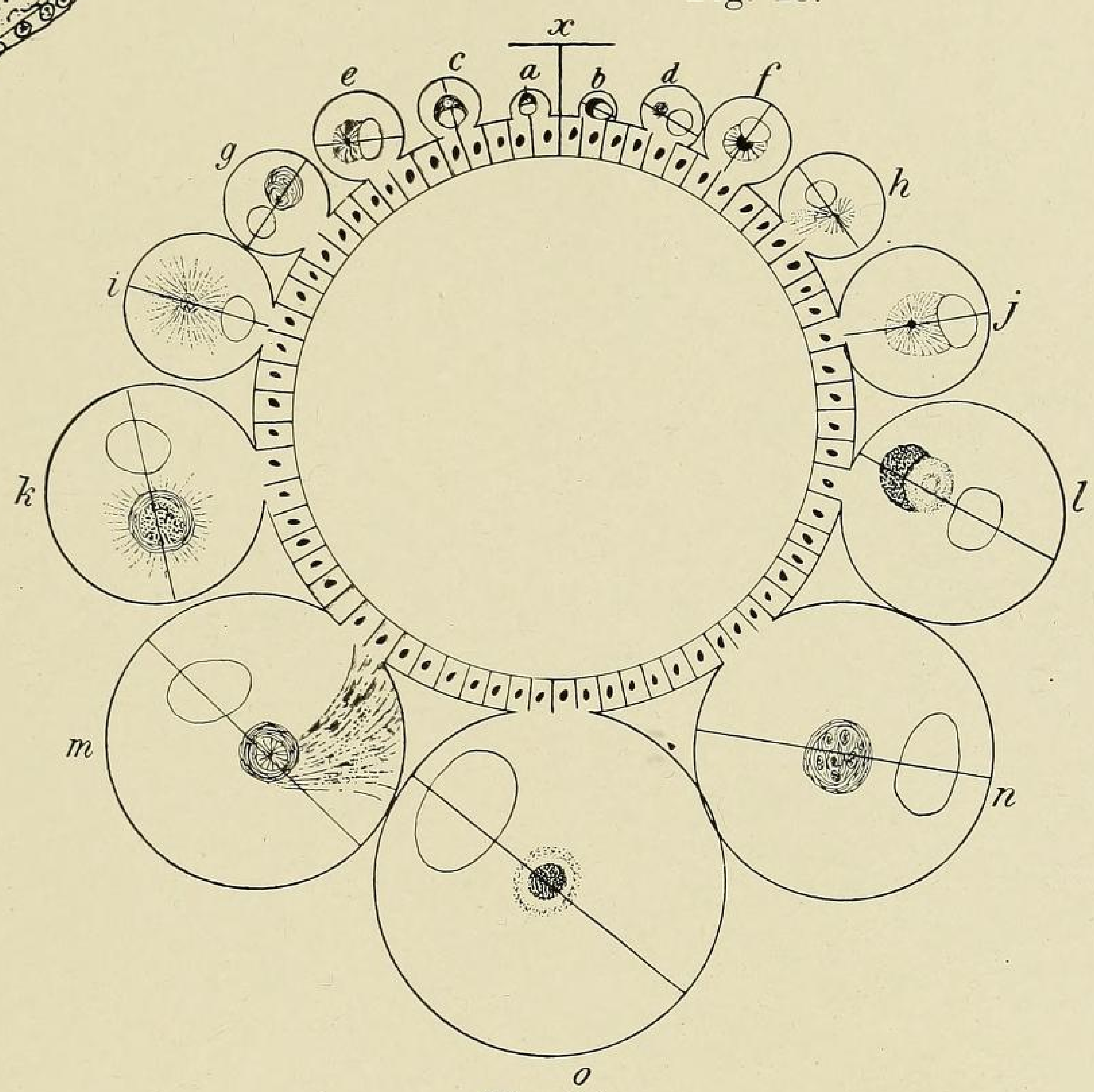

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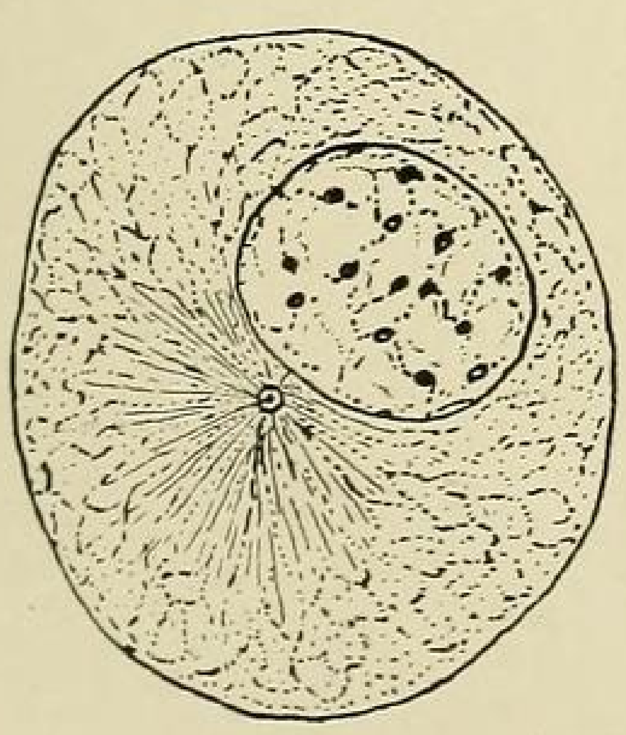

