The University of Chicago

FOUNDED BY JOHN D. ROCKEFELLER

THE OVARIAN EGG OF LIMULUS

A CONTRIBUTION TO THE PROBLEM OF THE
CENTROSOME AND YOLK-NUCLEUS

A DISSERTATION

Submitted to the Faculties of the Graduate Schools of Arts,
Literature, and Science, in candidacy for the degree of

DOCTOR OF PHILOSOPHY

May, 1896

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Volume XV. *November, 1898.* *Number 2.*

JOURNAL

OF

MORPHOLOGY.

THE OVARIAN EGG OF LIMULUS.

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JOHN P. MUNSON.

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INTRODUCTION.

THIS work on the history and morphology of the ovarian egg of the King Crab — *Limulus polyphemus* — has been done in the Zoölogical Laboratory of the University of Chicago, and in the Marine Biological Laboratory at Woods Holl, Mass., under the direction of Professor Whitman.

The object of the work has been to determine, so far as possible, the organization of the egg during its different stages of growth, and to give a connected history of its phases. The vitelline-body and centrosome have received special attention.

Much of the more valuable literature on this subject is of so recent date that it has not seemed advisable to encumber the paper with a historical compilation. Papers of special interest will be referred to in connection with my own observations.

Historical. — The main facts concerning the position and external form of the ovary have been known since 1828, when Strauss Durckheim made known the internal anatomy of Limulus.

Somewhat later, 1838, J. Van Der Hoeven also published an account of the ovary, its mode of branching, its ramifications throughout the cephalothorax, and the astonishing number of eggs produced.

Lockwood ('70) has treated in a popular, but exceedingly interesting way the habits of Limulus, and mentions many interesting facts in regard to its development.

Packard ('71) noted some points in the development of the ovary, and among other things called attention to the laminated structure of the egg membrane, which he calls a chorion.

Owen ('73) described and figured the ovary of Limulus, and showed the relation of the ovarian tubes to the terminal oviducts, as well as the relation of the right and left ovary to each other and to the underlying parts.

Among those who have devoted attention more directly to the nature of the ovarian eggs may be mentioned Ludwig, Gegenbaur, and Kingsley.

Ludwig ('74) called attention to the character of the germinal epithelium, and especially to the cell nature of the egg. He, however, based all of his conclusions on the observations of Gegenbaur ('58), who, he says, has had the good fortune of having a living specimen for dissection.

Kingsley ('92) has shown the relation of the egg to the germinal epithelium, and, from the point of view of oögenesis, has shown some of the similarities of Limulus to the spiders.

No one, however, so far as I know, has attempted to study the ovarian egg of Limulus with the more fundamental problems in view. I have been compelled to go over the whole ground and to reëxamine the observations previously recorded concerning the ovary. Where my description agrees with previous accounts, it has at least the value of a confirmation.

Material. — Material for the study of the mature eggs was obtained, through the kindness of Professor Whitman, from three female specimens that had been on exhibition in the aquaria of the United States Fish Commission at the World's Columbian Exposition in Chicago. In the following June, July, and August an ample supply of material was collected at Woods Holl, Mass., consisting chiefly of material from females having mature ovaries, and captured in the act of ovipositing. From some of this material, through natural and artificial fertilization, a large number of embryos were produced and raised to the desired age and size. Young Limuli, ranging from one-fourth inch to eight inches, were obtained in abundance at North Falmouth, Mass.

The results here presented have been confirmed and extended on material ranging in size from eight inches to the adult form, obtained in the latter part of October and the first week in November, off New Haven, in Long Island Sound, in water ranging from five to fifteen fathoms. The material has been very abundant and the series complete. My thanks for material are due to the following gentlemen: W. H. Munson, W. H. Packard, Dr. Watasé, and Professor Whitman. I would also acknowledge my obligations to Captain Barnes, of the Oyster Steamer, Roe & Co., of New Haven, Conn.

My former teacher, Prof. Sidney I. Smith, has very kindly enabled me to use the Yale Library; and Dr. Watasé has given me much encouragement in my work. I desire to express my appreciation of these favors.

Methods. — The best preserved material of the young forms was obtained by killing in (1) Kleinenberg's picro-sulphuric, (2) corrosive-acetic, and in (3) a mixture, in equal parts, of a ten per cent solution of nitric acid and picro-sulphuric. Material killed in this latter mixture was excellently preserved. It has the advantages of staining readily, and is especially suited to the double stain of Lyon's blue and lithium-carmine. By this means, the archoplasm and centrosomes are made very distinct. It was not so favorable for the study of the various phases of karyokinesis. For this, material preserved in Merkel's fluid was used.

