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A Comparative Study of the Structure and Origin of the Yolk Nucleus.

By

J. P. Munson.

(Awarded the Walker First Prize by the Boston Society of Natural History.)

With plates XXIX—XXXIV.

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I. Introduction.

Problems: — The question as to the comparative structure and origin of the yolk nucleus involves several other problems: 1. Is the yolk nucleus a normal element of the egg cytoplasm, or is it associated with pathological states of the egg, or is it an artefact due to reagents? 2. Is there a necessary connection between one form and another such that one can be derived from the other? 3. Is it an amorphous chemical substance or does it possess anything suggesting permanence of structure? 4. Does it arise de novo or is it a modification of something similar existing in the cytoplasm of the oogonium? 5. Does it arise from chromatin eliminated from the germinal vesicle? 6. Is it due to cells entering the egg? The answer to these questions will determine whether or not it is important.

Material: — Fresh ovaries of tortoise, spider, king crab, pigeon goosefish, cat, crayfish, and frog.

Methods: — Much experimenting has to be done before any results are obtained. The eggs of different animals require different treatment, and very often a new method has to be found for the successive stages of the same egg. In the matter of fixing, for instance, I find that the length of time is as important as the kind of fluid used. Different eggs, however, differ greatly as regards the behaviour of the yolk nucleus towards reagents. In some cases, as in the spider, almost any hardening fluid will show the yolk nucleus well preserved, often though the germinal vesicle and the rest of the cytoplasm be badly fixed. Poorly preserved material sometimes shows normal features that cannot be made out so clearly in more perfect fixation.

I have followed the plan of using much material and comparing results of different methods. In all those cases where astral rays are visible, the fixation has been most perfect.

The preparations from which my drawings are made, were exhibited at the Eighth International Zoological Congress, Graz, Austria 1910; and I have taken special pains to have my slides examined by those competent to judge. I find that even those who are not specially trained in the use of the microscope, experience little or no difficulty in seeing what I have represented in my drawings.

In the selection of types of animals for this study, I have necessarily been influenced by the ease with which the material could be procured.

I am very thankful to the Librarian of the University of Christiania, of the University of Berlin, and of the Naples Zoological Station for the many kindnesses shown me in my efforts to verify some of the data in connection with the literature used in the historical part of this work. I am especially indebted to the Librarian of the biological library of the University of Chicago, and desire to express, here, my hearty thanks.

It is hoped that no serious mistakes have been made in quoting the views of writers to whose works I have had access. The aim has been to give their own words, though it is realized that a false impression may even then be conveyed, when the extract is separated from what precedes and what follows.

My own observations, I relate in a separate chapter, to avoid the confusion which one experiences in trying to select the author's own work from the mass of quoted material.

To meet the requirement of the Prize Committee, my own work is referred to in the third person.

Historical:—The name yolk nucleus, is applied to a body or bodies found in the cytoplasm of eggs, differing somewhat from the rest of the cytoplasm. The following are some of the synonyms that have been used — the body of BALBIANI, Dotterkern, and paranucleus. MILNE EDWARDS called it the embryonic vesicle, and BALBIANI used the same term. O. SCHULTZE called it the vitelline nucleus; MUNSON called it the vitelline body, and cytocenter; and recently it has been called the egg centrosome and sphere.

The first account of the yolk nucleus seems to have been published in 1845 by VON WITTICH (97), in his Inaugural Dissertation. He published a second paper (98) on the subject in 1849. This being only a few years after the publication of the cell theory by SCHLEIDEN and SCHWANN, and fifteen years before MAX SCHULTZE gave us our present definition of a cell, and a similar period before GEGENBAUR (28) suggested the cell nature of the egg, a correct interpretation of this body could not be expected.

The entire literature on this subject, very considerable in amount, has with some notable recent exceptions, chiefly an historical value. But it is none the less interesting, in the light of modern cytology, when viewed in connection with recent views concerning heredity, cell organization, isotropism, epigenesis and preformation. It also touches the problems of the function of the nucleus, and its relation to the growth and differentiation of the cytoplasm.

As early as 1848, SIEBOLD (84) said: "Merkwürdig nehmen sich die Eier von *Lycosa*, *Thomiscus*, *Dimedes*, *Salticus* und *Tegeneria* aus, indem sie außer dem Keimbläschen, so lange sie noch nicht vollständig mit Dotter angefüllt sind, noch einen besonderen runden Kern von feinkörniger, aber fester Beschaffenheit enthalten." He makes the interesting observation that layers detach themselves from its surface, without any perceptible diminution of its size. His belief that the yolk nucleus plays an important role in the development of the egg, based on the observation that it appears early and disappears late is very interesting in view of what we now know of this body.

In the same year CRAMER (20) saw in small eggs of the frog, a granular body outside the germinal vesicle. This is what CRAMER says: "In dem freien Raum liegt die kleine Kugel von Körnchen, die früher von der Dotterhaut eng umgeben war . . . Wird das Ei etwas größer, dann erweicht die kleine Kugel, und immer flüssiger werdend verbreiten sich die Massen in einem eleganten Halbmond in der Höhle des Dotterraums."

Two years later CARUS (18) made similar observations on frog's eggs; and comparing the body with the yolk nucleus of spiders, concluded that yolk is formed on its surface. In 1849, VON WITTICH (98) wrote: "In den Eiern einiger Arten tritt nun, wie ich es bereits in meiner Inaugural-Dissertation beschrieb, und wie auch seitdem v. SIEBOLD beobachtet hat, neben dem Keimbläschen noch ein zweiter eigentümlicher Körper auf, über dessen Entstehung ich in meiner Abhandlung eine allerdings von SIEBOLD's Angabe abweichende Ansicht aussprach, bei der ich aber nach vielfältiger Beobachtung doch beharren zu müssen glaube."

COSTE (19) is often quoted as having seen the yolk nucleus in birds in 1847. In his large work, he refers to the cicatricula in birds, reptiles, and fishes, but there are reasons for doubting that he had seen the real yolk nucleus.

In 1853, LEUCKART (49) referred to the yolk nucleus in his famous article "Zeugung" as a very variable body. "Die Bedeutung dieses Körpers ist unbekannt."

Writers often quote BURMEISTER (16) as having discovered the yolk nucleus in eggs of *Branchipus* 1856. I have not succeeded in convincing myself that he actually saw the yolk nucleus.

LEYDIG (50) published a textbook on histology in 1857, in which he figured the yolk nucleus in Arachnids, with the confession that the meaning and use of this body is unknown to him.

In the same year, 1861, that MAX SCHULTZE defined the cell as it has since been conceived, GEGENBAUR (28) published his famous generalization that the egg is a cell. He, also, showed the presence of a yolk nucleus in the egg of birds.

In 1861, too, LUBBOCK (55) claimed to have seen the yolk nucleus in eggs of several species of Myriapods. He compares it to the body discovered by v. WITTICH for the first time in spiders. Because of its constancy, he seems to believe that it ought to have some important function; but is unable to assign any. Of *Lithobius* he says: "When the egg has attained a certain size, but before it has begun to darken, a small vesicle, about one third the size of the PURKINJEan vesicle, may generally be seen in it. It soon disappears and is replaced by a patch resembling that in *Julus*".

In the following year, LERBOULLET (51) described a body in the cytoplasm of eggs of crayfish. A radial arrangement of the yolk surrounding the body is suggestive in view of our present knowledge on that subject.

The impetus which GEGENBAUR had given to the study of the egg, and the problems which that study had already created, led BALBIANI (2) to make a comparative study of the eggs of different animals. He claimed to have found the body in *Helix*, and in representatives of most classes of animals, while in some species, he found no trace of it. He seems to have studied the living egg. Of *Tegeneria* he says: "At the moment when it becomes visible in the youngest eggs, it has the appearance of a little homogeneous and transparent vesicle, placed between the germinal vesicle and the egg stalk. It is at first much smaller than the germinal vesicle; but as it grows faster, it soon equals it in size. It consists of a great number of concentric layers forming a sort of capsule around the nucleus (central vesicle?), whose refringent aspect separates it distinctly from the cytoplasm of the young egg, which is still transparent. When the outer laminated layer is ruptured, the vesicle in the interior is found. This vesicle contains a pale and granular substance in which is seen a round capsule. In some forms of spiders, the laminated capsule is replaced by homogeneous or granular substance enclosing a central vesicle."

In 1872, EIMER (25) saw in the center of the egg of lizard, a spherical body which he regarded as the "Dotterkern", and which he considered to be a constant element in the egg of reptiles.

KOLESSNIKOFF (44) mentioned 1878 granular yolk nuclei in egg of several species of frogs and in egg of the toad.

He figured the yolk nucleus in eggs of fishes and BAMBEKE (6) has also seen it in fish eggs.

In 1877, the yolk nucleus was again described in the eggs of crayfish by REICHENBACH (76). It is said to consist of a central body with radial arrangement of the yolk granules.

In 1882, J. SCHÜTZ (82), in his Inaugural Dissertation, gave special attention to the yolk nucleus. His plates show many figures of it. It is represented chiefly as a spherical body, if I remember correctly. It is now some years since I saw his paper. I cannot very well describe it from memory. Recently I have not been able to secure a copy of it.

In 1822, also, JATTA (38) described a peculiar yolk nucleus in *Pholcus phalangioides* — "Il nucleo vitellino omogeneo, allungato, in arcato intorno alla vesicola germinativa e colarato intensamente in rosso."

In the same year NUSSBAUM (67) found bodies of various shapes in the cytoplasm of pancreas cells of *Salamandra maculosa.* Referring to these he says: "Dagegen wird man den Nebenkern der Drüsenzellen wohl mit dem von WITTICH entdeckten Dotterkern der Eier, dem durch von LA VALETTE ST. GEORGE zuerst bekannt gewordenen Nebenkern der Spermatocyten, den von LEYDIG aus der Epidermis von *Pelobates*-Larven beschriebenen Bildungen in eine Kategorie bringen dürfen."

The next year SARASIN (79) described a body in eggs of reptiles, consisting of a clear vesicle surrounded by little dark granules. He did not call it a yolk nucleus but only "Kern". He considered it to be a "Dotterherd" — a substance which transforms the secretion of the follicle cells into vitelline elements. EIMER (25) had described a similar body in the green lizard — a large body in the center of the cytoplasm of small eggs. He called it "Dotterkern". Two vitelline bodies were represented in a clear mass. From the latter he claimed fragments were detached.

OSCAR SCHULTZE (83), in 1887 figured and described a body in the cytoplasm of the frog's egg, as the yolk nucleus. It was close to the germinal vesicle, crescentshaped and resembled archoplasm.

In a series of articles from 1864 to 1893, BALBIANI (3) has given descriptions and figures of the yolk nucleus in eggs of various animals, including *Tegeneria*, *Clubiona*, *Geophilus*, skate, frog, estrel, hen, cow and the human ovum. In nearly all cases , it is represented as a spherical vesicle containing granules and surrounded either by a circle of granules, or by a layer of concentric fibers. In *Geophilus*, he represents it as a sphere surrounded by radial striations like the rays of an aster, but he also shows in the same egg, other large granular masses in the cytoplasm.

From the year 1893, up to the present time, observations on the yolk nucleus have been numerous; and in many cases, the granular, amorphous bodies in connection with it, or included in the cytoplasm of the same egg, have received more attention.

As early as 1878 WHITMAN (99) described amorphous substances in the egg of *Clepsine*, which owing to its position in the egg, he called polar rings.

In 1886, WILL (94) found the yolk nucleus in eggs of insects, which, however, had already been studied by BALBIANI in Aphids and in *Hymenoptera*.

In the following year BLOCHMANN (12) published a paper in which he mentions something in the egg of *Camponotus ligniperda*, suggesting the yolk nucleus. These are his words: "In den Eiern, die sich ungefähr in der Mitte der Eiröhre finden, bemerkt man eine sehr auffallende faserige

Differenzierung des Eiplasmas, welche man am besten mit dem Aussehen eines vielfach durchscheinenden geschlungenen Fadenbündels vergleichen kann. Zerdrückt man ein derartiges Ei, so ergibt sich, daß diese Struktur bedingt wird durch eine ungeheure Menge kleiner 0,12 mm langer Stäbchen, welche in regelmäßiger Weise angeordnet sind. Auch bei anderen Ameisen, z. B. *Formica fusca*, findet sich ähnliches, nur nicht in dieser auffallenden Deutlichkeit, wie bei *Camponotus*, indem die Plasmastäbchen viel kleiner, und nicht so regelmäßig angeordnet sind. Bei der beginnenden Dotterbildung verschwindet allmählich die erwähnte Struktur."

In the same year Stuhlmann (86) also published observations on several species of insects. In *Bombus terrestris*, he found yolk nuclei arranged near the periphery of the egg, somewhat like the ascidian test cells. He found also in insect eggs granular masses which do not fuse into a large one; and this he calls a diffuse yolk nucleus. Of the eggs of Hymenoptera he says: "Es bilden sich stets ganz kleine Konkretionen dicht an der Peripherie des Keimbläschens oder doch wenigstens in seiner unmittelbaren Nähe. Diese wandern nun vom Keimbläschen weg und legen sich in einer vollständigen Schicht an die ganze Eiperipherie, *Bombus*, oder sie bleiben mehr am oberen Eipol angesammelt, *Vespa*, *Trogus*, *Pimpla*, oder endlich sie konnten sich zu einer Anzahl etwas größerer im ganzen Ei verbreiteter Klumpen vereinigen, *Banchus*. Ich bezeichnete dies mit dem Namen diffuser Dotterkern. Es können nun auch die einzelnen kleinen Dotterkonkretionen sich zu einer einzelnen, großen gefärbten Masse vereinigen, die stets am hinteren Eipol lag. Dies Gebilde nannte ich den eigentlichen Dotterkern."

In 1887, Scharff (80) published observations on a granular ring, surrounding the germinal vesicle of fish eggs. This appearance is common in many eggs. It was seen in the living egg of *Limulus* by Munson (61) and it is sometimes called yolk nucleus.

In 1890, Holl (35) figured and described the yolk nucleus in the hen's egg, as a crescentshaped granular body, closely applied to the germinal vesicle, and having radial striations proceeding from it. Holl says it is visible in egg cells of 0,4 mm diameter, and that it is distinguished by its deep staining as compared with the network of the cytoplasm, which remains unstained.

Holl found spherical granules in the egg of the cat, which like similar bodies seen by Romiti (78), stain black in osmic acid.

Loewenthal (54) found that similar granules may be massed into a few or even a single large stainable body hardly inferior to the germinal vesicle in size.

Korschelt (46) had shown in 1889 that, in the egg of insects, there is a mass of stainable granules in the neighborhood of the germinal vesicle, and in 1892, Monticelli (60) announced the finding of a yolk nucleus in the ovum of Trematodes.

In 1983, Henneguy (32) wrote; "Chez les Rats, âgés de quelques semaines, dont les ovaires ne renferment que des ovules peu avancés, on constate, après fixation par le liquide de Flemming, que tous les jeunes ovules contiennent, à côté du vésicule germinative, un petit corps arrondi, nettement circonscrit et un peu plus coloré que le reste du protoplasme ovulaire."

An account of an irregular body in the cytoplasm of the egg of a fish was published in 1894 by Hubbard (37).

In 1893, Mertens (58) published observations on the egg of birds and mammals. He shows the presence in the egg of birds, including the fowl, a large spherical, granular body about the size of the nucleus, and occupying a position in the center of the egg. He found the same in the egg of a young cat and in a young human ovum. In the center of some of these, he found a deeply staining granule suggesting a centrosome in the midst of an attraction sphere.

The term yolk nucleus was applied by Calkins (17) to an irregular mass of granules partly surrounding the germinal vesicle of the egg of *Lumbricus*.

It is clear that the definite, spherical body originally described as the yolk nucleus in spiders and myriapods, and described again in 1893 by Balbiani (4) and by Henneguy (32), has now become thoroughly mixed up with any granular substance in the cytoplasm. When nothing more definite is found, even yolk granules are called yolk nucleus.

Foot in the following year, published observations on eggs of *Allolobophora*, with figures showing amorphous substances appearing as irregular patches throughout the egg cytoplasm, and described as more or less fluid, and capable of flowing from place to place. It was supposed to be allied to the polar rings observed by Whitman.

In 1897, Němec (66) published an account of the yolk nucleus in *Polyzonium*. In the young eggs, it is represented as a granular mass, in which there is a granule, the whole forming a cap partly enclosing the nucleus. Later this, he affirms, is differentiated into two distinct bodies, one of which assumes the form of an aster.

Munson (61) published in 1898, an account of the history of the ovarian egg of *Limulus*, in which he showed the various stages of the egg from the beginning in the germinal epithelium to the period of maturity, and its discharge into the ovarian tube. In the youngest eggs he showed the presence of archoplasm containing a centrosome; and traced the same through the ovarian history of the egg. Besides an attraction sphere and centrosome often forming a real aster, he showed the presence in the cytoplasm of amorphous masses resembling nuclei, which he designated as yolk nuclei reserving the name vitelline body for the egg attraction sphere.

In 1898 Bambeke (8) gave us an account of an elongated mass in the cytoplasm of the egg of *Pholcus*, staining deeply and partly surrounding the germinal vesicle as a ring or as a stainable band of substance near the periphery. Judging from his plates this corresponds to what I have called metaplasm in the egg of the tortoise.

In recent years, contributions to the subject have been numerous, and some of them of considerable importance, notwithstanding the fact that so much attention has been given to study of chromosomes.

It appears from the literature thus far considered, that the term yolk nucleus includes many dissimilar things in the cytoplasm. Says Stuhlmann (86) of eggs of Hymenoptera: "Here is therefore two entirely different kinds of yolk nuclei — "Denn als Dotterkern bezeichnen wir doch ein Gebilde, das von dem übrigen, normalen Dotter abweicht."

Wilson (95) in his work on the "Cell in Development and Inheritance" even goes so far as to speak of the yolk bodies in the egg of the newt, as yolk nuclei.

In 1900, Bouin (14) described, in egg of *Rana*, a dense mass in the cytoplasm attached to the germinal vesicle. In ovocytes he found it in form of crescent in which a central stainable granule could be seen.

In the same year Gurwitch (31) published an account of his investigations on the yolk nucleus in mammalian ova finding it always present in these eggs.

Winiwarter (96) published a paper in the same year on the yolk nucleus in the mammalian ovum.

In 1903 Skrobansky (85) figured and described the vitelline body in the human ovum and also in the cat.

In the egg of *Montis religiosa*, Giardina (29) found in 1904, near the germinal vesicle a dense mass in the cytoplasm at the point where chromatin is massed in synapsis, and in the center of this mass were stainable granules.

Interesting studies on the yolk nucleus in eggs of birds and mammals were published by Hollander (36) in 1904.

Munson (62) also published in this year an account of the yolk nucleus in the egg of the tortoise, *Clemmys marmorata*, showing in the cytoplasm a constant body in which were evidences of a center and aster, besides other masses of metaplasm scattered throughout the egg.

An important contribution was made by Loyez (53b) in 1906. He published figures of the yolk nucleus of *Anguis fragilis*, *Tropidonotus viperinas*, *Vipera aspis*, *Testudo graeca*, *Cistudo Europaea*, *Crocodilus*, *Coccothraustes chloris*, *Emboriza citrinella*, *Passer domesticus*, *Polyboroides Madagascariensis*.

In 1907 Lams (53) published very interesting observations on amphibian ova. He shows many fine figures of dividing oogonia with centrosome and sphere, the sphere being apparently continuous with the yolk nucleus of the oocyte.

Finally King (40) published a paper in 1908 in which she has this to say about the yolk nucleus in egg of *Bufo*: "In the egg of *Bufo* it is possible to trace the anlage of the yolk nuclei back to the primordial germ cells".

II. Original Observations and Inferences.

The Egg of the Tortoise. The oogonia lie in the connective tissue stroma of the ovary, pl. XXIX, fig. 1. In all of these oogonia, there is a very distinct body in the cytoplasm, always spherical, nearly one half the size of the nucleus. It often shows indications of concentric circles, with a clear area in the middle; or else a central granule suggesting a centrosome.

The body is connected with the nucleus, by a circle of larger cytomicrosomes, enclosing an area which differs somewhat from the cytoplasm outside this circle. The oogonia finally divide, and produce a nest of several smaller cells, one of which becomes the oocyte or egg; the others, the follicle cells.