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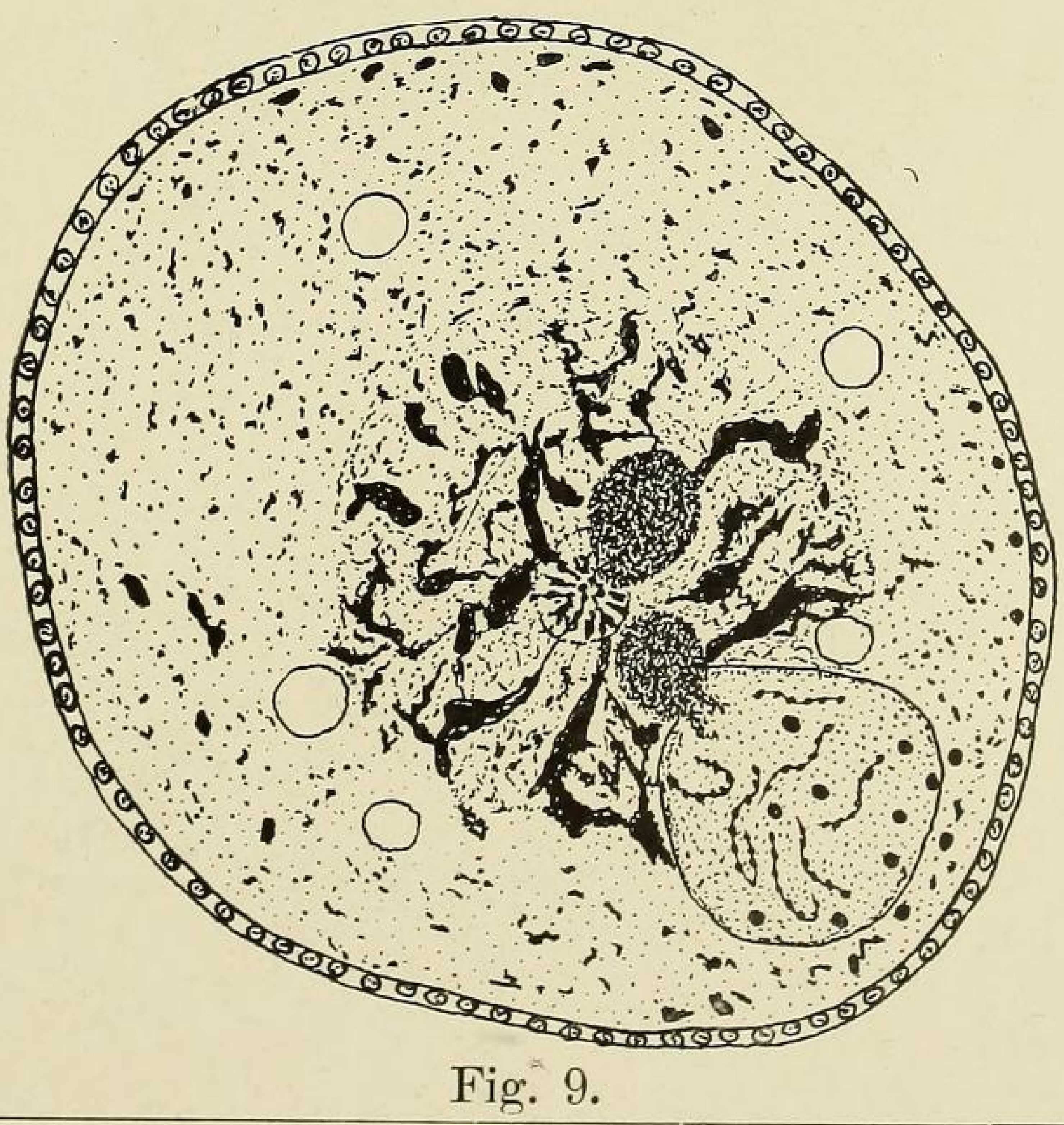