The ovaries of the adult animal successfully preserved in (4) Merkel's fluid are excellent for the study of the centrosomes and sphere. This fluid, however, does not always give equally good results, even when most carefully applied. Slight irregularities in the preparation of the mixture, as well as differences in temperature, may account for some of the differences in the effect; but physiological variations in the egg itself, especially those changes arising from a constantly increasing quantity of food material, are perhaps responsible for much of the variation.

In that stage of the egg immediately preceding its escape from the follicle, the following method was successfully employed: (5) one-fourth per cent aqueous solution of platinum chloride applied for twenty-four to forty-eight hours, and the eggs then passed through the various grades of alcohol. The eggs may also first be killed by leaving them a few minutes in Flemming's fluid and then transferring them to platinum chloride for forty-eight hours.

For the stages of the mature egg, after it gets into the ovarian tube, Kleinenberg's picro-sulphuric has been found most favorable, where attempts to imbed in paraffine have been made.

Owing to the difficulty of staining after Flemming's and Hermann's fluid, these have not been extensively used, although a number of the drawings have been made from preparations of material preserved in this way, as well as from material hardened in corrosive sublimate, corrosive-acetic, and picro-sulphuric.

Imbedding has been done in the usual way by means of paraffine. The mature eggs of the ovarian tubes were imbedded and sectioned in celloidin. To enable the imbedding medium to penetrate, a slit was made in the chorion by means of a sharp razor.

Previous to imbedding, the absolute alcohol was removed by means of chloroform, saturated with dissolved paraffine. To avoid the hardening effect of the chloroform on the yolk of the larger eggs, xylol and turpentine were substituted for the chloroform.

The paraffine sections, from five to ten μ in thickness, were fixed to the slide by means of water, Mayer's albumen fixative, or by the two combined.

Staining was done almost exclusively after the sections were mounted on the slide. The sections of the larger eggs in celloidin were stained with Delafield's haematoxylin diluted ten times with water and slightly acidulated with HCl. This leaves the yolk spheres unstained and facilitates the search for traces of the nucleus and the maturation spindle.

For the study of karyokinesis, Heidenhain's iron-haematoxylin was used. The archoplasm and centrosome in the younger eggs were studied by means of Heidenhain's iron-haematoxylin, either alone or followed with erythrosin, eosin, or acid fuchsin. Erythrosin and cyanin have also been used to good advantage; also borax-carmine, followed with picric acid; Delafield's haematoxylin, either alone or followed with picric acid; Weigert's picro-carmine; Ehrlich's haematoxylin, either alone or followed with erythrosin, eosin, and especially with acid fuchsin; eosin and nigrosin to a limited extent; the Biondi-Ehrlich mixture, and finally lithium-carmine and Lyon's blue.

These stains all give good results, but they differ in the extent to which they can be applied. The carmine stains have not been found useful on material killed in Merkel's or Flemming's fluids. To obtain the double stain with Lyon's blue, safranin has been substituted for the carmine on Merkel's material. In such cases the sections were first stained in Lyon's blue for twenty-four hours, after which they were stained for twenty-four hours in safranin; and previous to clearing in xylol, the sections were dehydrated in absolute alcohol, containing powdered copper sulphate. This method was reversed when lithium-carmine could be associated with the Lyon's blue. With the method above described, I have not found it necessary to watch the stain under the microscope with the care which Miss Foot ('96) seems to think is necessary. On the whole, Ehrlich's haematoxylin, followed with acid fuchsin, has been the method that I have placed greatest reliance on in the case of material killed in Merkel's fluid. Weigert's picro-carmine has also been found very useful. The specific effect of each is more profitably stated in connection with the description which follows.

It has been found very profitable to verify as many of the points as possible on the living material. Much uncertainty has been removed in that way.

OECOLOGY.

Oviposition. — Many of the observations of Packard ('70), Kingsley ('92), Lockwood ('70), Agassiz ('78), and others have been confirmed. Oviposition, at Woods Holl, takes place during the months of May, June, and July. The females at this season frequent a particular beach characterized by an abundance of medium-sized sand and the entire absence of rocks. They appear to come in with the tide. As stated by Kingsley and others, they are usually accompanied by one or more males, one of which has attached himself to the posterior margin of the female carapace, the other males occupying a similar position with reference to him and to each other.

If the male occupying the position described be seized and raised out of the water, he does not let go his hold, but lifts the much larger female out with him. If they are then dropped into the water, they continue the same gait as if they had never been molested.

The attachment of the male appears to take place in deeper water; but frequently isolated males may be seen moving over the shallow bottom off shore, apparently in search of females, which, when they approach, they appear to recognize at considerable distances. Isolated females are also met with; but oviposition in the absence of the male was not observed.