During this division, the large, conspicuous centrosome is greatly reduced and appears as a mere dot in the cytoplasm of the daughter cells.

As one of these cells begins to increase more rapidly than those destined to become follicle cells, the centrosome becomes more and more conspicuous, fig. 4. It consists of a central circle of microsomes, enclosing a central granule, fig. 7, the centrosome.

This original centrosome becomes embedded in, and consequently obscured by, a substance produced or arising in the neighborhood of the germinal vesicle. It often presents the appearance of a sticky, amorphous substance, staining differently from the contents of the germinal vesicle, and differently from any other part of the cytoplasm. I have called this metaplasm; and I take it to be the result of karyolymph united to, or acting upon food matter in the cytoplasm, that has not yet been assimilated.

This substance accumulates around the centrosome, giving rise to a large body at one pole of the germinal vesicle, and occupying a space about the size of the germinal vesicle, figs. 2, 5. I presume this corresponds to the latebra of the bird's egg.

There are evidences of concentric circles around the centrosome, having a definite relation to the germinal vesicle. There seems to be two different substances — one taking the stain much more readily and deeply than the other. The latter generally occupies the center, while the former is peripheral, fig. 6.

The less stainable portion is very finely granular, in preserved material; and I incline to the belief that in the living egg it is fluid, filling the spaces between the fibers of the astral rays, and coagulated by reagents into a finely granular precipitate. It may be that the two substances correspond to the white and the yellow yolk in bird's eggs.

The more stainable substance is either coarsely granular, or else appears as an amorphous sticky mass, surrounding the large central body as an irregular ring, figs. 5, 6; or else diffused, in isolated patches of varying size and shape throughout the cytoplasm, figs. 3, 9.

The large, feebly staining body often seems homogeneous, and may assume gigantic proportions in comparison with the rest of the cytoplasm and the germinal vesicle. Its form may be circular in section, or oval, but occasionally greatly elongated and irregular. But it always retains its connection with the nucleus, and it always occupies approximately the geometrical center of the egg. The germinal vesicle is consequently excentric: and being a constant feature, more or less conspicuous, it confers on the egg a distinct polarity, much as the latebra does in the hen's egg.

In many cases, perhaps in most cases, it is possible to make out a central, condensed, spherical body as in fig. 8, the centrosome; and often, also, a series of concentric rings around this, fig. 2, suggesting the zones of yellow and white yolk in the hen's egg. I take these zones to correspond to the concentric circles seen in the centrosome and aster of karyokinesis, and in leucocytes, pl. XXXIV, figs. 64, 68, 71, 74. This is seen in fig. 2 when the large, central body is surrounded by an other wide zone of the deeply staining substance in which there are several spherical vacuoles, seeming to be filled with a substance resembling the central body.

In fig. 5 is represented, near the center of the large mass, a circular opening containing a system of fibers like a nucleus, reminding one of the central nucleus or vesicle in the yolk nucleus of the spider, pl. XXX, fig. 20.

I do not see how such a perfectly shaped thing could be an artefact. I take it as an indication of the location of the centrosome, indeed probably the centrosome itself, which need not necessarily be a single granule, but in the relaxed uncontracted state, assumes the appearance of a nucleus.

In the later or advanced stages of the egg, this body assumes various shapes and appearances, owing to the variable distribution of the amorphous granules. It is apt to have the appearance represented in fig. 3.

There is never more than one of these, though, as in fig. 3, there may be several large, spherical or irregular masses of deeply staining substance in various parts of the cytoplasm. These, however, never assume the form of an aster like the cytocenter. Instead of forming large deeply staining masses, this substance may be scattered all through the cytoplasm as is shown in figs. 8 and 9.

In many of the larger eggs, the cytocenter has all the characteristics of a typical centrosome and aster, fig. 10. The most delicate preservation is required to see this. If good fixing has been secured, most of the differential stains make these bodies prominent.

Interpretation and Summary. There is in the cytoplasm of the oogonia of *Clemmys*, a large centrosome, which is greatly reduced when the division to form the follicle takes place. In the cell which becomes the oocyte, (the one usually which is centrally located) this centrosome persists as a typical centrosome and aster at one pole of the germinal vesicle.

It consists of a central granule, with a circle of microsomes surrounding it, and astral rays extending to a second and a third circle of microsomes. The latter circle intersects the germinal vesicle, and forms a body about the size of the germinal vesicle. It seems to be a receptacle into which fluids from the germinal vesicle, possibly karyolymph, is poured. This fluid acting on substances in the cytoplasm, either in the neighborhood of the germinal vesicle or around the astral body, causes chemical changes which result in a new deeply staining substance, figs. 2, 4, 5, 6. This new substance, metaplasm perhaps, is then diffused apparently by currents in the interfillar substance or cytolymph, forming larger or smaller aggregations.

On application of reagents, the karyolymph filling the cavity of the aster and central vesicle or centrosome, is coagulated. It then seems slightly granular and resembles in appearance and staining reaction, the matrix of the germinal vesicle. It has none of the characteristics staining reaction of chromatin, which is prominent in the nuclear reticulum of these eggs.

On coagulation, the karyolymph which has entered the aster and formed its matrix, obscures the delicate fibers constituting the framework of the aster. Only in rare cases can the centrosome itself be seen in the center of this coagulated mass.

There are, therefore, in the cytoplasm of the eggs of the tortoise, *Clemmys marmorata*, three parts which would be called yolk nucleus if seen alone: 1. the centrosphere; 2. extruded karyolymph, filling the meshes of the centrosphere; 3. the metaplasm.

The first or centrosphere is a continuation of the centrosome of the dividing oogonia. The karyolymph is extruded from the nucleus, where I have elsewhere suggested, it is produced in or by the chromatin. The metaplasm is a new chemical compound arising in the cytoplasm through the action of the karyolymph on some substance in the cytoplasm. It may be a synthetic food product which is gradually absorbed in the growth of the egg. The capacity of the egg cell for growth as compared with the follicle cells, may be due to the persistence of the centrosome in the former.

Whether my inference from appearances be correct or not in this important matter, there is no reason why we continue longer to regard everything differing from the rest of the cytoplasm as one body, to be designated by one general term, yolk nucleus.

Before proceeding to the description of similar things in other eggs, I would like to suggest that the identification of a part of the socalled yolk nucleus as the centrosome or centrosphere, ought not to be considered a radical assumption at the present time. The Nebenkern of sperm cells, more often the middle piece, has been declared to be the centrosome on far less adequate evidence than that which these eggs present. And on apriori grounds, the persistence of the centrosome in the cytoplasm of the oocyte ought not to be regarded with greater scepticism than an affirmation of its persistence in sperm cells. The fact may be after all, that there is yet much to be learned about the centrosome. The yolk nucleus may be the body which because of its greater size, may add to our knowledge of that minute dot usually taken to be the centrosome in its typical form.

What I have called the cytocenter, the centrosphere, has a constant relation to the nucleus and to the cytoplasm. It becomes the center of the vegetative pole of the egg; and together with the germinal vesicle, locates the egg axis: and consequently is involved in the determination of the two primary germ layers.

The Yolk Nucleus of Spider's Egg.

In the very young eggs of spiders, the yolk nucleus has the structure and general appearance of the centrosphere of leucocytes and the fertilized egg of *Ascaris*, pl. XXXIV, figs. 68, 71, 74. It consists of a central granule, the centrosome, surrounded by a circle of microsomes, this being again surrounded by another zone of granules, definitely limited by a circle of large microsomes; and outside this, again, two more such circles. Most of the cytoplasm at this stage is such a centrosphere, the astral rays of which become lost in the general cytoreticulum of the thin outer layer of cytoplasm, pl. XXX, fig. 11.

With the growth of the egg, the centrosphere assumes a variety of appearances as regards structural details. Owing to variable distribution of granules, and the vacuoles formed from extruded karyolymph, the concentric circles become compressed, roughly illustrated by a folded Chinese lantern, giving rise to the concentrically striated appearance so marked in the yolk nucleus of these eggs, figs. 12, 13.

It often remains closely applied to the germinal vesicle, figs. 12, 17. But as the egg grows, it may become separated from it, fig. 23. In such cases, however, it is sometimes possible to see radial bundels of fibers, doubtless aggregated astral rays, connecting the two, figs. 15, 16, 23.

On a superficial examination, the yolk nucleus looks like a spherical mass of archoplasm; but more careful study of favorable preparations reveals a structure, which, in most respects, points to the original type of centrosome, with a central vesicle, containing a deeply staining dot, the centrosome, fig. 24. This is surrounded by zones, difficult to represent in drawings and equally difficult to describe. Sometimes the zones seem granular; sometimes they seem fibrous, varying somewhat with the magnifying power used, figs. 19 and 20.

As these bodies are so large that their diameter exceeds the thickness of a single section, the appearance varies, of course in different planes in which the body is cut. But the structure seems to be much the same in all radii. In my drawings, I have represented the section showing the central vesicle. Doubtless different hardening reagents produce differences in condensation. I have, therefore, made all my drawings of this egg from the same kind of material.

The fibers of which this body is composed, are evidently continuous with the cytoreticulum of the general cytoplasm. Between the fibers are vacuoles or cavities, evidently corresponding to the meshes of the general cytoplasm, fig. 23.

Occasionally there seems to be a definite and comparatively thick limiting membrane, surrounding the body, as in fig. 19. But more commonly the concentric fibers pass imperceptibly over into the surrounding reticulum. In fig. 22, these fibers are seen to be massed at one side of the germinal vesicle, *g. v.*

The outer zone of concentric fibers may form a layer of rather uniform thickness enclosing the central granular portion, fig. 4. Occasionally a granular layer has developed in the center of the ring, fig. 18.

The central vesicle appears typically in figs. 15, 20, 22, 23, 24. Its similarity to a young germinal vesicle or to an ordinary nucleus is very striking. It is not at all surprising that early observers took it to be a real nucleus, as they often called it.

As this yolk nucleus originates from a typical centrosphere of the oogonium, fig. 21, and as it retains these characters for some time after the young oocyte has begun to grow, fig. 11, and as it is always present and never more than one in each egg, I take all the later forms to be modified centrospheres. The central granule in fig. 24 is, therefore, not a nucleolus as has been affirmed; but more probably a centrosome.

The different appearances of the concentric layers is due to difference in condensation, or rather to differences in expansion of the network, which needs only an accumulation of cytolymph and metaplasmic granules to be just like the rest of the cytoplasm. The fibers are there, and the meshes between them are there. Both are smaller, or let us say, less developed than in the rest of the cytoplasm. The fibers of the yolk nucleus are continuous with the fibers of the cytoreticulum, fig. 23. In fact it is a part of the cytoreticulum which has not yet expanded.

I venture to say, as I have said before, that the cytoplasm grows from this by a process of expansion.

Morphologically, this yolk nucleus bears the same relation to the cytoplasm as the chromosomes bear to the nucleus. Given chromosomes, and a nucleus develops by the formation of karyolymph in the vacuoles of the chromatin substance, which thus is made to assume the form of the nuclear reticulum. Similarly, given a yolk nucleus like that of the spider, and cytoplasm may be formed from it by the formation of vacuoles, fig. 19, and a consequent expansion due to the mechanical pressure of an ever increasing cytolymph. Appearances suggest that the fibers also actually grow by intussusception both in thickness and in length.

There is, in other words a typical centrosphere forming the framework of this body; and, as has already been noted, the karyolymph bathing this body as it comes out from the nucleus, forms one of the constituents of the culture medium, the metaplasm, from which the living substance grows.

The yolk nucleus is not an amorphous, dead substance of little or no significance as has been assumed. It is the germ, so to speak, of the living part of the cytoplasm; and as it represents a centrosome and a sphere on a large scale, it gives an insight into that body, which the study of the little dot, usually called the centrosome, could never give.

From what can be seen of the structure and history of the yolk nucleus in the egg of spiders, one would not hesitate to predict that this body, when introduced with the nucleus in fertilization, might regenerate the cytoplasm of the sperm, just as the chromosomes form a male pronucleus. The discrepancy between the amount of cytoplasm in the egg und the sperm would thus really be of no consequence, as far as the hereditary qualities are concerned. Judged by appearances in the yolk nucleus, the centrosome is the cytoplasm packed into a very small area, the convenience of which is evident in the sperm cell.

The distribution of the amorphous granules in the yolk nucleus varies, being sometimes massed in the center, giving rise to the form represented in fig. 14. The granules obscure the delicate fibers in the center, which make up the essential part, the centrosphere.

Yolk Nucleus (Vitelline Body) in Egg of Limulus.

In very young *Limuli*, fig. 25, when the ovarian tubes can first be seen, the oogonia form the lining cells of the tubes. Some of these become oocytes; which, as they grow, push out the basement membrane, through openings between muscle fibers. In larger tubes when slit open and spread out flat, these openings appear as regular oval areas surrounded by the muscle fibers, and connective tissue fibers.

There is then visible, in properly preserved and stained material, at one pole of the nucleus of the oogonia, a body looking like archoplasm. Sometimes it is spherical; sometimes it partly encloses the nucleus as a crescent. In Lyons blue and saffranin, the crescent alone is deep blue, all other parts of the egg being red. As the oocyte begins to grow, and as it pushes out the wall of the tube, the blue body becomes more prominent, fig. 25.

Superficial examination gives one the impression that the blue body is a sticky, amorphous mass. But more careful examination, with the highest magnifying powers, and with proper adjustment as regards illumination, reveals a central vesicle with a little dot in it, the centrosome.

It is easily seen, also, that in the surrounding cytoplasm, there is a regular, radial arrangement of the fibers; that these proceed from the center; and in fact, form an aster, the rays of which are continuous with the cytoreticulum. It is, therefore, a typical centrosphere, very similar to that seen in figs. 64, 68, 71 and fig. 74. Nothing but preconceived ideas regarding the origin of the centrosome de novo, — a claim which has not yet been proven — would prevent us from inferring that this body originates from the centrosome of the dividing oogonia. It has all the characteristics of a true centrosphere. It maintains these characteristics, (though often much modified by accumulating yolk granules), in later stages of growth of the egg, figs. 26, 27, 32, 39, 40—63, 67, 70, 73.

In fig. 42, the body is shown as it appears in an egg about one half the size of the fully matured egg. It is drawn with low magnification; and it is a true picture of what everyone can see for himself in my preparation.

The section of an egg represented in fig. 46 is drawn with a higher magnifying power. It is a platinum chloride preparation, beautifully preserved. It shows a large body in the center of the cytoplasm connected with the germinal vesicle in the same way as is the similar body in the egg of the tortoise, pl. XXIX. The zones are distinctly separated as if by a membrane; and the radial fibers within the body, are exceedingly fine and closely arranged, giving a silken effect. Stained with the Biondi-Erlich mixture, its color is a golden brown, while the rest of the cytoplasm is reddish.

Taking the forms mentioned as the typical forms most clearly suggesting the centrosphere, it is possible to understand the many other strange forms met with in these eggs. The body represented in fig. 31 appears as a bluish green body from Lyons blue, the rest of the egg showing the red of saffranin. It contains vacuoles with dark granules resembling nuclei.

In fig. 34 is represented a vitelline body like that of spiders. A distinct astral arrangement of the fibers is visible around the main portion, which stands out large and conspicuous.

Very often the central, tangled mass of fibers, enclosing one or several granular vacuoles, is surrounded by a zone of large blue granules. These are seen most clearly in material stained with hematoxylin, fig. 37; but also in other stains, as acid fuchsin, fig. 33.

As in the spider, the central felted mass consists of very fine fibers enclosing minute meshes, which sometimes form large vacuoles, fig. 35. The minute fibers and meshes pass imperceptibly into the general cytoreticulum of the surrounding cytoplasm. If this felted mass were entirely expanded, it would resemble the rest of the cytoplasm, in all respects, except that a system of radial fibers would doubtless become visible as in fig. 32. In this case the central body is stained bright red with acid fuchsin, while the surrounding granules are blue from the hematoxylin stain. As there is never more than one body like this in each egg, I cannot consider it anything but a centrosome, attraction sphere and aster. The astral rays are distinctly visible throughout most of the cytoplasm. In fact one gets the impression that the cytoplasm is a huge aster. Compare figs. 26 a and 33 with figs. 39 and 40.

That this body has the structure of an aster as distinct as that in any fertilized egg, may be seen by comparing fig. 37 from the oocyte of *Limulus*, with fig. 68 from a fertilized egg of *Ascaris*.

If this typical centrosome and aster in the ovarian egg originates de novo from amorphous granules, scattered throughout the cytoplasm, and often said to originate from nuclear chromatin, it should be possible to find more than one of these in an egg. But that is never the case.

Such strange forms as that seen in figs. 45, 47, 49 and in figs. 65, 66, and in fig. 36, are probably due to unequal distribution of yolk granules, causing condensation of fibers, which present different appearances according to the plane in which it is sectioned. The typical aster like that of fig. 73 where no distortion has been produced by yolk granules or metaplasm, seems to be a sphere, the same in appearance in whatever plane looked at.

In the large eggs, when definite yolk bodies make their appearance, the sphere and aster are obscured. But its presence is still indicated by a large, solid granular body surrounded by a clearer ring, fig. 28; or else by a large ring of deeply staining yolk granules occupying the center of the cytoplasm, and having a relation to the germinal vesicle, fig. 48, similar to that seen in the youngest oocytes, fig. 25. Something similar can be seen in the large eggs of the tortoise.

As the yolk accumulates in and around the center, the germinal vesicle is crowded nearer to the periphery of the egg, between which and the germinal vesicle a clear spongy protoplasm appears, fig. 48. The latter finally spreads out over the surface of the egg, as the germinal vesicle approaches the surface. This point is the animal pole; while the center determines the vegetative pole, as in the spiders and the tortoise.

There is in this egg, too, a distinct polarity, which must be attributed to definite structural elements persisting throughout the growing period of the ovarian egg. I have elsewhere shown (65) that this polarity does not reside in the nucleus alone, but in the nucleus and cytoplasm combined. The yolk nucleus, as here described, being fundamentally an attraction sphere, consisting of a central body, circles of granules and astral rays, represents that part of the cytoplasm by which that persistence can be accounted for.

As in many other eggs, so in this, a ring around the germinal vesicle sometimes appears. I have seen this very clearly also in the living egg. Such a ring is represented in fig. 38. It is clearer or lighter than the outer zone of cytoplasm, and it is bounded by a thin layer of fibers, which seem to proceed at one pole of the germinal vesicle, from an obscure sphere, around which there are indistinct evidences of astral rays.

In this case the germinal vesicle is normal, having chromatin arranged in the usual way in these eggs, viz., as a nuclear reticulum with a large, distinct nucleolus in which there is a vacuole.

But cases are frequently met with in which the inner zone is particularly dark and granular, especially when stained with hematoxylin; but distinct also, when stained with picrocarmine or a variety of other stains, fig. 43.

In many cases, as here, the ring is broadest at one pole; and often forms an irregular mass of closely packed granules, or else, more or less scattered irregularly throughout the central part of the cytoplasm.

Always in these cases, so far as my observations on this egg go, the germinal vesicle is large and spherical, showing no evidence of shrinkage. But it seems to be entirely devoid of chromatin, there being no nucleolus and only very slight traces, if any, of the stainable substance corresponding to the nuclear reticulum of the more usual forms, such as is shown in figs. 38, 46.

I have been inclined to consider this a pathological condition of the egg. It is not an artefact, as it occurs in the best preserved material, and the entire egg shows no evidence of shrinkage of any kind, that might be attributed to bad preservation.

If the eggs showing these features, fig. 43, are normal, and if the inner granular zone is due to extruded chromatin, it is certainly difficult to have much faith in the individuality of the chromosomes. The contents of the distended nucleus looks like a colorless precipitate, such as one might expect from the action of acids on solutions.

Aside from the absence of chromatin, of which I have no explanation to offer, the granular ring may possibly be accounted for as was the metaplasm in the egg of the tortoise; namely, a combination of karyolymph from the germinal vesicle with unassimilated food in the cytoplasm derived from an external source. In this case, it would be the secretion of the lining cells of the ovarian tubes, which, in *Limulus*, as in spiders, serve as follicle cells, so far as nourishing the egg is concerned. This granular substance may be the metaplasm used by the astral rays as food.