Fig. 9.

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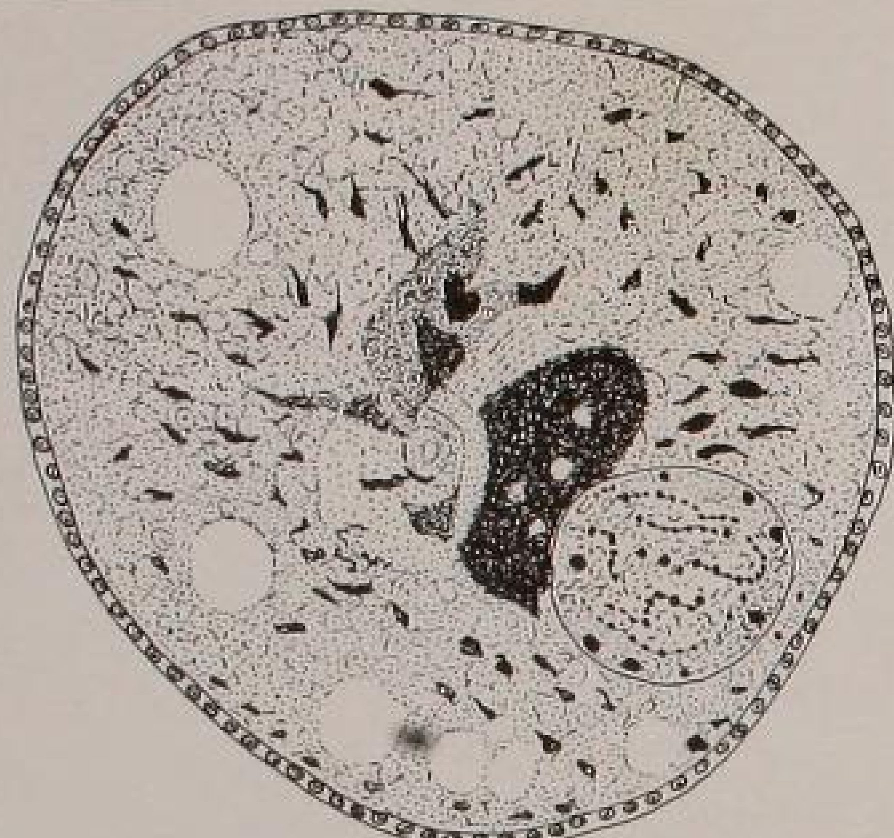