I can confirm Lockwood's observation to the effect that they deposit their eggs at the point reached by the highest tides. But Kingsley is also right when he affirms that there are exceptions to this rule. Thus I have found them ovipositing at a point where I was in some doubt as to whether the eggs would ever be exposed to direct rays of the sun. On the other hand, the nests can be found at the point reached by the high tides, even where no superficial evidence of their presence is visible, — an evidence that they are numerous at that point. During oviposition the animals may be covered with as much as a foot or more of water; but they usually approach so near shore that their carapace is only partly covered.

The act of ovipositing is apparently accompanied with considerable activity and excitement, which is indicated by an accumulation of air bubbles on the surface of the water, forming a distinct line, extending in the direction of movement of the animals. By pursuing this line, they can be traced for considerable distances. In ovipositing, the female is partly buried in the sand, and only slight movements are visible from above; but the appendages are evidently in rapid motion, excavating a deep cavity from which the finer sand becomes sifted out, and into which the eggs are discharged. The eggs thus come to lie in the midst of sand peculiarly resembling the eggs both in size and appearance.

Careful examination of these nests would seem to indicate that the terminal oviducts are discharged into each nest.

It seems probable that most, but not all, of the eggs contained in the ovarian tubes are laid during one season. The females captured on June 1 usually show a turgid condition of the ovary. At the end of the laying season, on the other hand, the ovarian tubes are nearly collapsed.

On the other hand, there is much evidence to show that a female Limulus does not oviposit every year, and that females having the ovarian tubes filled with eggs may, even in a state of nature, carry these over at least one season. On the twenty-fifth of October, in Long Island Sound, females were taken by me whose ovaries were turgid with what appeared to be mature eggs, and which could not be distinguished from those examined at Woods Holl at the beginning of the spawning season. Others presented ovaries in which only comparatively few eggs had arrived in the ovarian tubes. The condition of ovaries taken from the females that had been on exhibition at the World's Fair was that of the former, *i.e.*, the ovarian tubes were filled with mature eggs. This being on November 1, it was concluded that the eggs had been retained because of the confinement of the animals during the season when oviposition takes place. The above observation shows that, in their native element, females with ovaries in a similar condition, at the same season of the year, are abundant, and that neither the fullness of the ovarian tubes, nor the apparent maturity of the eggs, are sure indications of the time when oviposition will take place. Yet, as Kingsley has observed, there are reasons for believing that there is no oviposition in confinement. The ovaries of the female Limuli kept in the large floating aquaria of the Marine Biological Laboratory at Woods Holl were always filled with mature eggs, even after the spawning season was over.

The movement of Limulus is a uniform, gliding one. Oblivious of everything except the business which occasions its visit, it pursues a more or less direct path for the beach, which is most favorable to the concealment and subsequent development of its eggs. Any attempt at concealment or betrayal of fear, by a hasty retreat, is not to be observed. If, on its first arrival off the beach, it be disturbed, it cannot be induced to deposit its eggs, but endeavors stubbornly to make its way back into deep water. The male still clings to the female, but I have in vain, for hours, endeavored to secure freshly laid eggs by urging them towards the favorable point of oviposition.

Moulting. — Lockwood found a soft-shelled specimen in the month of February, and concluded that, while the young moult four or five times the first year and adults usually only once, in the month of August, there might be two moults a year even in the case of the adult. As several soft-shelled ones were observed by me on the twenty-eighth day of October and the first of November, it may be supposed that the moulting period is not fixed to definite seasons, but that it may take place at any time, according to the physiological condition of the animal.

Although the soft-shelled females observed by me were of a size not inferior to the largest hard-shelled specimens found to possess an ovary filled with mature eggs, yet the eggs in these soft-shelled ones were scarcely visible to the naked eye. The larger eggs, however, when exposed to favorable light, showed a decided reddish-pink coloration, indicating the second stage of yolk formation. Numerous specimens, apparently fully grown, appeared to have moulted at an earlier period. These were distinguished from the hard-shelled ones, having mature eggs, by the translucency of the carapace, and their consequent brown appearance in contrast to the black appearance of the hard-shelled ones. The carapace in the former was further distinguished by many distinct internal markings not visible in the hard shells; and, unlike the latter, the eggs had not yet been discharged into the ovarian tubes, but many of them, apparently, had reached follicular maturity. In no case did I find one recently moulted containing mature eggs. All females that were observed ovipositing at Woods Holl had hard shells.

Habitat. — Limuli, ranging from eight inches to the adult forms, were found in abundance on the last of October and the first of November, off New Haven, in Long Island Sound, in water ranging from five to fifteen fathoms. It was the general opinion among the oyster fishermen, who are engaged in dredging oysters during the greater part of the winter months, that Limulus goes into deeper water later in the season. But very little reliance can be placed on their observations; for, although they had been engaged in dredging starfish previous to November 1, yet they seemed to be ignorant of the presence of Limulus at that time, till they were asked to take notice of them.