That the vitelline body and the aster originate de novo, from these amorphous granules, I cannot believe; for I have shown that they are a direct continuation of the centrosome and aster of the dividing oogonia.

The aster and centrosome (vitelline body) in the cytoplasm, is one thing. Amorphous masses of metaplasm, like that seen in fig. 43, and a similar substance in the egg of the tortoise, figs. 2, 3, 6, 9 is an other thing. To my mind, they bear the same relation to each other as an amoeba bears to the food which it takes in.

Real Nuclei in Cytoplasm.

I find a third body in the egg cytoplasm, which is also distinct; namely, real nuclei, figs. 58 and 59. These bodies alone show the specific staining reaction of chromatin. With the triple Biondi-Erlich stain, they retain the green, while all else in the section is red. They differentiate beautifully in picrocarmine and in borax carmine and hematoxylin. What I have called metaplasm in the turtles, egg, and also in that of *Limulus*, fig. 43, never gives the specific stain of chromatin so far as my experiments go.

In my mind, there is not a shadow of doubt that the little bodies, shown in figs. 58 and 59, are real nuclei. Besides their staining reaction, they have the general form of nuclei. They often occur in great numbers, especially near the center of the egg. Eggs in which these nuclei are found show evidences of degeneration. Their presence in the cytoplasm is doubtless evidence of a diseased condition of the egg. In many cases, the yolk and cytoplasm seem normal, fig. 59; but the germinal vesicle is always wanting in such eggs. The distinctly pathological features appear later, when the yolk granules begin to disappear in irregular patches, as if devoured by nuclei. Strands of clear protoplasm then become visible; and in these, the nuclei are imbedded, fig. 58. The outlines of the egg become irregular; the cell membrane greatly folded and pierced with holes; and the whole egg seems finally to be absorbed.

Cases are often met with where the egg is evidently being absorbed, though no such nuclei can be detected.

Whether these nuclei come from outside as leucocytes, or from epithelial cells of the egg stalk, serving as food for the egg and surviving the digestive process, or whether they arise from fragmentation of the germinal vesicle, would be a very interesting subject for investigation. But there is no reason to suspect that they have anything to do with the formation of the vitelline body. Their occasional presence in what at first sight seems to be normal eggs, was to me a very perplexing matter, till I was able to convince myself, that even where the cytoplasm seems normal, their presence and the absence of the germinal vesicle, is evidence of beginning degeneration. The whole matter, as far as the question of this origin of the yolk nucleus is concerned, is put in a new light, as soon as later stages of degeneration, when these real nuclei are present, can be examined.

While the central vesicle, both in the spider and in *Limulus* and also in the tortoise, has the general appearance of a nucleus, it never gives the specific chromatin reaction that these nuclei give. The same may be said of the other bodies, as those in figs. 31, 35, 41, 45, which while they often look like nuclei, never give the specific chromatin reaction. I have, therefore, called them vacuoles; and the granules which they contain, I have regarded as metaplasm, — a form of food material which may be stored as reserve food, giving rise to yolk; or, as occasion demands, may be again absorbed by the living, growing substance.

Yolk Nucleus in Egg of Pigeon.

If the yolk nucleus in the egg of the tortoise is what I have intimated we should expect to find something similar in the egg of the pigeon, representing the birds, which are so closely allied to the reptiles. It is interesting to find that this relationship, which has been based on somatic characters, proves to be equally close when the ovarian eggs of the two forms are compared. The statement may be made also regarding the egg of *Limulus* and that of the spider. There is a similarity in the cytoplasm, when seen through the microscope, as unmistakable, one is tempted to say, as that which a naked eye examination of the bodies of the two creatures reveals.

In fig. 51, is represented an oogonium of the pigeon's ovary, a section of which is represented in fig. 50. The oogonium is oval. It has a large nucleus with distinct chromatin bodies arranged as a network. The cytoplasm is very thin except at one pole of the nucleus. At this point, a centrosome and archoplasm spreading out over the nucleus can be seen. After some divisions, the oogonia cease dividing; and some of the cells are differentiated into follicle cells, surrounding the growing oocyte. I have studied this point carefully in *Clemmys*; and I have found nothing in the pigeon's ovary that would warrant a different interpretation.

In this section of the pigeon's ovary represented in fig. 50, several oocytes of different sizes appear. They are all surrounded by a follicle. Like the oogonium which has no follicle, the oocytes are oval; the nucleus, now the germinal vesicle, is excentric.

In osmic acid preparations, a dark crescent stands out prominently in the thicker part of the cytoplasm. The horns of the crescent, partly surround the germinal vesicle. Higher magnifying powers reveal in the thickest part of this crescent, a round, light area. In favorable preparations, a round dot, the centrosome, fig. 53, is distinctly visible, in the center of the light area. Closer examination shows the whole stained body to be an attraction sphere, with astral rays and concentric circles of microsomes, fig. 52.

I take this structure to be a continuation of the similar structure seen in fig. 51. Without some positive proof of spontaneous generation, I do not feel justified in giving it another interpretation. The actual proof of this conclusion could be had, if it were possible to see the oogonium in fig. 51 actually grow into the oocyte in fig. 52.

The structure in all is certainly identical, with slight differences readily accounted for by different amounts and disposition of granules; as well as by the variable condensations incident to hardening and staining the preparations.

Anybody who has examined a hardboiled hen's egg will not find it difficult, perhaps, to concede the possible connection of this centrosome and sphere with the central area of concentric circles of white and yellow yolk, the concentric zones in the mature egg being probably foreshadowed by the concentric circles of large microsomes of the aster, fig. 52. I find, in fact, in all eggs in which the yolk nucleus is prominent, distinct indications of regular stratification of the cytoplasm, as in the bird's egg.

I am not aware that the female centrosome has yet been demonstrated in the mature hen's egg. But if my suggestions are true, it ought to be found in the center of the latebra. If we compare the ovarian egg of the pigeon with that of the tortoise, the conviction that the latebra of the bird's egg develops out of the yolk nucleus, is irresistable. My preparations suggest that this is indeed the attraction sphere of the oocyte.

The connection between the germinal vesicle of the bird's egg and the latebra is similar to that between the germinal vesicle and the yolk nucleus in the egg of the tortoise. This connection also serves to explain the development of metaplasm, — (a kind of yolk, possibly corresponding to the early stages of yellow yolk in the hen's egg) — around the yolk nucleus in the tortoise. As I have already intimated, it forms a path or channel along which the karyolymph from the nucleus is conveyed to the expanding centrosome and sphere.

The constancy of the yolk nucleus in these eggs, precludes the interpretation that it is a transient feature, an accidental condensation, or a fortuitous concourse of atoms and molecules in an amorphous substance. There is a structure in the cytoplasm of eggs, revealed by the microscope, which enables one to recognize relationships between eggs of different classes of animals.

As in the case of the tortoise, the spider and the kingcrab, the yolk nucleus (centrosphere) determines the vegetative pole of this egg; and together with the germinal vesicle, with which it stands in the most intimate relation, it determines the egg axis. Through this body, therefore, there is an unbroken succession from the primitive germplasm, handed down from the preceding generation to the development of the primary germ layers, from which all organs finally develop.

The Frog's Egg.

I have not studied the frog's egg very carefully with reference to the presence or absence of the yolk nucleus in the very young egg. But I have found indications of a body resembling that in the pigeon's egg. It tends to form a ring around the germinal vesicle somewhat like that seen in the kingcrab, fig. 38. In this ring are irregular, stainable masses, between the inner and outer zone resembling the metaplasm in the egg of *Clemmys*.

At one pole, there is an aggregation of such granules both outside the germinal vesicle and inside. Many of them look like deeply staining nucleoli. That these nucleoli come out bodily from the germinal vesicle, I hesitate to believe.

The Cat's Egg.

In favorable preparations of the ovary of the cat, eggs can be found showing a yolk nucleus (centrosphere) plainly. Such an one is represented in fig. 56. These are beautiful preparations, with no indication of shrinkage inside or outside the egg. The germinal vesicle is uniformly spherical, and contains a nuclear network of chromatin in which there is a comparatively large spherical, and deeply staining nucleolus. At one pole of this germinal vesicle, there can be seen by proper manipulation of the illuminating apparatus of the microscope, a round area, nearly as large as the nucleus, and slightly more translucent than the rest of the cytoplasm. Careful focusing shows a ring of granules in the center, and a little granule in the center of the ring. I take this to be the yolk nucleus a real centrosphere.

Unlike the eggs previously described, the cat's egg develops no true yolk spheres, such as are found in the mature egg of the pigeon, kingcrab and tortoise. Neither is there in the early stages any of those irregular masses in the cytoplasm which I have designated as metaplasm. The presence of metaplasm in eggs developing true yolk spheres later, points to some connection between that metaplasm and the true yolk bodies.

In the cat's egg, the cytoreticulum is not so distinct, the cytoplasm is more uniformly granular. But as the yolk nucleus (sphere) is really part of the living substance, it has the same index of refraction. It confirms the statement of Balbiani, that in eggs having little yolk, it remains inconspicuous, because, in the living egg, it like the rest of the cytoplasm, is transparent; and by the influence of reagents, it changes like the rest of the cytoplasm. Consequently it remains as indistinct as ever. This to me is only another proof that it is a structural part of the cytoplasm, rather than a foreign, amorphous mass of chromatin or other substance.

The Yolk Nucleus in Egg of Fish.

In the egg of the goosefish, a similar body is conspicuous, figs. 54, 55, 57, 60. It has the appearance of a large lump of archoplasm, located at one pole of the germinal vesicle, where the latter is slightly indented. The horns of the archoplasmic mass extend out on either side of the germinal vesicle, fig. 57, and may even form a ring around it, fig. 54.

In the widest, central part of this archoplasm, is a denser, spherical body, which stands out prominently even in hematoxylin stains, fig. 55. In the center there is a clear vacuole or vesicle, fig. 57. But this may contain a deeply staining body suggesting the centrosome, figs. 54, 60. There are indications of indistinct, concentric rings, surrounding the central body; but astral rays are not distinctly visible in my preparations.

There is only one of these in each egg; and as it bears the same constant relation to the germinal vesicle as that seen in the other eggs described, I have no hesitation in identifying it as the vitelline body or centrosphere.

As I have not studied the fully developed eggs, I can say nothing as to the ultimate fate of this body. I can find no excuse, whatever, for assuming that this body originates from follicle cells, from fragments of the germinal vesicle, or from migrating nucleoli. There is nothing about it to suggest that it owes its origin to chromatin entering the cytoplasm from the nucleus. Rather, as I have suggested in the case of other eggs, especially that of the tortoise, it owes its prominence to an infiltration, between the constituent fibers, of karyolymph; which, when coagulated by reagents, makes it look more like a homogeneous mass. Consequently the finer details visible, for instance in some eggs of the kingcrab, are less evident. Possibly other methods of preparation, than those I have tried might bring the finer details into view.

It is conceivable that, when this archoplasm, partly or wholly surrounding the germinal vesicle, becomes vacuolated by the formation of metaplasm, it would be converted into a network enclosing granules. It would then resemble that broad granular ring around the germinal vesicle, so often seen in the eggs of fishes. The sphere would then be obscured and possibly be invisible altogether.

As in the other eggs, so in this, there is an axis differing from all other axes, that, namely, connecting the vitelline body (sphere) and the germinal vesicle. That is also indicated by the indentation of the germinal vesicle, where it is in contact with the sphere.

The Yolk Nucleus in Egg of Crayfish.

Of all the eggs examined, the crayfish egg, seems on a superficial examination, least likely to reveal any trace of a yolk nucleus, such as I have described in other eggs. The larger eggs seem to be radially symmetrical. The germinal vesicle is usually surrounded by a ring of less granular protoplasm, which sends out processes into the surrounding yolk.

But in many younger eggs, an indentation of the nucleus at one pole is visible. This indentation is often so marked as to give the germinal vesicle the appearance of a crescent, and occasionally the form of a horseshoe, fig. 77.

The finely granular protoplasm, surrounding the nucleus, fills the cavity in the nucleus. Owing to the extreme minuteness of the fibers and the granules in this part of the cell, with all those methods that I have employed, it is difficult to make out any definite structure.

But when the indentation is not so pronounced, as where there is only a notch in the otherwise circular germinal vesicle, a more condensed portion can be detected in that notch. A slightly lighter circular vesicle can be seen in the center of this, often close to the nuclear membrane, as in fig. 75. Surrounding it, are distinct indications of concentric circles, sometimes visible only part way around.

Surrounding this again is a large body of undifferentiated protoplasm, conforming to the outlines of the germinal vesicle. This taken in conjunction with the latter, completes an oval area occupying the center of the more granular cytoplasm outside. In this protoplasm, appearing at first view like homogeneous archoplasm, it is possible to see, with the highest power, a distinct indication of very delicate, closely packed striations.

The central part of this centrosphere may be partly embedded in the indentation of the nucleus, as in fig. 76. It is possible that in cases like that shown in fig. 77, the sphere may be wholly imbedded in the stalk of protoplasm extending into the interior of the nucleus.

In cases where the plane of the section does not coincide with this stalk, a circular area of homogeneous archoplasm occupies the center of the germinal vesicle; in which case the germinal vesicle looks, in section like a ring. The central mass of archoplasm, or let us say homogeneous protoplasm is always, so far as I have seen, connected with the main ring outside the germinal vesicle, sometimes, however, only by a very slender string or stalk.

It is possible that further investigation with methods of fixing better suited to this egg, than those I have used may reveal the centrosome as a constant element of the cytoplasm of this egg.

III. General Survey of the Literature.

Origin Structure and Significance of the Yolk Nucleus.

It appears from the literature that the yolk nucleus is to be found in the eggs of representatives of all classes of animals. Yet it is by no means admitted to be universally present. Even in spiders, where it is most prominent, Balbiani (4) admitted, that in a number of species he found no trace of it. Yet he says: "I was fortunate enough to find the body in a great number of representatives of almost all classes of vertebrates and invertebrates".

Thompson (89) wrote of the yolk Nucleus in frog's egg: "I have in general found it present, and think it more probable that it may be destined to form the external and larger corpuscles of the yolk".

Says Henneguy (32): "Ce n'est que chez quelques animaux que je suis arrivé à trouver la vésicule de Balbiani d'une manière constante dans tous les jeunes ovules; chez d'autres, je n'ai pu l'observer qu'exceptionnellement; chez beaucoup enfin je n'ai pu la voir".

Of the yolk nucleus of spiders v. Wittich (98) said in 1849: "Ob derselbe früher, gleichzeitig oder später als das Keimbläschen entsteht, wage ich nicht zu entscheiden. Immer sah ich sie übereinander, und zwar so, daß das Keimbläschen im fundus folliculi, jener zweite Körper aber seinem Halse zu gelagert ist".

LUBBOCK (56), studying principally the myriapods, refers to the constant presence of the yolk nucleus as one reason for assigning to it an important function, though, on the whole, he does not attach to it much importance.

In this matter as in many of his other observations, BALBIANI (4) is suggestive. Thus, he admits seeing the yolk nucleus in the egg of the dog, of the cat, of the squirrel, of the cow, and in the human ovum; but he says, the study is difficult because its refraction is the same as that of the vitellus. It is necessary, he says, to examine young follicles, where the vitellus is still homogeneous and transparent; and then he warns us not to use any reagents that will affect the transparency of the vitellus.

The failure to find the yolk nucleus in many cases, can be attributed first to the fact that, with the exception of MUNSON (61), no real, systematic and persistent attempt has been made to trace the history of the body in a single egg, haphazard observations being relied on to reveal its presence in as many different eggs as possible. The history of the yolk nucleus resembles the history of biological science, in that it has passed through a natural history stage of mere seeing and naming. A second reason for failure is as stated by BALBIANI, that in transparent protoplasm it too is transparent; and in opaque protoplasm rendered so by reagents, it, too, is equally affected leaving it as invisible as ever.

This ought to afford a warning not to attach much importance to statements as to the absence of this body. For while the investigator's inability to adapt his methods to the subject may thus be indicated, no positive proof is given of the absence of such a structure.

In the literature, the following cytoplasmic inclusions have been called yolk nuclei: 1. a single large spherical body, with fibrous capsule enclosing a vesicle filled with granules or with a more or less transparent substance; 2. several, scattered, small bodies resembling ordinary cell-nuclei in shape, size, and staining reaction; 3. amorphous masses of stainable granules encircling the nucleus; 4. scattered masses of stainable substance supposed to be more or less fluid; 5. single, definite masses either spherical or crescent-shaped, resembling archoplasm, closely applied to the germinal vesicle, or removed from it so as to occupy approximately the center of the egg; 6. definite attraction spheres, with astral rays and centrosome.

It is difficult to believe consequently that LERBOULLET (51), CRAMER (20) and REICHENBACH (76), though often quoted, in this connection, have described the real yolk nucleus. The latter, speaking of developing crayfish eggs, showing segmentation nuclei at the periphery says: "In dem ausgeflossenen Inhalt des Eies beobachtet man noch ein eigentümliches Gebilde, welches sich auch in späteren Stadien, wo bereits die Gastrula in der Entstehung begriffen ist, vorfindet. Es ist dies ein kugeliges Bläschen von 208 μ Durchmesser, mit scharfen Konturen, in dessen Inneren zahlreiche vacuolenartige Gebilde und fettige Dotterelemente sich vorfinden. Stets ist es von einer hofartigen Protoplasma-Ansammlung umgeben. Über seine Bedeutung konnte ich mir kein Urteil bilden, aber ich glaube, daß es dem von einigen Forschern beschriebenen Dotterkern in den Eiern gewisser Arachniden homolog ist".

Speaking of round granular bodies found in the cytoplasm of eggs of *Rana*, which King (40) has recently described also in toads as vitelline bodies, but which Goette saw nothing of Hertwig (34) says: "Mir scheint es einzig und allein mit der Bildung der Dottersubstanz in Beziehung zu stehen, und eine eigentümliche, lokale Ansammlung von Nährstoffen darzustellen".

On the other hand, Balbiani (4) says: "By reason of the homology which exists between the yolk nucleus and the centrosome, it is probable that the two elements have a similar origin."

On the one hand, therefore, the yolk nucleus resembles yolk or metaplasm; on the other, the aster and centrosome. The problem of the yolk nucleus involves first the problem of cell metabolism and cell physiology in general; second, the problem of cell morphology and protoplasmic organization. Where these two sets of problems unite as they do in this body, there is sure to be conflict.

In view of this, the statements of observers regarding the origin of this body, and its significance acquires additional interest, partly because many of the early observers were ignorant of these problems as we now view them, and partly because of a naïve element arising from the prominence, at that time, of problems which are now regarded as settled.

Many of the early writers like Leydig (50), frankly admit they do not comprehend the significance of the yolk nucleus. v. Wittich (98) says: "Die Bedeutung dieses Gebildes vermag ich bis jetzt noch nicht anzugeben ... Gleichzeitig mit dem Anwachsen dieses Körpers, das ein bestimmtes Maß nicht zu überschreiten scheint, beginnt eine allmähliche Verflüssigung vom Centrum aus, dieselbe greift immer mehr um sich, so daß zuletzt die äußerste Schicht eine immer noch dickwandige Kapsel bildet, die erst bei fortgesetztem Druck an einer Stelle platzt und ihren ganz flüssigen Inhalt entleert."

Siebold (84) believed that it plays an important role in the development of the egg, because, he says it appears early and disappears late.

According to Carus (18), it is the center of formation of the plastic substance of the egg, while the nutritive parts seem to collect around the germinal vesicle. The granular body seen by Cramer (20) in the young transparent egg of the frog, and also seen by Carus, was compared by them to the yolk nucleus of Arachnids, observed by von Wittich (97); and was thought to form on its surface the vitelline granules. Balbiani (3) also said that it is around this body, whenever it is to be seen, that the granules of the cytoplasm, which constitute the plastic part of the egg, or the germ, are formed.

Lubbock (55) regarded it as a condensed portion of the cytoplasm. In myriapods, he described it as a clear vesicle, often enclosed by a circle of little, bright granules, which are sometimes scattered throughout the vesicle. The protoplasm surrounding it becomes condensed, and in it, granules are formed, which spread throughout the cytoplasm, and forms on the periphery of the egg a continuous layer which constitutes the germ.