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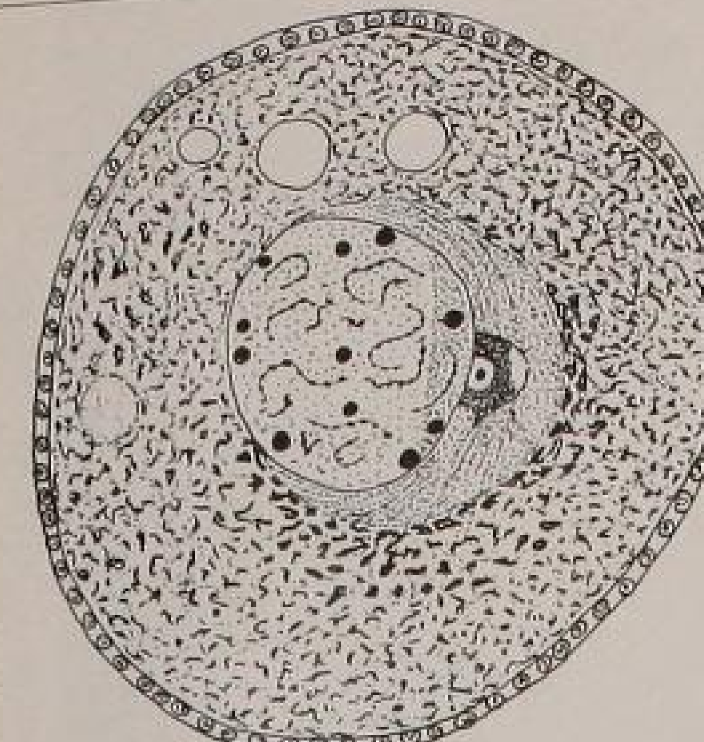

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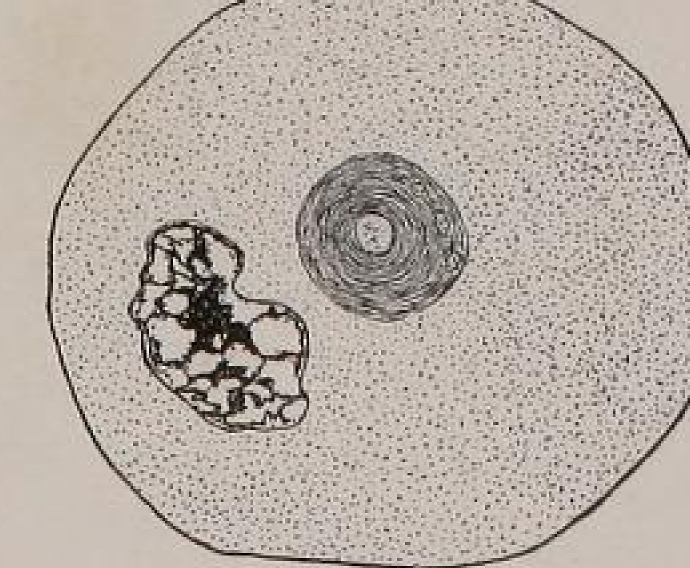