In both vertebrates and invertebrates, the yolk nucleus has been described as a clear vesicle, varying in size, and surrounded by a zone of concentric fibers, or by a zone of granules or both. In the frog's egg, Reichenbach (76) found that the central body is surrounded by a radial arrangement of the yolk, which is of special interest in view of the fact that the centrosome and aster had not yet been discovered.

The central vesicle is often called a nucleus. Thus Henneguy (32), quoting Balbiani, says of this body in the frog: "It is always a granular mass, containing a little clear nucleated vesicle which is the nucleus." Says Balbiani (3): "La vésicule embryogène est une cellule, puisqu'elle est formée d'une masse du protoplasma, avec un noyau et un nucléole. Ces deux derniers éléments ne sont généralement pas difficiles à apercevoir, mais le protoplasma se confond souvent avec celui de l'œuf parsqu'il possède la même réfringence."

Because of its similarity to the nucleus of an ordinary cell, a similarity especially striking in many cases where there are several scattered throughout the cytoplasm, it has been surmised that it results from cells wandering in either as follicle cells or as leucocytes. Such appearances of which the ascidian test cells are good examples, led many reliable observers to doubt the correctness of Gegenbaur's generalization that all eggs are single cells.

At this stage of development of the subject, the problem of the yolk nucleus becomes intimately involved in the problem of nutrition of the egg, and its great accumulation of yolk as compared with ordinary cells. At the same time, also, the discovery of the phenomena of parthenogenesis adds a new impetus to speculation as to the probable meaning of the yolk nucleus. It was then suspected that parthenogenesis in aphids, depends upon it. Balbiani suggested that the follicle cells, penetrating the egg, act as a spermatozoon, the presence of the yolk nucleus in insect eggs being evidence of such a follicle cell in the egg. Balbiani (3) claimed to have seen the follicle cells in the egg of the cat. He says: "La vésicule embryogène naît par bourgeonnement de l'une des cellules épithéliales qui entourent l'œuf dans le follicule de Graaf." To strengthen his argument he quotes Pflüger and Lindgren who claimed to have seen cells passing through the zona radiata of the cat. Balbiani (4) gives figures showing in one a large yolk nucleus lying at the periphery of the egg, close to the follicle; and in another figure, he shows how this supposed cell has entered the egg, leaving behind it a path or canal. He shows the same in the egg of *Gephilus*, where a large nucleus connected with the follicle has pushed in the outer protoplasm of the egg, and developed around it a rudimentary aster.

In the egg of spiders, where there is no follicle, Balbiani claimed that the yolk nucleus is due to the entrance of a cell of the egg stalk. He claimed with v. Wittich, that the yolk nucleus in spiders is first seen in the neighborhood of the stalk. The writer has investigated this question, and finds that both were mistaken in these observations. Munson (61) has also shown that in *Limulus*, whose ovary resembles that of the spider, there is no constant relation between the position of the vitelline body and the point of attachment of the egg, not even in the earliest stages of the egg. Munson has also figured a yolk nucleus attached to the periphery of the egg by a band of fibrous protoplasm; but it shows no connection with the stalk, and is given no such interpretation.

In connection with the above theory of Balbiani it is of some interest to recall the socalled paracopulation cells of eggs of Daphnids described by Weismann and Ischikawa (92). These investigators found that, in those eggs which are parthenogenetic, only one polar body is given off; while, in those that require fertilization, two polar bodies are extruded. In the fertilizable winter eggs, of six species of Daphnids, belonging to four genera, there is found, in the egg during ovarian development, a cell which like a foreign intruder moves slowly about. It arises, according to them, from extrusion of nuclear substances into the body of the egg cell, develops into a real paranucleus, and finally becomes surrounded by a cell body.

The subsequent history of this cell is that it fuses with one of the cleavage cells after development has begun. They claim that this is common in the animal kingdom, but they do not pretend to give any further explanation of it.

Interesting, also, in its bearing, first on the question of origin and nature of the yolk nucleus, second in its bearing on parthenogenesis, but mainly because it shows to what extent interpretation of observations is influenced by the larger problems prominent at the time, is the work of STUHLMANN (86). He says: "Es ist mir nun gelungen, an einer Reihe von Insekteneiern sicher einen Austritt von großen Ballen aus dem Keimbläschen zu konstatieren, die sich nachher im Eiplasma auflösen. Später verschwindet das Keimbläschen vor unseren Blicken, bis wir endlich am oberen Eipol den Furchungskern wiederfinden." STUHLMANN calls attention to the work of GROBBEN (30) and also to WEISMANN to show that maturation takes place in parthenogenetic ova. He seeks to show that the germinal vesicle in such eggs, behaves like an amoeba, giving out pseudopodia containing nucleoli and chromatin granules which are constricted off from the germinal vesicle, and appear for some time in the cytoplasm, as "Reifungsballen" and are finally dissolved. He also finds bodies resembling real nuclei near the periphery of the egg, which he admits may be derived from inwandering follicle cells. But they differ from the first named "Reifungsballen". The latter give rise, in some eggs, to granular masses, which he calls diffuse yolk nucleus, or they may give rise to one or two or more large spherical bodies, the true yolk nucleus.

STUHLMANN (86) seems to believe that parthenogenesis is common; and that many eggs can develop partly parthenogenetically; in support of which he cites LEUCKART (49) on frog's egg, OELLACHER (69), HEUSMANN, JOURDAN (39) and OSBORNE (70). He seems to assume that the "Reifungsballen" are given off as a preparation for such development. He says: "Die Reifungsgeschichte der Eier von *Banchus* hat uns aber die interessante Tatsache ergeben, daß das Auftreten der Dotterkerne unabhängig von dem Austritt der Ballen ist, da letzterer Vorgang ersterem hier vorangeht. Das sind entschieden voneinander ganz unabhängige Bildungen . . . Bei der Bildung des Dotterkerns konnten wir zwei Stadien unterscheiden: Zuerst werden kleinere Ballen in der Nähe des Keimbläschens gebildet, welche dann später zu einem am hinteren Eipol liegenden Dotterkern verschmelzen."

The entrance of chromatin substance from the nucleus into the vitellus has been affirmed by FOL, ROULE, WILL, LEYDIG, VAN BAMBEKE, HENNEGUY, SCHMIDT, KOHLBRUGGE, LOYEZ and many others. Some of these also admit that yolk material from the follicle cells enters the egg.

It is often claimed also by competent observers that real cells in the cyloplasm of eggs may be a normal occurrence; that eggs may devour neighboring cells as an amoeba or a paramaecium eats other cells, the latter retaining their identity for some time in the cytoplasm. Says LOYEZ (53b): "Remarquons tout d'abord que, dans les très jeunes ovules, on voit quelquefois dans le cytoplasma, de préférence vers la périphérie, des noyaux de petites cellules folliculaires, les uns sont absolument intacts et normaux; d'autres sont plus ou moins altérés, en voie de dégénérescence ou transformés en un globule."

Thus also VAN BENEDEN (11) says: "The character of the deutoplasm varies much. Sometimes one finds it in the egg represented by real cells, provided with a nucleus and a nucleolus. This fact is easy to prove in many Trematodes, such as *Amphistoma*, *Polystoma* and many others. MAX SCHULTZE observed them in *Prorhynchus*, and LEYDIG in his treatise on comparative histology emphasized the same fact in several Annelids, as *Piscicola*. WEISMANN and BESSELS have seen them in insects, the former in Muscides, the latter in Lepidoptera. But little by little, these cells become disorganized in the egg and their contents set free".

It may be well to recall in this connection that previous to 1861, yolk bodies were thought to be cells. They were so considered by AGASSIZ and CLARK (1, b).

For a long time HIS maintained that the egg in fishes is nourished by leucocytes, the follicle being in fact formed by leucocytes which penetrate the egg and being dissolved form the outer part of the cytoplasm, the cicatricula being thought to be the only remnant of the original egg protoplasm.

Says DOFLEIN (23): "Das Ei von *Tubularia* entsteht durch Verschmelzung einer Anzahl von kleinen Zellen. Der Kern derjenigen Zelle, welche als die kräftigste in den Verband eintritt, unterdrückt die übrigen Keimzellkerne. Seine Individualität persistiert, indem er zum Eikern wird." It is needless to say that the theory of HIS has long since been abandoned.

LUBBOCK (55) quotes HUXLEY as follows: "It will be observed that all these authors consider the winter ova or ephippial ova and the ordinary ova to be essentially identical only that the former have an outer case. The truth is that they are essentially different structures. The true ova are single cells, which have undergone a special development. The ephippial ova are aggregations of cells (in fact larger or smaller portions, sometimes the whole of the ovary), which become enveloped in a shell, and simulate true ova." This aggregation of several cells (one of them putting on the appearance and performing the function of a PURKINJEan vesicle), and the whole becoming enveloped in a shell, is, however, the ordinary, and only method of egg development in many lower animals. In the Trematodes, Cestodes, and the great number of the Turbellaria, the yolk and the PURKINJEan vesicle are formed in two separate organs."

Speaking of insect eggs, LUBBOCK (56) says: "We cannot, therefore, class as false eggs those which arise from more than one cell. Perhaps it would be better to distinguish the two classes as compound and simple or unicellular."

PRENANT (73) says of *Plathelminthes*: "In flat worms, the separation of the nutritive and the formative vitellus is still more narrowed. With them, the nutritive yolk belongs exclusively to special cells — vitelline cells furnished by one particular gland, the vitellogene. These cells arrange themselves around the formative cell, the germigene. Thus results a composite egg."

WILSON (95) says of hydroids that the egg may actually feed upon surrounding cells, taking them bodily into its substance, or fusing with them, and assimilating their substance with its own. In such cases the nuclei of the food cells long persist in the egg cytoplasm, forming the socalled pseudocells, but finally degenerate, and are absorbed by the egg. It would here seem as if a struggle for existence took place among the young ovarian cells, the victorious individuals persisting as the egg; and this view is probably applicable, also, to the more usual case, when the egg is only indirectly nourished by its brethren. He cites DOFLEIN (23) in support of this. A similar generalization as to a struggle for existence among germ cells has been expressed by MUNSON (64), based on numerous observations on both egg and sperm cells. He refers, however, to cases where this struggle results in the elimination of the germ cells. This seems to be a common phenomenon.

It seems that in some cases cells entering the egg cytoplasm may persist for some time as the test cells of truncates, where there is little room for doubt in regard to their origin. WILSON (95) refers to the observations of FLODERUS (26) in Ascidians, and to OBST's observations, of similar import, in eggs of Molluscs.

Early writers like LUBBOCK did not regard the fertilized egg as a single cell, and where several cells fuse and persist in the egg, he would admit the propriety of speaking of compound eggs. Yet he says of Myriapods, the eggs of *Lithobius*, *Cryptopus*, *Geophilus*, *Arthronomalus*, *Polydesmus* and *Julus* are simple, the vitelline vesicle occurring in some of them being probably homologous with the yolk nucleus of spiders. He apparently did not suspect that the yolk nucleus has its origin in cells, entering the egg from without. The question of the origin of the yolk nucleus was not considered seriously at that time, as the problem of the centrosome did not then exist.

There is no doubt that egg cells are nourished by other cells, but how far such cells can destroy the unity of the egg or how far they influence the polarity of the egg, is a matter deserving more attention than it has received. Munson (65) has shown that in *Limulus* the position of the yolk nucleus bears no constant relation to the point of attachment of the egg, where the granular food material secreted by the lining epithelial cells of the ovarian tubes, accumulates.

In *Myzostoma*, according to Wheeler (93), the ovarian egg is accompanied by nurse cells which fuse at opposite poles of the egg, and give rise to a spongy protoplasm corresponding to the vegetative and the animal pole of the egg.

That eggs may resemble amoebae and devour other cells as protozoa do, seems to be pretty well established. Weismann tries to explain the need of this on the grounds that the nourishment received from the blood is insufficient. The eggs may grow to a certain size from that source alone, but to grow larger, they devour the smaller cells around them. In Daphnids, according to Weismann (90), the food cells may first dissolve into a Protoplasmalösung which then is devoured by neighboring cells, and they in turn dissolve, yielding their substance to the growing egg.

Degenerative Processes.

While the entrance of cells may be considered normal in many cases, there is no doubt that it is in many cases a result of degeneration of the egg; and, unless degeneration be considered a normal process, must be regarded as pathological.

According to Weismann (90), the eggs of Daphnids undergo spontaneous degeneration, and are sometimes absorbed by neighboring epithelial cells. In 1849 Leydig (50) described degenerative eggs. He says: "Außer den so also veränderten primitiven Eiern trifft man aber in demselben Eierstocke auch viele Eier, die eine wohl rückgängige Metamorphose anfangen."

Will (94) described in insect eggs, wandering cells which he assumed to be food cells, but two years later he interpreted this as the beginning degeneration of eggs that have not been discharged.

Wagner (89b) speaking of the dog's ovary says: "Die ungeheure Zahl von Eiern wird durch das Zugrundegehen einer Menge derselben vermindert." He seems to regard the degeneration as due to absence of a nucleolus. Instead of it, there are in the germinal vesicle irregular, shining, striated, crystalline bodies. Both the germinal vesicle and the yolk may undergo the same change.

PFLÜGER (71) referring to the egg of the cat and of the cow, says that a cat becomes ten years old; and during that time, at most two hundred eggs have left the ovary, while in youth several thousand were present. Therefore very many must have degenerated. Physiology, he says, must regard this as normal; for, in order that one follicle may develop, several must perish; for all would perish, if nature allowed all to be equally developed. He refers to fatty degeneration as follows: "Durch die beschriebene fettige Entartung werden die Follikel mit Epithel und Ei ergriffen; allmählich klärt sich das Gewebe des GRAAFschen Bläschens wieder auf; seine Struktur ist aber undeutlich geworden, und schließlich deuten nur hellere, runde Flecken die Stelle an, wo ein Follikel lag." He says the yolk is often in the form or balls or spheres resembling cleavage cells. The yolk dissolves. The most interesting cause of this was cells. They were found attached to the yolk balls. He describes these as granulosa cells, that send processes through the zona where they are connected inside the egg with vesicles. As the peripheral yolk dissolves, these inner vesicles become loosened and lead an independent existence. He observed this in eggs of cats from four to ten months old, and in a certain sense, he considers it normal. "Es handelt sich also hier um die eigentümliche Tatsache, daß eine im Grund krankhafte Erscheinung als notwendiges Glied in das Bereich des gesunden Lebens sich einfügt."

SCHNEIDER (81) gives an extended account of degeneration of egg and sperm cells in leeches, birds, mammals and other animals. He says it occurs in fishes and amphibia. According to him degeneration of the egg is most apt to occur in those animals in which the period of oviposition is limited to a definite period of the year. In eggs carried over from one season to the next, degeneration may take place, by fatty degeneration and by inwandering leucocytes. He says: "Diese Zellen setzen sich nun an die von dem Eierstock abgelösten Eier und dringen in dieselben ein. Das erste Eindringen kommt selten zur Beobachtung ... Nach und nach dringen mehr Zellen in das Ei, welche darin wachsen, fressen und sich mit kleinen Körnchen und einem größeren fettartigen konturierten Körper füllen. Das Ei zerfällt immer mehr, es bilden sich scharf umschriebene Ballen darin, welche ungefähr wie Furchungskugeln aussehen. Endlich wird die Membran faltig und schließlich wird sich das ganze Ei wohl auflösen."

These observations correspond very closely to those of Munson (61). He found eggs of *Limulus* crowded with real nuclei, giving all the staining reactions of chromatin. He also raises the question whether these nuclei are due to phagocytes or whether they result from fragmentation of the germinal vesicle which is no longer to be found in such eggs. At first the yolk containing these nuclei appears normal. Later the yolk granules disappear, and the nuclei lie imbedded in the protoplasm, but cell boundaries separating the nuclei are not to be seen. The entire egg is finally absorbed or transformed into cells resembling those lining the walls of the ovarian tube.

Munson (63) has also seen degenerating spermatogonia in the butterfly. No cells appear within the spermatogonia or spermatocytes, but they crumble to pieces, not from fatty degeneration as Schneider assumes, but from starvation. In a later paper Munson (64) explains the degeneration of spermatogonia as being due to an abnormal condition of the nuclear chromatin. It was found that those degenerating cells do not secrete the karyolymph in the chromatin which according to him is necessary to digestion; and consequently, as he says, the cell dies from starvation due to indigestion. According to this view leucocytes may enter the cell after it is diseased, and remove it as phagocytes do other foreign bodies. In the case of the spermatogonia, they simply crumble to pieces and furnish a "Protoplasmalösung", as Weismann called it, serving as food for other cells.

Extrusion of Nuclear Material.

Many observers have noticed that the germinal vesicle is often surrounded by a ring, which is either lighter or more granular than the rest of the cytoplasm. And this, again, raises the question, first as to the origin of yolk; and second, as to the relation of this inner ring to the yolk nucleus. As early as 1863, Pflüger (71) distinguished an inner and an outer zone of yolk in mammalian ova, and the clear zone seen by O. Schultze (83) seems to correspond with what Brass (15) called the "Nährplasmaschicht".

Schultze (83) figures four eggs of frog with a zone around the germinal vesicle, and a yolk nucleus between the inner and the outer zone, suggesting the observations of Bambeke (7) and of Munson. He says: "The membrane of the germinal vesicle was irregular, and around it, a clear zone had arisen." The granules of the yolk nucleus began to separate first at the periphery of the egg. Thereupon granules separated from the yolk nucleus . . . these spread in a dark granular zone around the germinal vesicle . . . The clear zone may be present in eggs that have a complete yolk nucleus. Its appearance is therefore not due to the yolk nucleus."

Eimer (25) saw two zones in fish eggs, the outer of which he called the "Rindenschicht".

Bambeke (7) found that the cytoplasm of the egg of *Leuciscus*, *Hipocampus*, and *Lota* is divided into an inner zone and an outer zone. The division of the cytoplasm into two zones was also seen by Lancaster (47) in Molluscs. Will (94) found a ring around the germinal vesicle in insects.

van Bambeke (6) found a granular zone surrounding the germinal vesicle in the fish.

Of reptile eggs, Loyez (53b) says: "One sees around the germinal vesicle a clear zone which Sarasin (79) has compared to the latebra in birds. Němec (66) found a ring partly enclosing the germinal vesicle in very young eggs of Myriapods, as Bambeke (5) had done also in the spider's egg. In the egg of fishes Scharff (80) figures a dark zone of granules surrounding the germinal vesicle. Such a dark granular ring around the nucleus has been described also, in spermatogonia by Auerbach (1). Munson (61) saw a dark ring around the germinal vesicle of *Limulus* both in the living egg and in preserved material. Munson has also seen a clear zone around the germinal vesicle, and as the clear zone seems to precede the dark, he attributed it to the entrance of karyolymph into the cytoplasm. Cunningham (22) regarded the clear zone around the germinal vesicle of fish eggs as an artefact, Carnoy and Lebrun and Lubosch (57) attribute it to faulty fixation. Lams (52) shows in fig. 27 a living oocyte with an outer granular zone containing the "Masse vitellogène" in which is the vitelline body with its central corpuscle. Around the germinal vesicle, he represents a nongranular fibrous zone.

According to van Beneden (11), the deutoplasm may be intimately mixed with the cytoplasm, but also at times separated, and of different constitution, as when produced as a secretion in special organs, the vitellogenes of Cestodes, Trematodes, Distomes, and Turbellarians. Since this secreted deutoplasm is sometimes a granular mass of amorphous substances, sometimes composed of cells, it needs not mix at once with the protoplasm, but forms a ring in the center of which the real egg cell is found.

Coste (19) compared the cicatricula of bird's egg to the entire alecithal egg of lower animals, and recently Riddle (77) has attempted to show that yolk is deposited in layers as a precipitate from without.

According to Beddard (10), the yolk in egg protoplasm is probably derived from the surrounding follicle cells. Munson (61) found in *Limulus* cases where yolklike substances secreted by the lining cells of the ovarian tube accumulate outside the egg membrane after the latter is formed, and that it cannot be distinguished from the granules of the cytoplasm of the egg.

Kohlbrugge (43) gives as origin of yolk two kinds of elements, cytomicrosomes and karyosomes from the germinal vesicle. He recognizes two zones of yolk formation — one at the periphery under the influence of follicle cells, the other at the center under the influence of the germinal vesicle.

Munson (61) expressed the opinion that besides substances that enter the egg from the outside, granules issue, like little drops from the living substance itself. He expresses the view that the inner dark zone is not an artefact, because both he and Giardina (29) have seen it in the living egg. He maintains that it is a normal occurrence, but agrees with Hertwig (34) when he maintains that it is not due to extruded nucleoli. Munson (64) claims that the inner dark zone is due to the action of karyolymph on food entering the egg from outside — the first step in assimilation or digestion.

It has been maintained by Will (94) of insects, by Fol of Ascidians, by Roule and Balbiani of Myriapods, that diverticula of the germinal vesicle containing chromatin are pinched off from the germinal vesicle and move to the periphery where they form the follicle cells. Later Will abandoned this view.

The fact that His tried to explain these zones as the effect of inwandering leucocytes has already been referred to.

Experiments on infusoria have shown the importance of the nutritive function of the nucleus. Referring to observations of Will, Blochmann, Schäfer and Leydig on the extrusion of nuclear fragments forming the yolk nucleus in the cytoplasm, Henneguy (32) remarks that both he and Löwenthal (54) have seen in the young ovule of the cat a stainable corpuscle resembling a nucleolus, leave the germinal vesicle.

Sarasin (79) observing concentric rings in the cytoplasm of *Lacerta* concluded as did Riddle (77) that these are results of a periodicity in the growth of the egg, corresponding to variations in the nutrition of the animal.

The wellknown connection between the latebra and the germinal vesicle in the hen's egg, and the connection of these with the alternate zones of white and yellow yolk are suggestive. Lams (52) found the attraction sphere in the center of a protoplasmic mass called masse vitellogène. The latter seems to correspond to what French writers call couche vitellogène in birds. Loyez (53b) has compared this to the cytocenter described by MUNSON (62) in the tortoise. KOHLBRUGGE (43) regards a similar body in the egg of *Mabuia multifasei* the analogue of the nucleus of PANDER in birds. According to him it is deutoplasm derived from the germinal vesicle.

SCHARFF (80) seems to believe that the inner, granular zone is due to extruded nucleoli. According to him, pouches are formed all over the germinal vesicle like pseudopodia of amoeba, into which the nucleoli are drawn; and these pouches thus constricted off, present the appearance of minute vesicles or cells containing a nucleus. OSIANNSKOV has mistaken them for real nuclei. Sometimes the vesicles thus formed do not contain any nucleolar matter and remain unaffected by stains. Like the others, they move towards but do not quite reach the surface, leaving a cortical layer of protoplasm which is the Rindenschicht of HIS. SCHARFF seems to believe these bodies are finally converted into yolk.

WILL (94) found dark bodies in the cytoplasm which cause the cytoplasm to take chromatin stain. Thus a stainable ring is found around the germinal vesicle, which is finally almost devoid of chromatin. Later this extruded chromatin is converted into yolk bodies.

BAMBEKE (7) figures flamelike stainable bodies or substances coming out from the germinal vesicle, staining like chromatin, and surrounding the germinal vesicle as a ring. Speaking of the boundary of the zones he says: "Il s'agit ici, non d'une couche limite séparant deux parties constituantes du corps protoplasmique cellulaire ou ovulaire, ni de rempli d'une membrane, mais d'une condensation sous forme de faisceau du réticulum cellulaire."

In his earlier publications BALBIANI regarded the yolk nucleus as due to cells entering the egg, but later he maintained that it was due to extruded nuclear material. Finally (4) he came to the conclusion that it is the centrosome and sphere.

Says HENNEGUY (32): "Cet élément provient donc, chez les Vertébrés, de la vésicule germinative, comme M. BALBIANI l'a constaté pour les Invertébrés, chez les Géophiles. C'est très probablement une partie de la tache germinative ou une tache germinative entière qui sort de la vésicule pour pénétrer dans le vitellus."

HOLL (35) says; "Eine eigentümliche Form des Dotterkerns fand ich einige Male: sie besteht darin, daß derselbe in Form einer ringförmigen Masse um die Kernwand auftritt, und nach allen Seiten die gefärbten Strahlen in den ungefärbten Zelleib entsendet, und die Umwandlung des letzteren herbeiführt. Ist dies geschehen, so bildet sich eine neue, dichte sich stark färbende Masse um die Kernwandung, welche neuerdings tiefer gefärbte Strahlen als früher allseitig entsendet und die Umwandlung des soeben erzeugten Netzwerkes in ein neues dichteres, sich noch mehr färbendes herbeiführt."

Says STUHLMANN (86): "Bei der Bildung des Dotterkerns konnten wir zwei Stadien unterscheiden: Zuerst werden kleinere Ballen in der Nähe des Keimbläschens gebildet, welche dann später zu einem am hinteren Eipol liegenden Dotterkern verschmelzen."

As regards the number of yolk nuclei a few writers claim to have seen more than one in an egg. But it is always difficult to determine which form they refer to. LOYEZ (53b) found in small eggs of *Platydoctylus muratis*, two yolk nuclei in the same egg; as had also EIMER in egg of *Lacerta viridis*. LOYEZ says they may result from division of one yolk nucleus. HOLL (35) says: "Anstatt eines Dotterkernes, der also mit seinen Ausläufern in wesentlicher Beziehung zum Protoplasmaleib der Zelle steht, und der immer vorhanden ist (im Gegensatze zu Angaben, daß er nicht immer angetroffen wird, und ein bedeutungsloses Gebilde ist), können auch zwei, ja drei untereinander verbundene Dotterkerne auftreten, die immer in der Nähe der Kernwandung liegen."

HENNEGUY (32) says: „Je n'ai jamais trouvé qu'un seul corps dans un ovule."

Interpretation of Observations on Yolk Nucleus.

Speaking of Araneae, KORSCHELT and HEIDER (45) say that the yolk nucleus is not sufficiently understood, and LEUCKART (49) said: "Die Bedeutung diese Körpers ist unbekannt; weder Bau noch Bildung bietet einen sicheren Anhaltspunkt." Says CARUS (18): "Bei der ungemeinen Veränderlichkeit des Dotterkernes war es schwer, zu einem klaren Verständnis seiner Natur zu gelangen."

KOHLBRUGGE (43) believes the yolk nucleus may arise from follicle cells dissolved in the cytoplasm or from extruded nucleoli.

BALBIANI was supported by SABATIER in the belief that the yolk nucleus is derived from the germinal vesicle, being the male part of the egg which is absorbed by the yolk — a process of maturation, calling to mind the theory of MINOT regarding the meaning of the first polar body.

HENNEGUY (32) concludes with JULIN as follows: "C'est un organ ancestral qui, avec les éléments nucleolaires de la vésicule germinative, correspond au macronucleus des Infusoires, le micronucleus étant représenté par un réseau chromatique prenant seul part aux phénomènes de fécondation."

STUHLMANN (86) agrees with SCHUTZ (82) in regard to its significance. "Der Dotterkern stellt eine Concretion von besonderem, von dem gewöhnlichen Dotter verschiedenem Nahrungsmaterial dar, das zu irgend einer Zeit vom Ei resorbiert wird."

LUBBOCK (56) did not seem inclined to attribute much importance to it, though its presence is so constant, he says, that it ought to have some significance.

STUHLMANN (86) says that it may dissolve early or may be present even in the mature egg. According to KISHINOUYE (42) it is still to be found in the two and four cell stage near the nucleus of the cleavage cells of spider's eggs. The same observation has been made by BALBIANI.

Says BLOCHMANN (12): "Über das endliche Schicksal dieser Kerne bin ich bis jetzt noch zu keinem vollständig sicheren Resultat gekommen. Sie gehen, wie es scheint, allmählich zugrunde, ohne irgend einem später in dem reifen Ei sich findenden Gebilde den Ursprung zu geben." Says HENNEGUY (32): "Cet élément disparaît donc d'assez bonne heure, avant que l'œuf ait atteint la moitié de sa taille définitive."

CARUS (18) speaking of frog's egg says: "Von der Peripherie dieses Körpers löst sich nun ebenso wie beim Dotterkern des Spinneneies eine Körnchenschicht nach der andern los und mengt sich der Eiflüssigkeit bei. Ich stehe deshalb nicht an dieselbe für den Dotterkern des Froscheies, zu erklären. Mit der Vollendung des Eies ist seine Funktion beendet, und während er in der Entwicklung des Eies trotz der Abgabe von Körnchenschichten seine Größe nicht verändert, ist im vollendeten Ei keine Spur mehr von ihm aufzufinden."

MONTICELLI (60) speaking of the yolk nucleus in ova of *Distomum* says that he does not agree with BALBIANI and SABATIER when they identify the yolk nucleus as a centrosome and attraction sphere, but rather with SCHULTZ, STUHLMANN and v. IHRING that the yolk nucleus has no intimate connection with the germinal vesicle and no part in the process of fertilization, but that it is a cytoplasmic product, a nutritive differentiation probably acting as a center in the formation of yolk.

LOYEZ (53b) says: "Je n'ajouterai rien de plus à ce qui a été dit précédemment au sujet du noyau vitellin. C'est une question qui est encore loin d'être resolue. On tend de plus en plus à reconnaître l'existence de cet élément dans les oocytes jeunes, ou il se présente comme le reste de la sphère attractive et du centrosome, mais comme il disparaît plus tard, il est difficile de lui attribuer un rôle dans la formation du vitellus."

STUHLMANN (86) says: "Man kann also wohl den diffusen Dotterkern als eine ontogenetische und phylogenetische Vorstufe des eigentlichen Dotterkerns betrachten, wenigstens bei den Hymenopteren ... Der

Dotterkern der Hymenopteren usw. bildet sich in der Nähe des Keimbläschens, unter dem Einflusse desselben, aber nicht aus ihm."

The latter view corresponds to that expressed by MUNSON (61) who found in the egg of *Limulus* that the granular zone around the germinal vesicle was a temporary effect due to the action of karyolymph on food material, the karyolymph being considered as a digestive fluid. He found that in the spermatocytes of the butterfly (MUNSON [63]), the nutrition of the cell is interfered with when the nucleus fails to secrete the karyolymph. He also found a clear zone around the germinal vesicle which he interpreted as evidence of the entrance of the clear karyolymph into the cytoplasm since little remained in the germinal vesicle when this extrusion had taken place.

MUNSON (65), however, distinguishes between this granular yolk nucleus and what he calls the vitelline body, the latter being interpreted by him as the centrosome of the dividing oogonia. His researches and many later ones yet to be cited render this interpretation very probable.

According to HOLLANDER (36) there are two different elements found in the egg of birds and mammals: first elements of nuclear origin; second an attraction sphere described as a condensed mass outside of the germinal vesicle — the couche palleale of VAN BAMBEKE and the couche vitellogène of VAN DER STRICHT in which one can make out a central body.

PRENANT (73) seems to have had a suspicion of the same fact; for in speaking of the body of BALBIANI in arachnids, which BALBIANI called the "vésicule embryogène", PRENANT says: "Since then some bodies undoubtedly analogous to this body have been found in various eggs — vitelline nucleus of O. SCHULTZE (amphibia); sphere attractive of E. VAN BENEDEN; archoplasmic sphere and centrosome of BOVERI; and corpuscule polaire of VIALLETON. PRENANT says undoubtedly they are analogous bodies."

The importance of this question is seen in the following statement by WILSON (95), which also reveals the attitude of that distinguished writer on this problem: Speaking of the vitelline body of MUNSON, he says: "MUNSON's observations show that this body first appears in the very young ova as a crescent applied to the nucleus precisely as in *Molgula* or *Lumbricus*, but containing one or more central granules . . . and if it be a true attraction sphere in the one case, we must probably so regard it in all." WILSON failing to offer any reason for this supposed necessity introduces the problem of a de novo origin of the centrosome — a theory which is based entirely on negative evidence. MUNSON (61) has clearly shown that the crescent shaped body in young ova of *Limulus* is the archoplasm and centrosome, which show no trace of chromatin as is claimed by some other observers, and which persist in the oocyte giving rise to the yolk nucleus (vitelline body) of larger eggs.

The final conclusion of BALBIANI (4) 1893 is expressed as follows: "Le noyau vitellin (Dotterkern) des Aranéids est l'homologue du Nebenkern (centrosome de PLATNER) des cellules séminales et du centrosome des cellules somatiques."

Miss KING (40) by a peculiar confusion of terms says of the vitelline body in *Bufo*: "Judging from its staining reaction, this body is not chromatin; neither is it the centrosome, since the same section of the cell may show both of these structures."

GURWITSCH (31) speaking of mammalian ova says: "Wir können somit mit gutem Rechte den Dotterkern der unreifen Säugetiereier dem Idiozome der Samenzellen vollständig homologisieren — also: Der Dotterkern ist in jeder Eizelle voll ausgebildet vorhanden."

Of the same eggs WINIWARTER (96) says: "Le rapprochement avec une sphère attractive devient encore plus manifeste si l'on songe que les spicules que j'y ai observés, ont été signalés par MEVES dans une formation reconnue avec certitude comme sphère attractive et d'un stade correspondant."

In 1898 VAN DER STRICHT (87) said: "Pour ce qui concerne l'oocyte humain, il est incontestable que au point de vue morphologique le noyau vitellin présente une résemblance frappante avec la sphère attractive . . . Il résulte de ce qui précède que le noyau vitellin de l'oocyte humain est une formation, qui au point de vue physiologique doit être considéré comme un centre, qui tient sous sa dépendance la genèse du deutoplasma." He has also said: "Elle correspond évidemment au centrosome de BOVERI ou bien au corpuscule central, plus la zone médullaire de la sphère attractive de VAN BENEDEN."

SKROBANSKY (85) figures the vitelline body of BALBIANI in the human ovum and also in the cat, resembling very closely some of the forms figured by MUNSON in his work on *Limulus*. SKROBANSKY seems to regard yolk bodies which make their appearance later as the disintegration product of the yolk nucleus, since they appear when the former disappears.

In 1885—1889 RABL (74) said: "Wenn wirklich die Attraktionssphäre, beziehungsweise das Polkörperchen, ein Bestandteil jeder Zelle ist, so müssen wohl auch das unbefruchtete Ei und das Spermatozoon dieses Organ besitzen. Aber gerade die neueren Untersuchungen haben darüber nichts von Belang zutage gefördert."

The numerous recent investigations make these words of RABL no longer true. But these same recent researches tend to show that RABL's conception of a cell represented in his text figure one, is probably the correct conception, and very emphatically support the conclusions of Munson (61) expressed in 1898 in his work on *Limulus* as follows: "I will only invite a comparison of some of the forms represented in the plates with the sphere in sperm cells of the salamander as figured by Rawitz, by Mewes, and in nerve cells by Lenhossék. Such a comparison will only serve to strengthen the conviction, that the vitelline body is indeed a sphere, which not only possesses the typical form of a centrosphere, the many forms of the real aster found in dividing cells, in leucocytes, and in the fertilized egg of *Ascaris megalocephala*, but also the less typical forms observed in sperm cells as Nebenkern, and in the resting ganglion cells."

„The vitelline body in the ovarian egg of *Limulus* is genetically the centrosome and sphere of the dividing oogonia, and continues to be the centrosome and attraction sphere of the growing ovarian egg."

"It would seem that the attraction sphere, centrosome and vitelline body are the primitive basis or center of growth of the cytoplasm."

M. Loyez (53b) has figured a yolk nucleus showing astral rays in egg of *Testudo graeca*, other stainable bodies in the cytoplasm he believes come from the germinal vesicle. In sections of a young oocyte of *Choradrius hiaticula*, he has figured a fine sphere, with centrosome in the center of the "Masse vitellogène". In young ovules of canard, he also shows fine spheres with astral rays.

Hollander (36) claims to have fulfilled the requirements of van der Stricht (87), who insisted that to prove the yolk nucleus to be a centrosome it would be necessary to show its origin from the centrosome of the oogonia, and also to show that it becomes the centrosome of the maturation spindle. He has shown the centrosome in oogonia of bird's eggs, the division of the oogonia and the origin of the yolk nucleus from the centrosome of karyokinesis. According to his own account, also, he has shown the yolk nucleus in all oocytes during period of growth. In later stages he has seen the masse vitellogène in the center of which one occasionally sees the true vitelline body.

In this connection Lams' (52) work is also interesting. He says: "J'identifie le corps vitellin avec la sphère attractive qui subsiste dans l'ovule après la dernière mitose des oogonies." Also: "Le corps vitellin de la grenonille décrit jusqu'ici par tous les auteurs sous l'aspect d'un amas granuleux situé dans le cytoplasm ovulaire, pris de la vésicule germinative, n'est pas le corps vitellin véritable: c'est la masse vitellogène, et celle-ci renferme en son sein le corps de Balbiani proprement dit."

It may now reasonably be said of the yolk nucleus what Platner (72) said of the Nebenkern, namely: "Der Nebenkern, dieses von la Valette St. George zuerst beobachtete, vielfach bestrittene und noch öfter mißdeutete Element ist damit aus der Sonderstellung, welche er bisher einnahm, herausgetreten, und muß in eine Reihe gestellt werden mit der von van Beneden in den Furchungszellen von *Ascaris megalocephala* beschriebenen "sphère attractive", mit ihren "corpuscules centraux", mit dem Boverischen "Archoplasma" und den Periplasten Vejdovskys. Ich bin mit van Beneden der Ansicht, daß sich ähnliche Elemente wohl noch in allen Zellen nachweisen lassen werden."

IV. General Summary.

1. If we mean by the term, yolk nucleus, anything in the cytoplasm which differs in any respect from the egg cytoplasm in general, there are at least four different bodies included in that term: 1. real nuclei; 2. karyolymph; 3. metaplasm; 4. the centrosphere or vitelline body.

2. Eggs may devour other cells. But if the egg is normal, such cells soon dissolve and leave no permanent trace that could account for the yolk nucleus. This statement does not seem to apply to ascidian test cells.

3. When real nuclei, giving the staining reaction of chromatin are found in the cytoplasm, it is evidence of beginning degeneration of the egg — not a normal but a pathological effect.

I have elsewhere expressed the view that these nuclei are an indication of a regressive metamorphosis of the egg, when it is retained in the ovary beyond the normal time of its discharge. The nuclei may be due to phagocytes, or since there are no distinct cell boundaries, they may be due to a fragmentation of the germinal vesicle, which no longer exists in those eggs.

Spermatocytes die and disintegrate without the entrance of phagocytes; and in those cases the trouble seems to be in the nucleus, which evidently ceases to produce the karyolymph.

4. The karyolymph is produced by the chromatin, causing vacuoles, which in the normal cell give rise to the nuclear network from the chromosomes after karyokinesis.

In eggs, the karyolymph comes out from the nucleus, and may form a clear zone around the germinal vesicle. But more commonly, it passes into the sphere at one pole of the nucleus.

5. In either case when the karyolymph comes in contact with unassimilated, ingested food, such as a cell, or other proteid substances in

solution, it acts as a ferment or digestive fluid, causing chemical changes resulting in metaplasm.

6. The metaplasm when formed or in the process of forming may surround the nucleus giving rise to a deeply staining ring. More commonly it is formed around the centrosphere (see plates) where it often obscures the latter making an unusually large and often irregular body.

The metaplasm may form either within the sphere or at its periphery.

It then becomes distributed in patches throughout the cytoplasm or as larger dark masses. It seems to flow with the currents of the cytolymph.

Instead of these masses giving rise to a sphere, they are gradually absorbed as food by the sphere.

7. The sphere is the organized part of the yolk nucleus, and it is an organic part of the living substance of the egg. It is the living framework of the body originally described as the yolk nucleus in spiders.

8. The true yolk nucleus (vitelline body) is a centrosphere, not a mere accidental aggregation of granules, nor an artefact due to reagents.

Many things in the cytoplasm of eggs designated by writers as yolk nuclei is not the true yolk nucleus (vitelline body) at all. Very often they are mere metaplasm, deutoplasm or yolk masses that may appear in the neighborhood of the centrosome, but not necessarily, for that reason, produced by the yolk nucleus or centrosome.

9. Typically, the yolk nucleus (vitelline body) is an aster with a centrosome and concentric circle as in dividing cells or in leucocytes.