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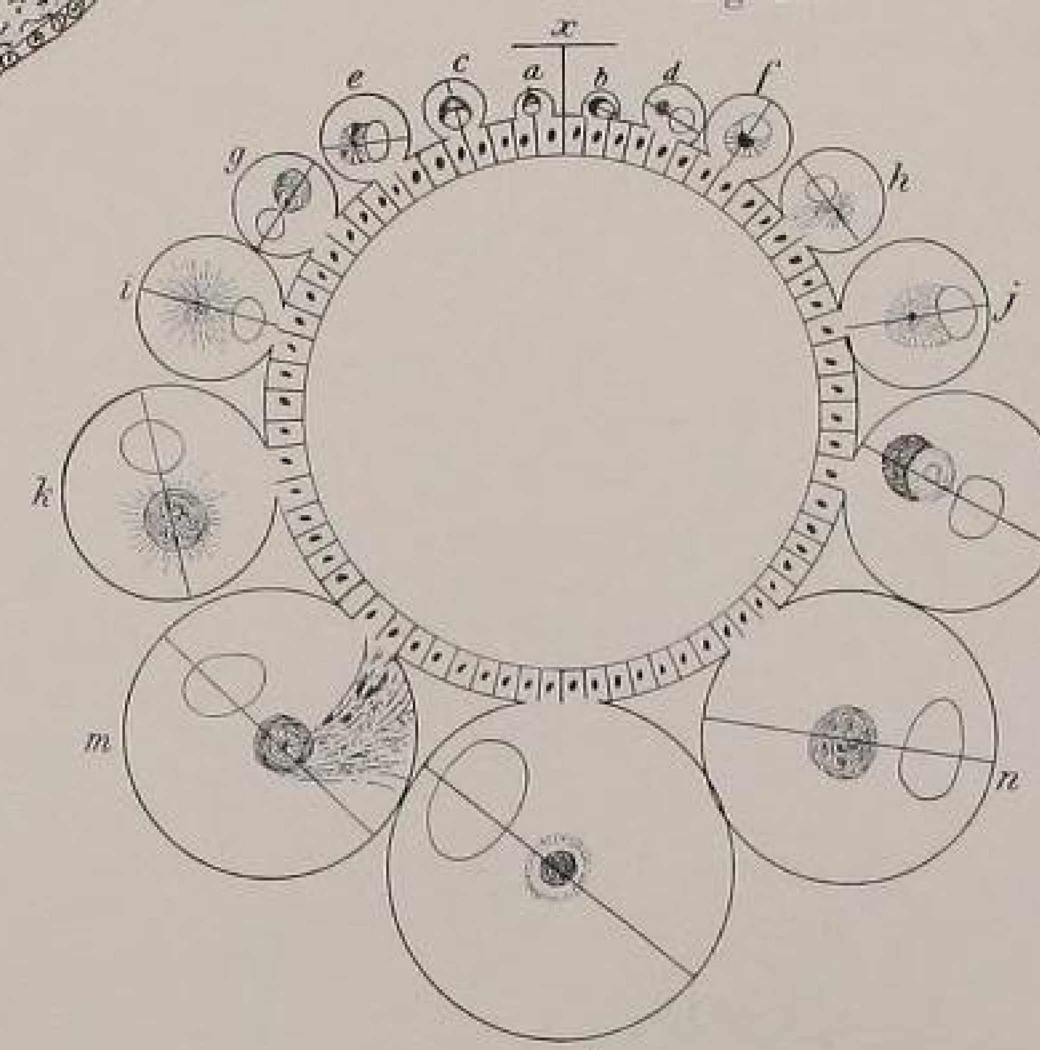

Fig. 34.

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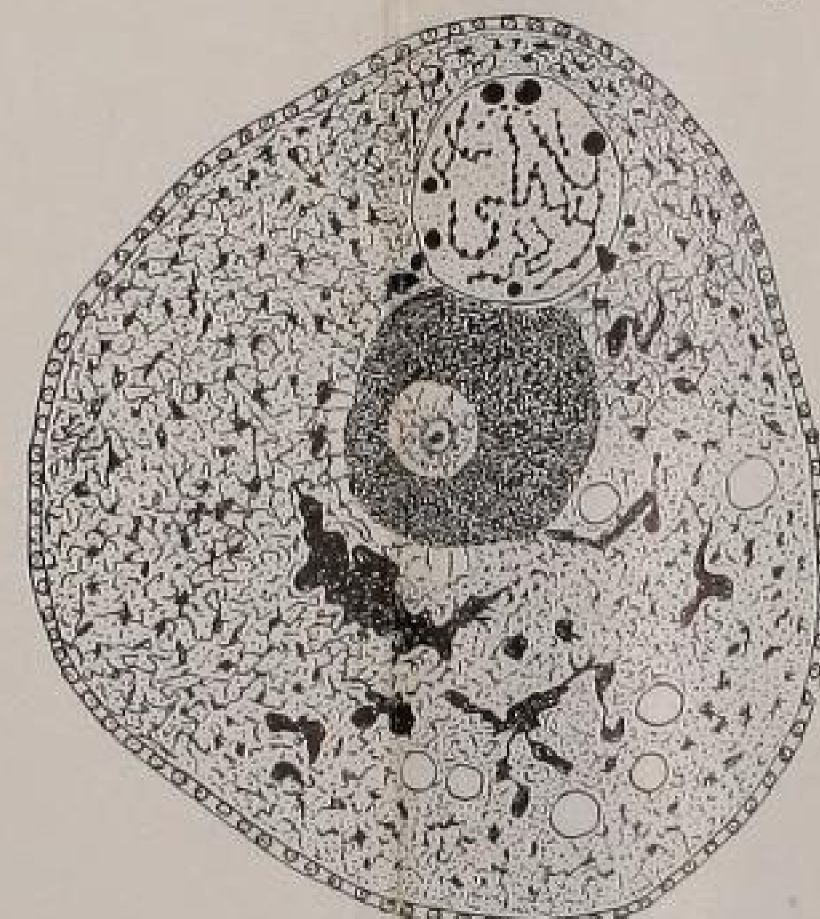