10. The growth of the cytoplasm of eggs is largely due to actual growth of this body. But partly also a mechanical expansion due to the accumulation of yolk. Growth seems to be by intussusception of metaplasm resulting in the formation of true yolk bodies usually laid down in zones around the sphere, which in some eggs becomes conspicuous as the latebra or nucleus of Pander.

11. Metaplasm is absorbed by this body, and hence the cytoplasm grows from this point as a center.

12. The relation of this body (vitelline body or yolk nucleus) to the germinal vesicle is such as exists between the centrosome and chromosomes after karyokinesis.

13. Its connection with the germinal vesicle and its peculiar structure is such that it serves as a reservoir into which the karyolymph is poured, and consequently becomes the seat of assimilation and growth.

14. The yolk nucleus (vitelline body) is often found to be an expanded aster, with radial fibers extending to the periphery nearly, and concentric

rings. These rings correspond on the one hand to the rings observed in leucocytes and in *Ascaris* and on the other hand, correspond to the concentric layers of yellow and white yolk in the bird's egg, the central part being the "Anlage" of the latebra in bird's eggs.

15. Rejecting all real nuclei and amorphous masses of yolk granules and metaplasm, and confining the term yolk nucleus to the centrosphere like that of spiders first described under that name, we have to conclude that this vitelline body is derived from the centrosome of the dividing oogonia. Only indirectly as food can metaplasm be said to take part in its formation.

16. Since it is often visible as a single body in late stages of the growing oocyte, it affords evidence of persistence of the centrosome for several years in some eggs. It affords evidence of structure in the cytoplasm which together with the germinal vesicle, causes a polarity in the egg, which presumably cannot be ascribed to chemical action, nor to the effect of gravity or other external influences; for it determines the vegetative pole of the egg, since it is the center of growth, and consequently the center around which the greatest amount of yolk is deposited.

17. As regards its origin de novo, it shares the fate of the centrosome, but affords evidence of the permanence of that body as a cell organ. The origin of centrosome de novo has not yet been proven. Published accounts of the disappearances of centrosomes are being discredited. Disappearance of such a body in a mass of yolk granules need not mean anihilation by any means.

18. The yolk nucleus as defined (vitelline body) does not arise from extruded chromatin, nor from migrating nucleoli, nor from leucocytes or devoured cells. It is the morphological center as it is the physiological center of the cytoplasm. It may be a center of low oxidation and a center of fermentation since it is in it that the karyolymph usually does its work of synthesis, which is suggested by the origin of metaplasm in its vicinity.

19. Its many strange forms shown in the plates are due: (1) to increasing amounts of yolkgranules in its neighborhood; (2) to the formation of vacuoles and the resulting compression of concentric zones of which it typically consists; (3) to the variable state of tension or relaxation of the astral rays, which become conspicuous when aggregated, but inconspicuous when at rest, like the cytoreticulum with which it is continuous.

20. As the macronucleus in Infusoria gives the staining reaction of chromatin, the yolk nucleus of eggs cannot very well be homologized with that body as Henneguy and Julin have done.

21. The mitosome in the sperm cells of *Papilio rutulus* originates from the remnant of the spindle in the last karyokinetic division of the spermatocyte. It cannot therefore be the homologue of the yolk nucleus. The vitelline body (yolk nucleus) must rather be compared to the sperm centrosome sometimes said to form the middle piece, sometimes as in *Papilio*, the head piece. If this be called the Nebenkern, it compares very well with the yolk nucleus of egg cells.

22. It may perhaps be wise to admit that we do not yet know all that is to be known about the centrosome; that our knowledge of that minute dot may be increased by a careful study of the yolk nucleus.

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Explanation of Plates.

Plate XXIX.

Fig. 1. Ovary of *Clemmys marmorata*, c. 2 × 1/12. C = camera, 2 = ocular, 1/12 = objective, Bausch & Lomb.

Fig. 2. Ovarian egg of *Clemmys*, 2 × 1/6, c.

Fig. 3. Ovarian egg of *Clemmys*, 2 × 1/3, c.

Fig. 4. Ovarian egg of *Clemmys*, 2 × 1/6, c.

Fig. 5. Ovarian egg of *Clemmys*, 2 × 1/6, c.

Fig. 6. Ovarian egg of *Clemmys*, 2 × 1/6, c.

Fig. 7. Oogonium of *Clemmys*, 2 × 1/12, c.

Fig. 8. Oocyte of *Clemmys*, 2 × 1/6, c.

Fig. 9. Oocyte of *Clemmys*, 2 × 1/6, c.

Fig. 10. Cytocenter of large egg of *Clemmys*, 2 × 1/12, c.

Plate XXX.

Fig. 11. Ovarian egg of spider, 2 × 1/12, c., B. & L.

Fig. 12. Ovarian egg of spider, 2 × 1/12, c.

Fig. 13. Oocyte of spider, 2 × 1/12, c.

Fig. 14. Yolk nucleus of spider's egg.

Fig. 15. Yolk nucleus of spider's egg and part of g. v.

Fig. 16. Yolk nucleus of spider's egg and part of germinal vesicle, 2 × 1/12, c.

Fig. 17. Oocyte of spider, 2 × 1/12, c.

Fig. 18. Yolk nucleus of spider's egg, g. v. = germinal vesicle, 2 × 1/12, c.

Fig. 19. Yolk nucleus of spider's egg, 2 × 1/12, c.

Fig. 20. Yolk nucleus of spider's egg, 2 × 1/12, c.

Fig. 21. Oogonium of spider, 2 × 1/12, c.

Fig. 22. Yolk nucleus and germinal vesicle, g. v. of spider's egg, 2 × 1/12.

Fig. 23. Yolk nucleus and germinal vesicle of spider, 2 × 1/12.

Fig. 24. Yolk nucleus of spider's egg, 2 × 1/12, c.

Plate XXXI.

Fig. 25. Oogonia and oocytes of very young *Limulus*, when oocytes are forming for first time, 2 × 1/12, c.

Fig. 26. Oocytes of *Limulus*, showing two forms of vitelline body or yolk nuclei, 2 × 1/6, c.

Fig. 27. Vitelline body or yolk nucleus from oocytes of *Limulus polyphemus*, $2 \times {}^1/_6$, c.

Fig. 28. Vitelline body, egg of *Limulus*, $1 \times {}^1/_6$.

Fig. 29. Vitelline body, egg of *Limulus*.

Fig. 30. Vitelline body, egg of *Limulus*, $1 \times {}^1/_6$, c.

Fig. 31. Vitelline body (yolk nucleus) of *Limulus*, $2 \times {}^1/_{12}$, c.

Fig. 32. Aster and centrosome of ovarian egg, *Limulus*.

Fig. 33. Vitelline body (yolk nucleus) of *Limulus*.

Fig. 34. Vitelline body (yolk nucleus) of *Limulus*.

Fig. 35. Vitelline body (yolk nucleus) of *Limulus*, $2 \times {}^1/_{12}$, c.

Fig. 36. Vitelline body (yolk nucleus) of *Limulus*.

Fig. 37. Vitelline body (yolk nucleus) of *Limulus*.

Plate XXXII.

Fig. 38. Ovarian egg of *Limulus*, showing ring around the germinal vesicle, $2 \times {}^1/_6$, c.

Fig. 39. Ovarian egg of *Limulus*, showing vitelline body (yolk nucleus) with centrosome and aster, $2 \times {}^1/_6$, c.

Fig. 40. Oocyte of *Limulus*, showing vitelline body in form of aster, $2 \times {}^1/_6$, c.

Fig. 41. Vitelline body (yolk nucleus) of *Limulus*, $1 \times {}^1/_{12}$, c.

Fig. 42. Large ovarian egg, showing the sphere and aster of *Limulus*, $1 \times {}^1/_3$, c.

Fig. 43. Ovarian egg of *Limulus*, showing dark granular ring around germinal vesicle.

Fig. 44. Vitelline body (yolk nucleus) of *Limulus*, $1 \times {}^1/_6$, c.

Fig. 45. Vitelline body (yolk nucleus) of *Limulus*, $2 \times {}^1/_6$, c.

Fig. 46. Ovarian egg of *Limulus*, (gold chloride), showing minute structure of vitelline body, concentric zones seen separated by a line, $2 \times {}^1/_6$, c.

Fig. 47. Vitelline body (yolk nucleus) of *Limulus*, $1 \times {}^1/_6$, c.

Fig. 48. Ovarian egg of *Limulus*, $1 \times {}^1/_3$, c.

Fig. 49. Vitelline body (yolk nucleus) of *Limulus*.

Plate XXXIII.

Fig. 50. Ovary of pigeon, in section, showing oocytes and yolk nucleus, centrosome and aster.

Fig. 51. Oogonium of pigeon, showing centrosome.

Fig. 52. Ovarian egg of pigeon, showing yolk nucleus as aster and centrosome, $1 \times {}^1/_6$, c.

Fig. 53. Oocyte of pigeon, showing spherical body with a distinct centrosome.

Fig. 54. Ovarian egg of goosefish, with yolk nucleus, $2 \times {}^1/_6$, c.

Fig. 55. Oocyte of fish with yolk nucleus.

Fig. 56. Ovarian egg of cat with yolk nucleus and centrosome.

Fig. 57. Egg of fish with large yolk nucleus, looking like archoplasm.

Fig. 58. Cytoplasm of degenerating eggs of *Limulus*, containing real nuclei, granules partly absorbed.

Fig. 59. Cytoplasm of ovarian egg of *Limulus*, containing real nuclei, beginning degeneration.

Fig. 60. Egg of fish with large yolk nucleus, $2 \times {}^1/_{12}$.

Fig. 61. Vitelline body (yolk nucleus) of ovarian egg of *Limulus*.

Plate XXXIV.

Fig. 62. Viteline body (yolk nucleus) of *Limulus*.

Fig. 63. Ovarian egg of *Limulus*, showing aster.

Fig. 64. Segmenting egg of *Ascaris*, showing asters like those of the ovarian eggs of *Limulus* fig. 63, fig. 70, fig. 73.

Fig. 65. Vitelline body of *Limulus*.

Fig. 66. Vitelline body of *Limulus*.

Fig. 67. Ovarian egg of *Limulus*, showing aster.

Fig. 68. Fertilized egg of *Ascaris* showing centrosome.

Fig. 69. Vitelline body (yolk nucleus) of *Limulus*.

Fig. 70. Oocyte of *Limulus*, showing aster and centrosome.

Fig. 71. Testis cells of *Amblystoma*, showing aster like those of ovarian egg of *Limulus*, figs. 70, 73.

Fig. 72. Vitelline body (yolk nucleus) of *Limulus*.

Fig. 73. Aster and centrosome (vitelline body and yolk nucleus) in ovarian egg of *Limulus*, resembling those of fertilized egg of *Ascaris*, figs. 64 and 68 and also those of testis cells figs. 71 and 74.

Fig. 74. Testis cell of *Amblystoma*, $1 \times {}^1/_{12}$, c., resembling the yolk nucleus in egg of *Limulus polyphemus*.

Fig. 75. Ovarian egg of crayfish, $2 \times {}^1/_6$, c.

Fig. 76. Germinal vesicle and yolk nucleus of crayfish.

Fig. 77. Germinal vesicle and cytoplasmic ring of egg of crayfish.

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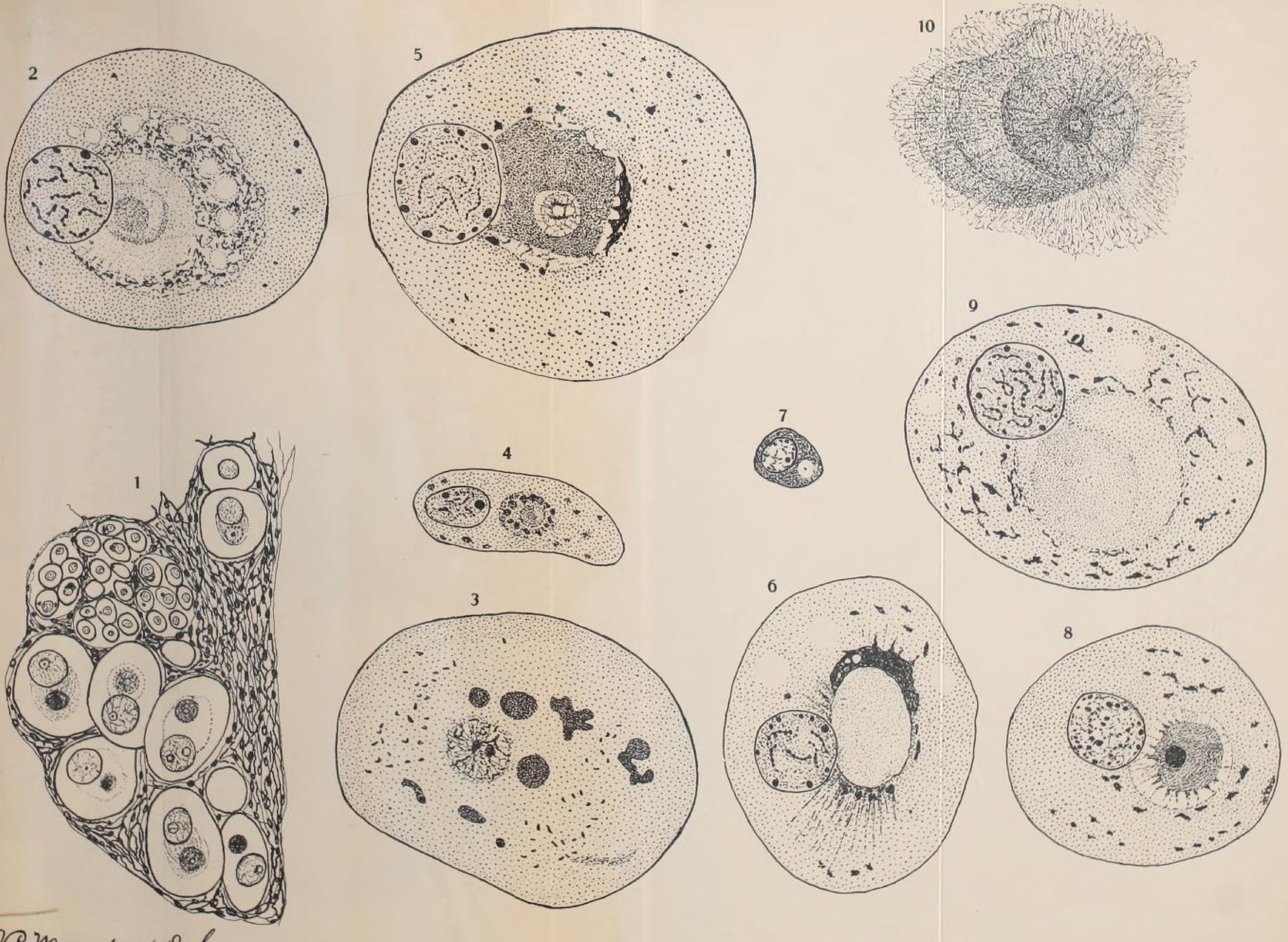

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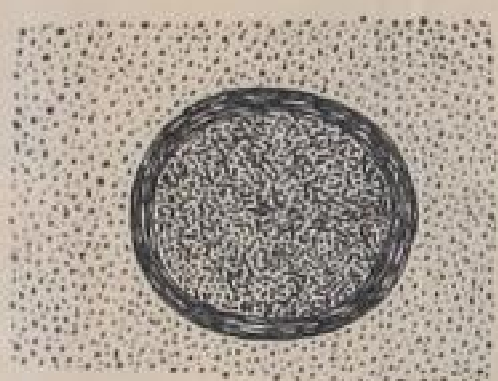

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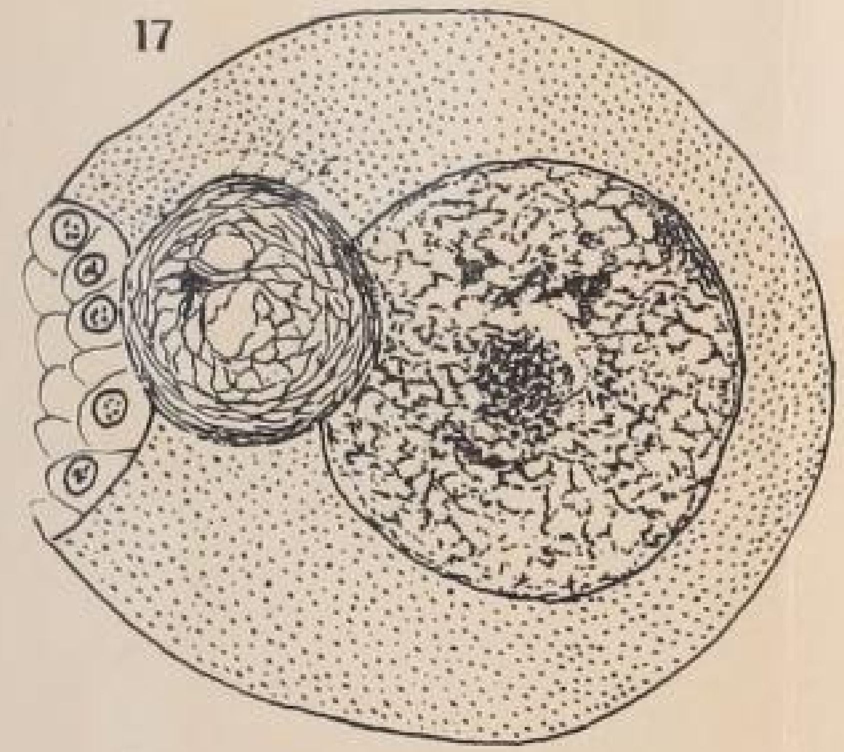

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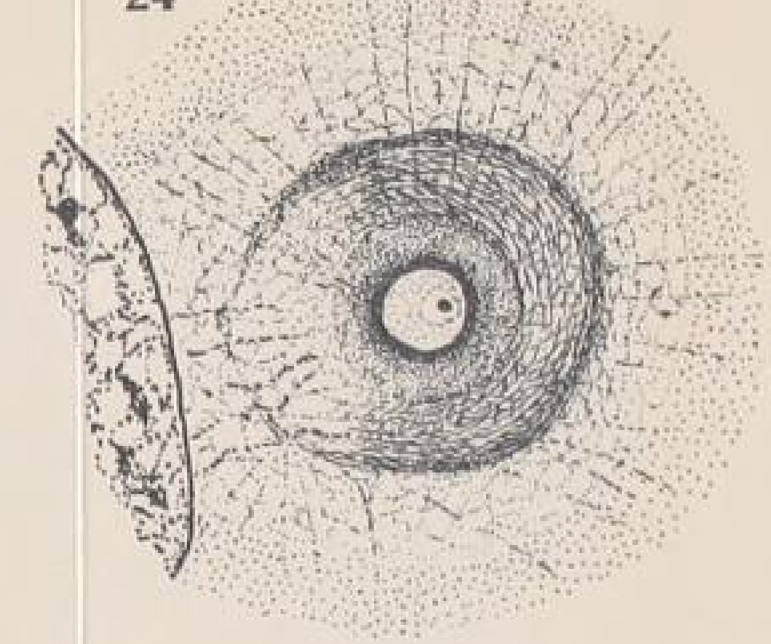

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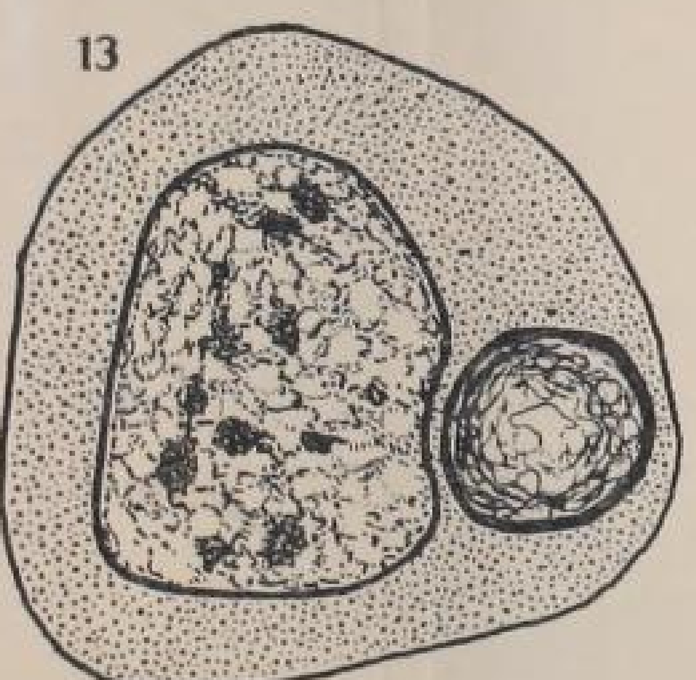

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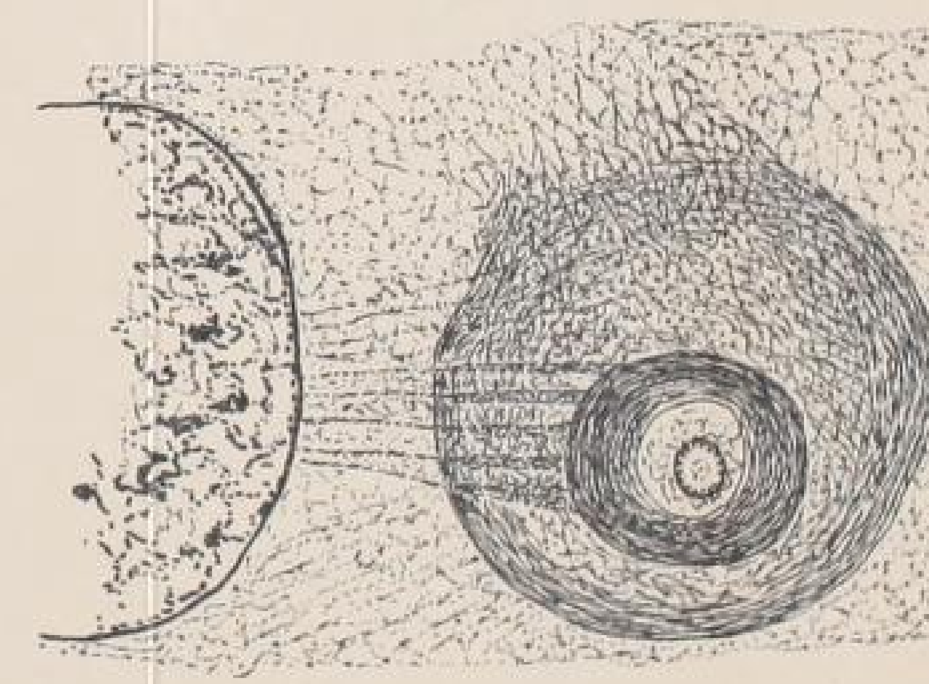

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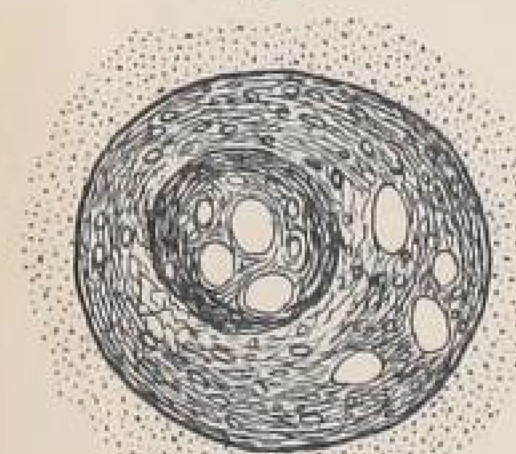

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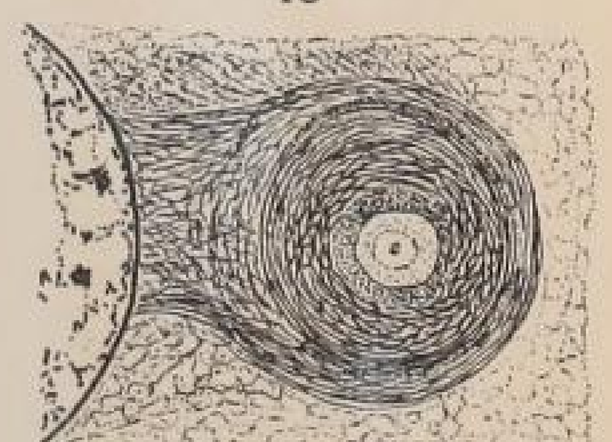

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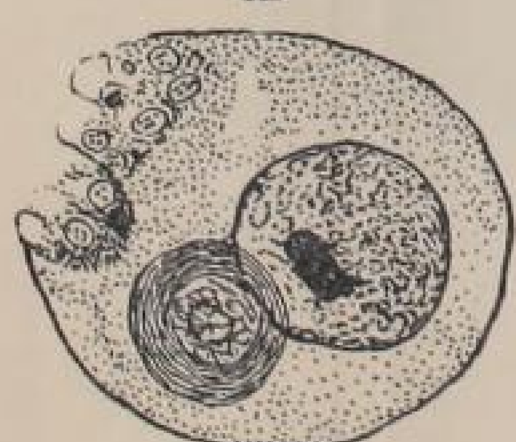

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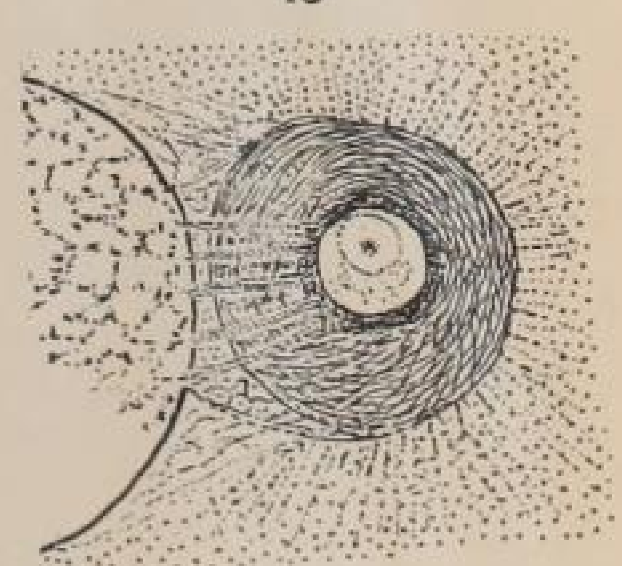

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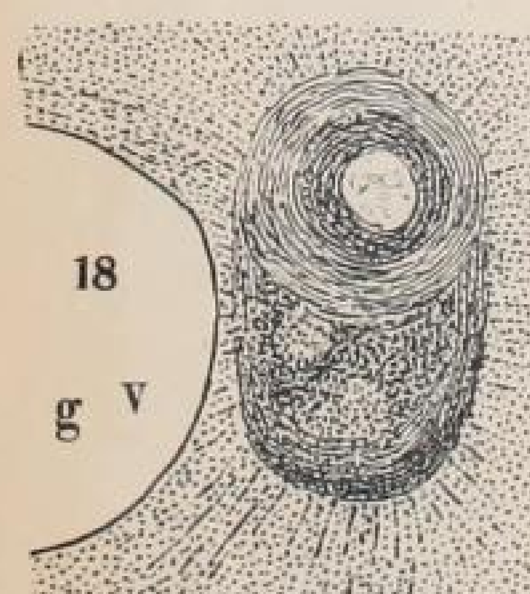

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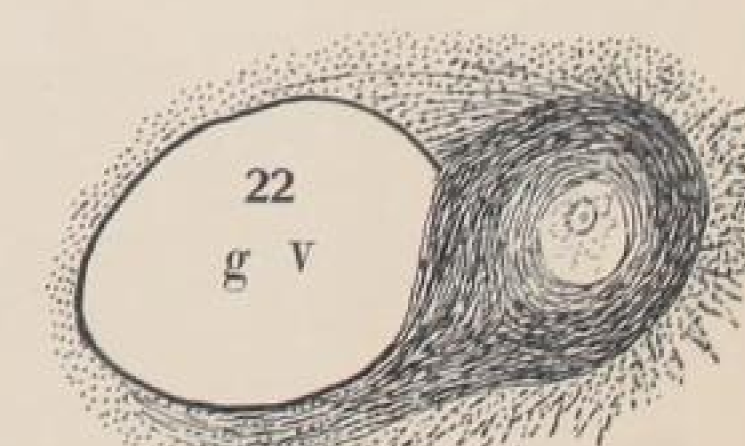

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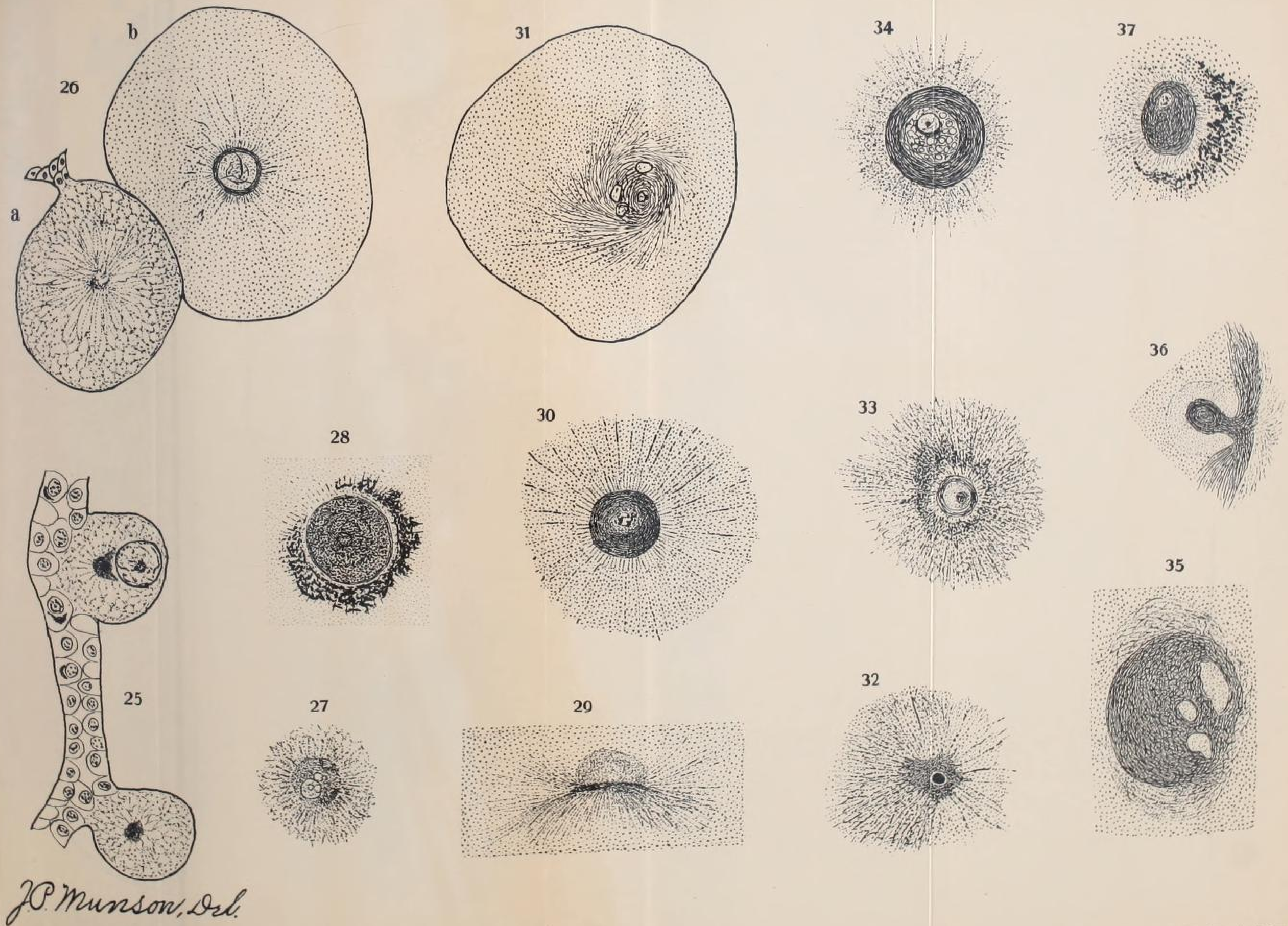

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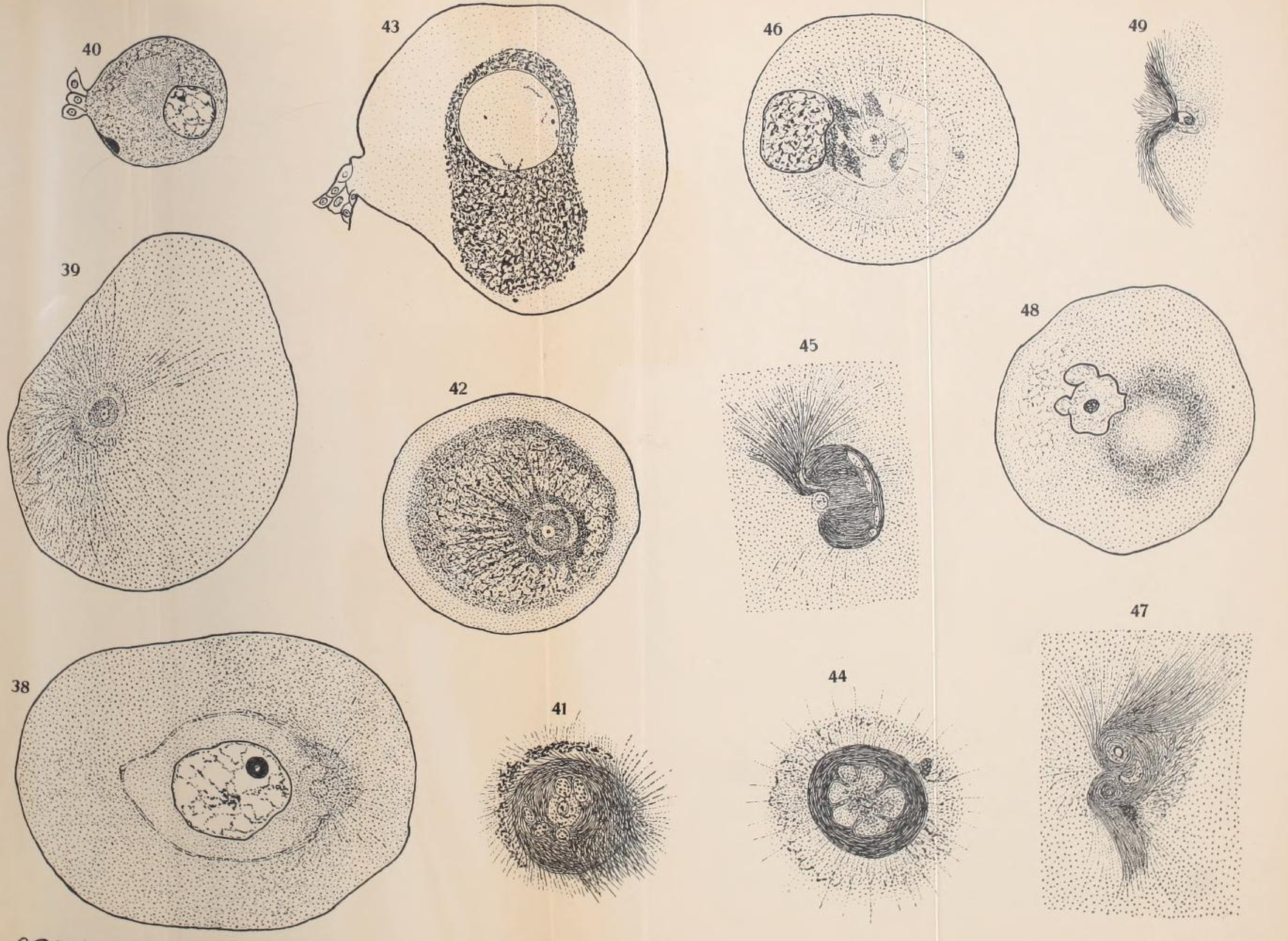

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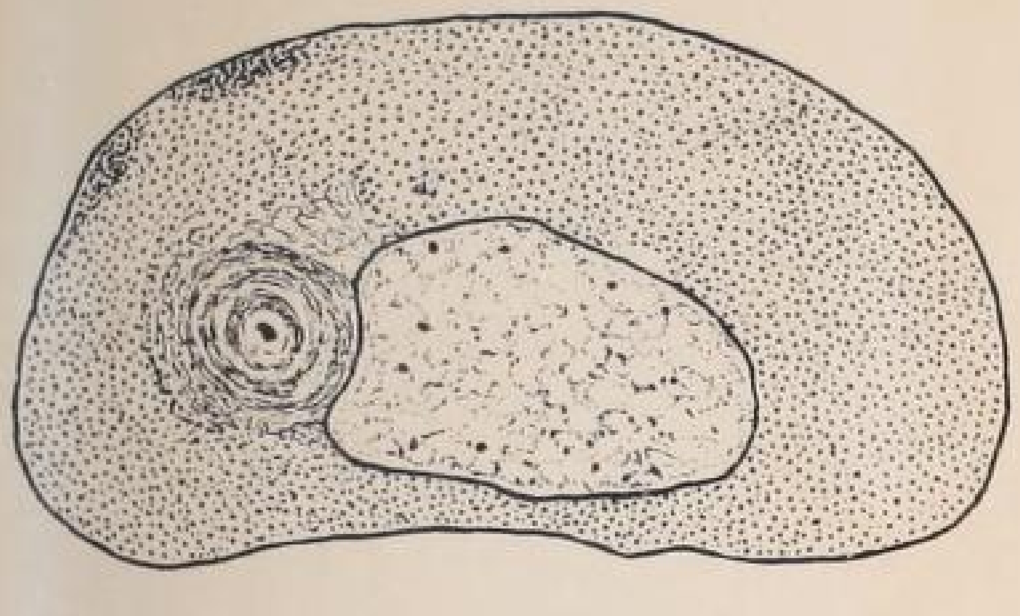

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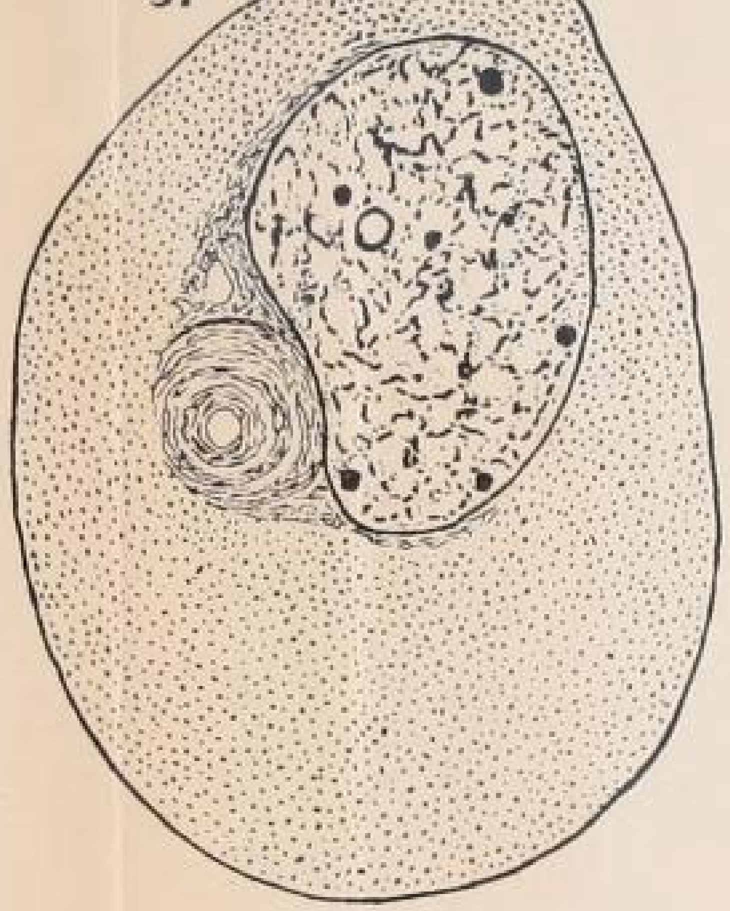