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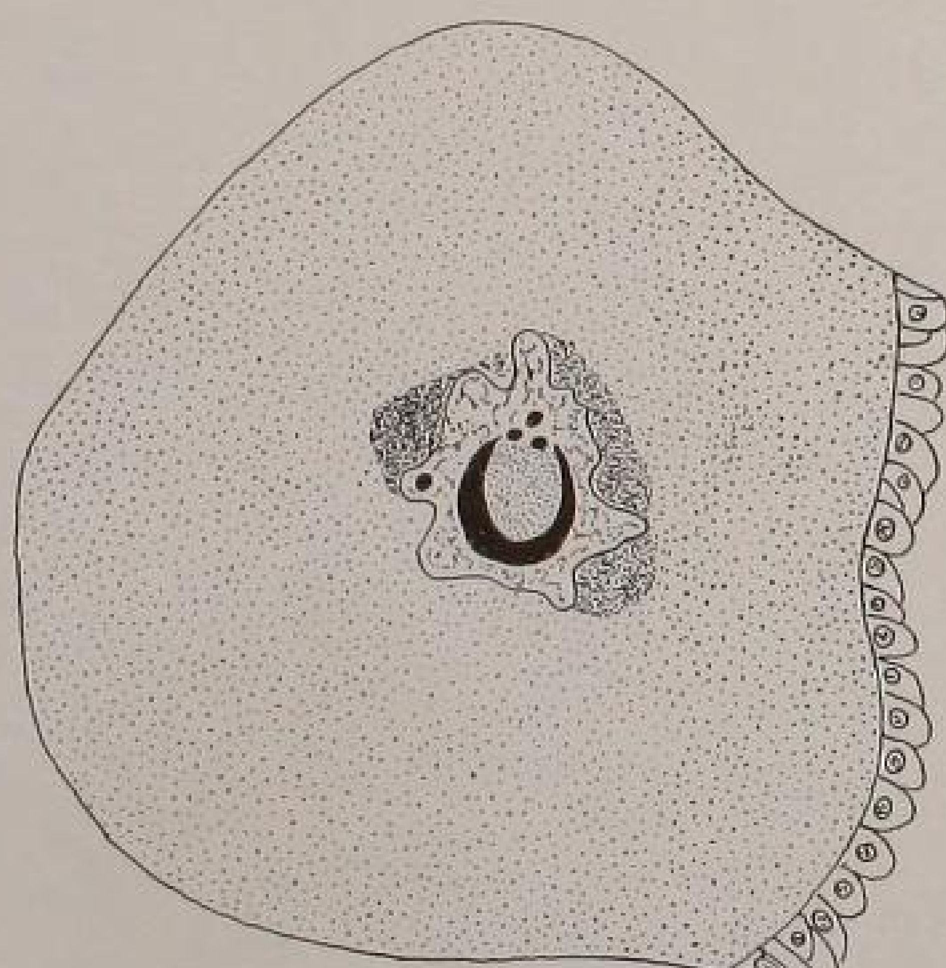

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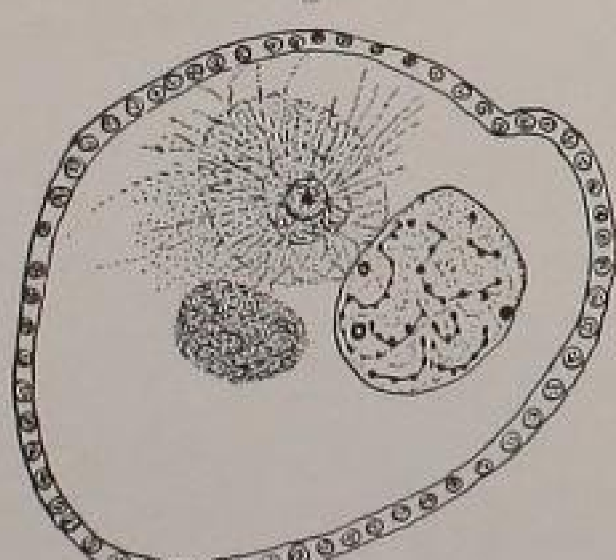

Fig. 11.

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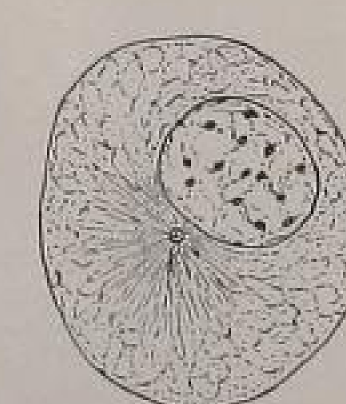

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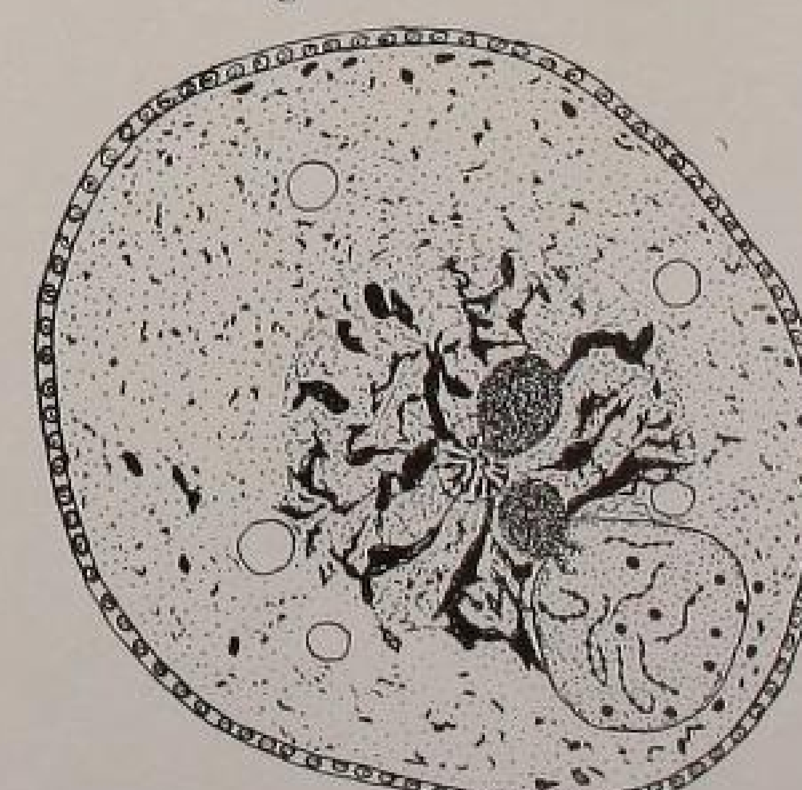

Fig. 9.