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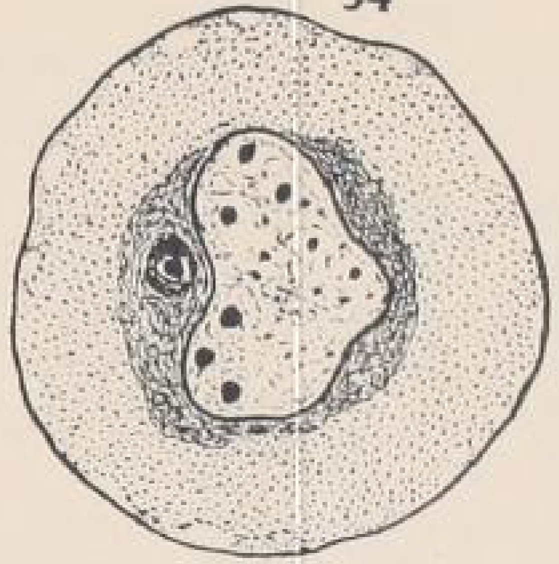

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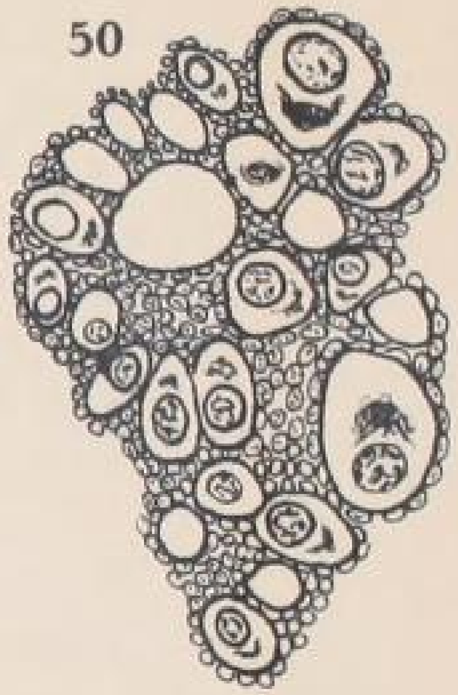

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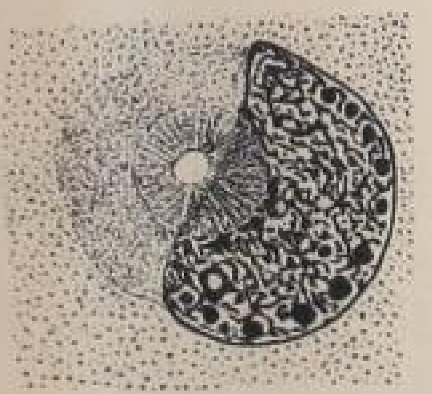

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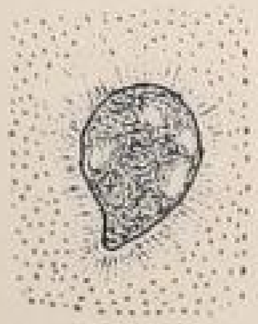

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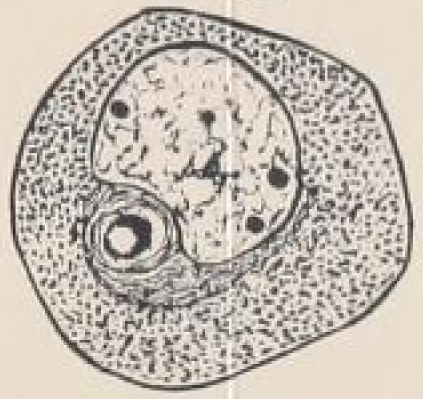

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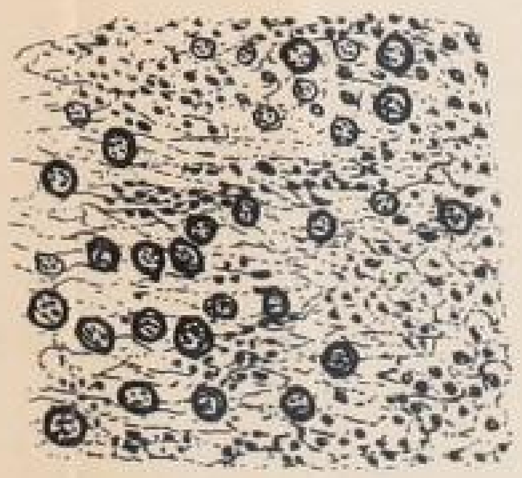

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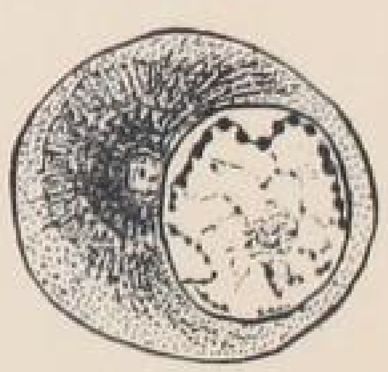

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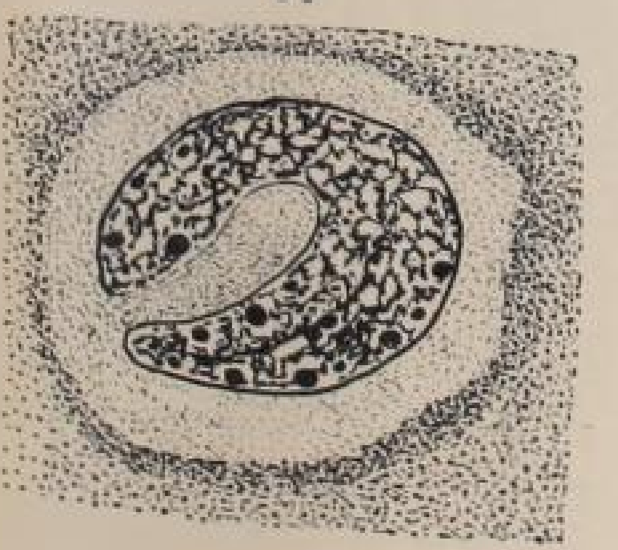

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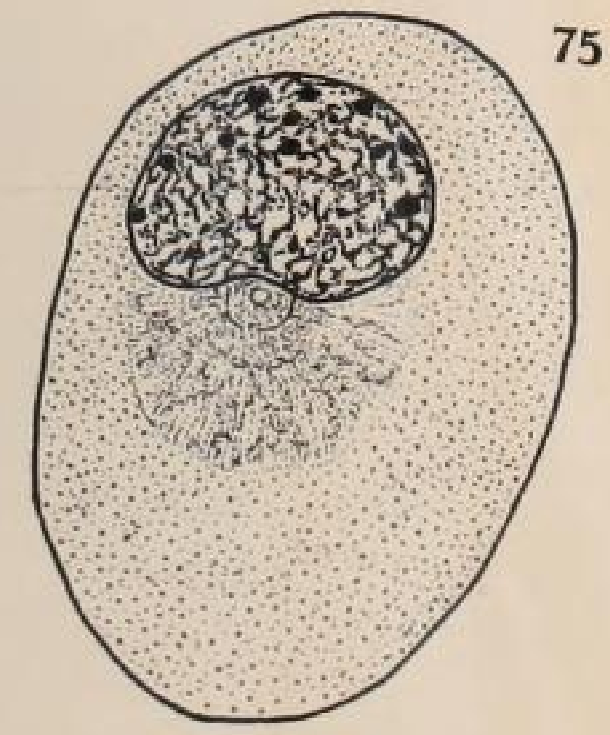

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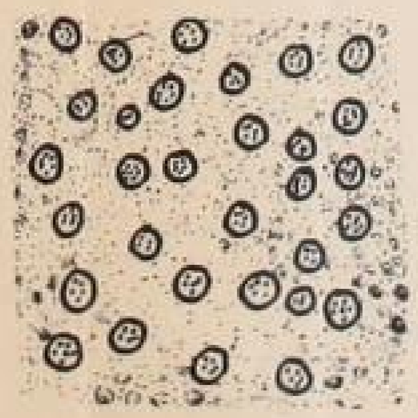

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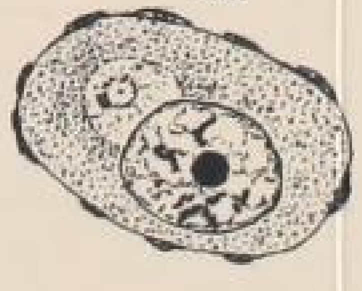

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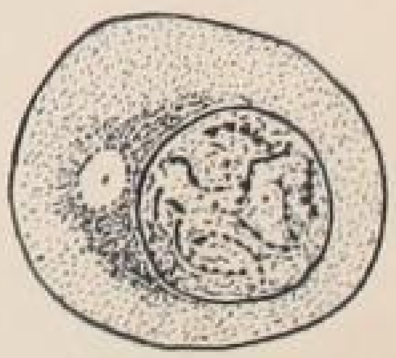

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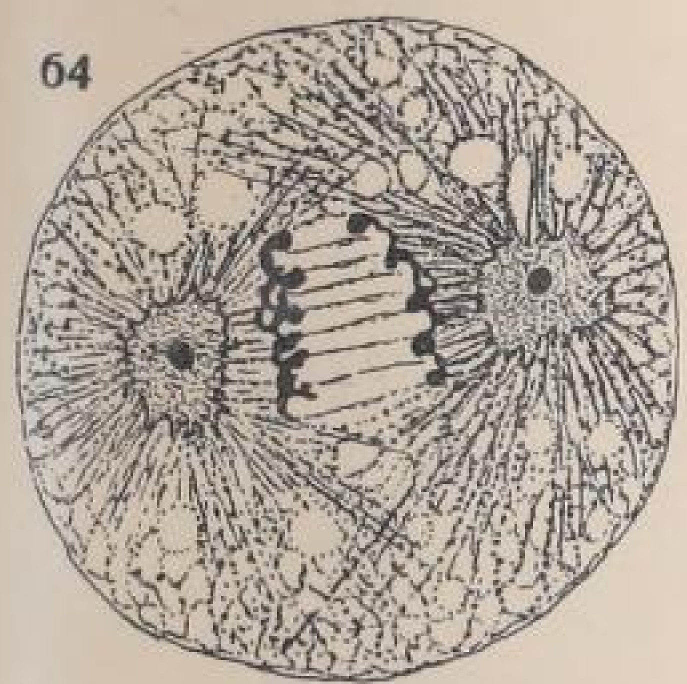

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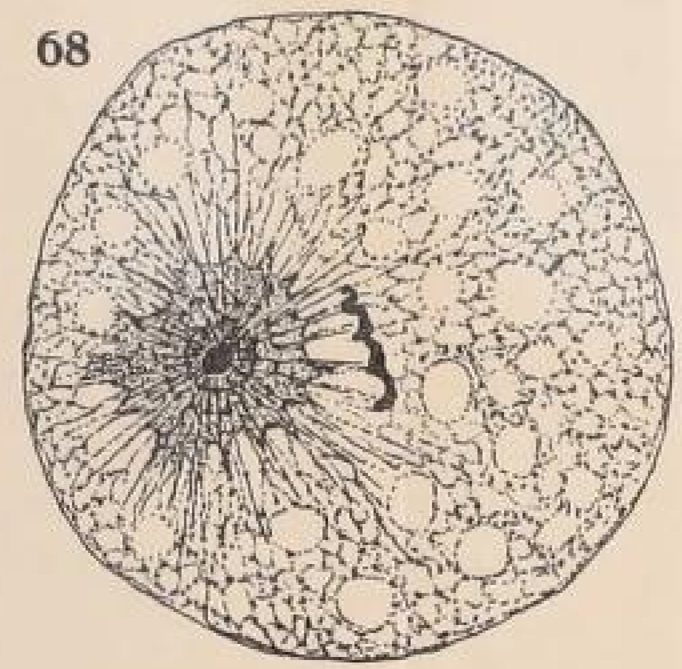

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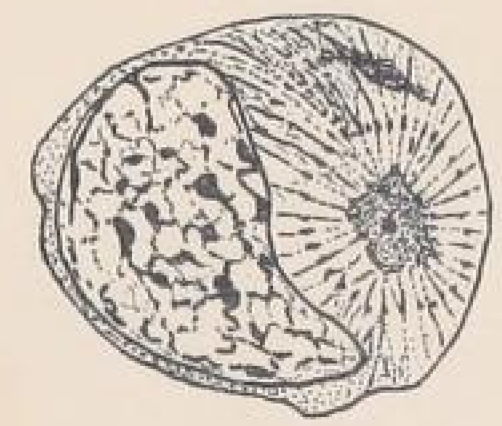

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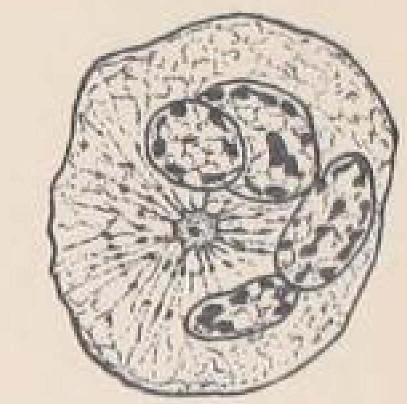

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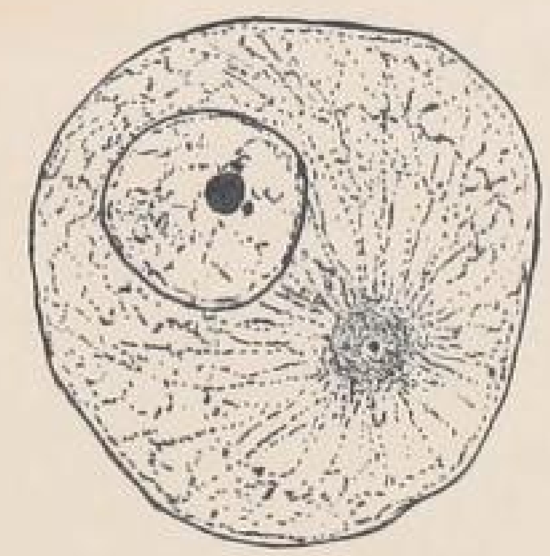

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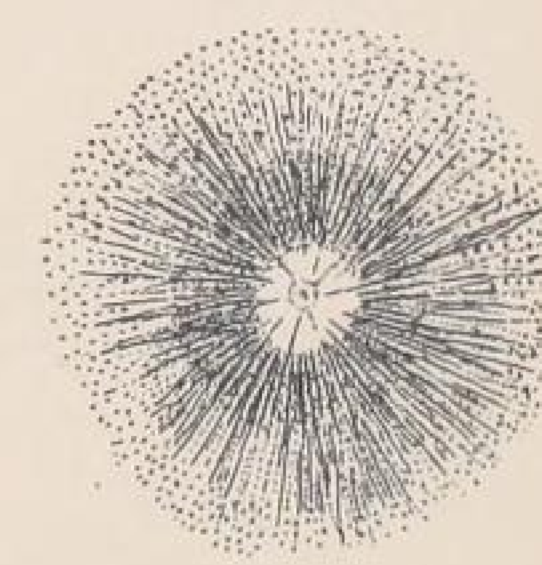

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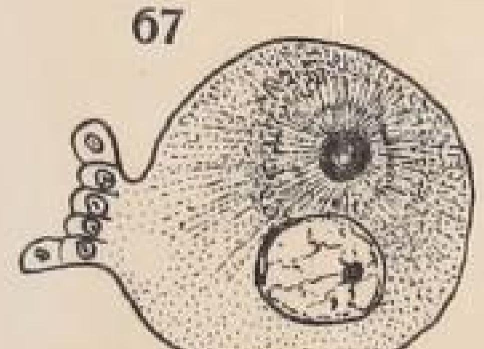

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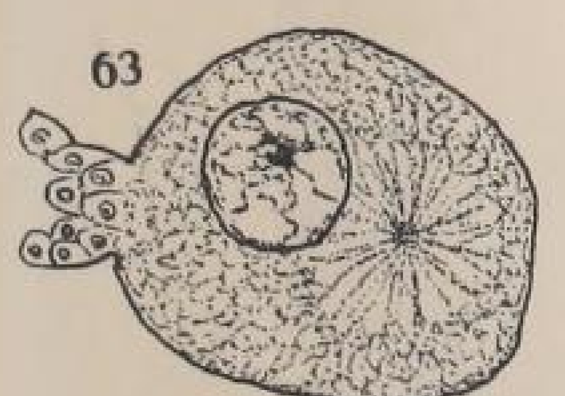

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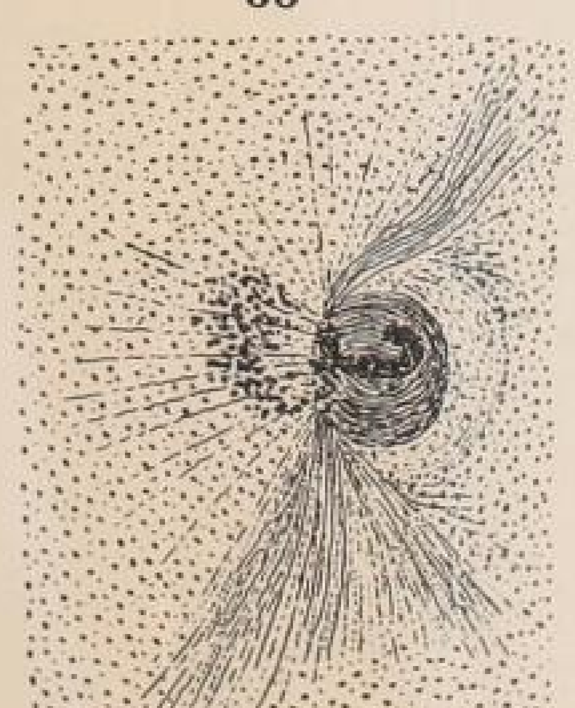

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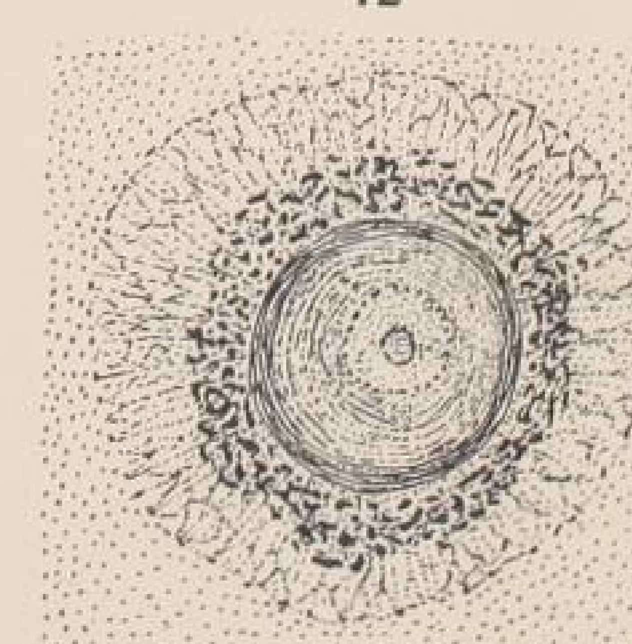

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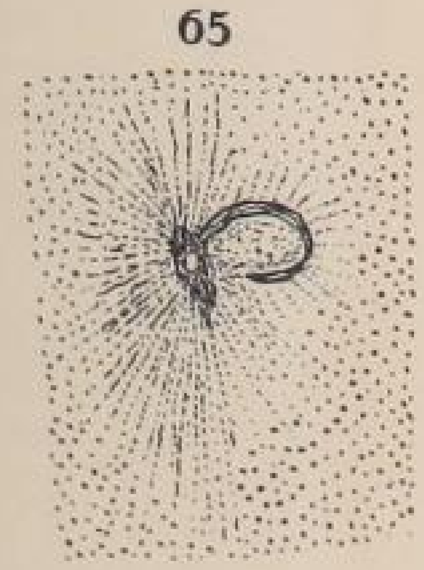

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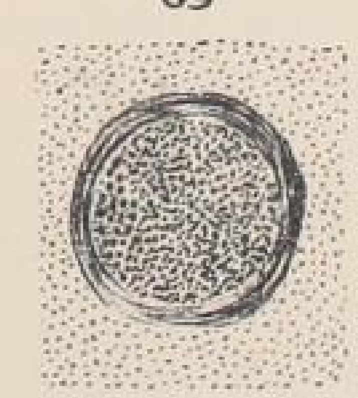

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