The earlier stages of Limulus, ranging from one-fourth inch to eight inches, were abundant at North Falmouth, Mass., in the month of August. The place where they are found is a large, level expanse of loose sand that is left entirely exposed on the retreat of the tides, and receives fresh water from a stream flowing into the estuary, as well as from several fresh-water springs along the shore. The sand is rich in clam shells and soft-bodied animals, and the abundance of organic material is evidenced by the black coloration of the sand in which the young Limuli live.

At low tide they lie quietly buried, just below the surface, and no tracks or markings reveal their presence. As soon as the incoming tide has covered the sand, however, the Limuli begin to move about, not on the surface of the sand, but just beneath the surface, being always covered with the uppermost layer of sand. This upper layer has the usual color of sand, while just below it is black. As the little Limuli plow their way along, the upper layer is pushed aside and a black track appears. Immediately after the incoming tide covers the sand, these black lines appear running in every direction. The beginning of the black line marks the resting place of the little Limulus during the absence of the tide; the end marks the distance which it has traveled. At this point it can always be found. The search for these little creatures is, therefore, a comparatively easy one, notwithstanding their protective coloring and their subterranean mode of locomotion. This mode of locomotion is evidently useful to them as a means of protection from the many enemies that infest the neighboring eelgrass. Many little Limuli, departing from the path which nature has marked out for them, can be seen to have fallen victims to these enemies.

Food. — One large female Limulus was found nearly buried in the mud in about three feet of water. An examination showed that it was enjoying its dinner, which consisted of a worm.

In Dr. Lockwood's vivid description of the habits of Limulus, an instance is cited where it had been observed caught by a clam, which, it was supposed, the Limulus had been trying to consume. From the oyster growers at New Haven, and especially from Captain Barnes and his crew, it was learned that, while the starfish is their dreaded foe, the horse-fish (which is the fisherman's name for Limulus) is perfectly harmless so far as oysters are concerned. I was delighted, however, after traveling from Chicago to New Haven in order to secure material late in the season, to find that they were abundant on these oyster beds.

Vitality of the eggs. — At Woods Holl, in the summer of 1894, the eggs, laid while the animals were observed in the act, were taken from the nest as soon as the animals were about to leave. The animals were also taken. Owing to the secretion of the oviduct covering the eggs, they adhered more or less firmly to the sand, with which they were intimately mixed, and formed balls. These eggs and sand were put into a dish, No. 1, containing sea-water. A considerable quantity of eggs were taken from the ovary of the same female, and placed in a dish, No. 2; and, after treating them with the contents of the male genital ducts, the dish was rotated; the eggs arranged themselves in a single layer, and adhered firmly to the wall of the dish. In the bottom of another dish, No. 3, were placed a number of glass slides, and eggs from the same source similarly treated. They were fixed to the slides in the same way. In a dish, No. 4, eggs and sand from another nest were placed, and, like the others, supplied with sea-water. Dish No. 1 was moved only sufficiently to change the sea-water occasionally. Dish No. 2 was treated in the same way. The glass slides in No. 3, with the eggs adhering, rested on supports, and were frequently turned, so that the eggs were alternately above and below the slide. The sand and eggs in dish No. 4 were vigorously stirred several times a day. In all four dishes the eggs developed. At the end of two months no perceptible difference, so far as the development of the eggs was concerned, could be observed. By means of the glass slides, the experiment of Patten ('94) was confirmed; but the changes in the first indications of cleavage observed by him have no perceptible influence on the development of the eggs.

The above experiments were begun on June 25. The eggs were not exposed to sunlight, and the development was slow and irregular. On the first of September many of the embryos had hatched, while many of the eggs showed no sign of development, and indeed appeared as if they were more or less decayed. Embryos, however, continued to be produced, and the eggs and sand were removed to Chicago in jars, where the development continued, the evaporated salt water being replaced from time to time with ordinary fresh tap water. After preserving as many of the different stages as seemed desirable and no more embryos being hatched, the jars were put to one side and neglected till a few days before Christmas, when a hasty examination showed that the water had evaporated and all signs of life had disappeared. In order to clean the jars they were filled with tap water and allowed to soak over night. The next day I could scarcely believe my own eyes when I found the bottom of the jars swarming with little Limuli.

THE OVARY.

1. *Position and general appearance.* — The ovaries of Limulus (Pl. XIII, Fig. 2, *ov.*) communicate with the exterior by means of two horizontal slits, situated on the posterio-dorsal side of the operculum, on each side of the median line, and approximated to within one-fourth of an inch of each other. Each external meatus (*g.o.*) is guarded by an upper and lower thickened lip, that is somewhat prominent externally ; and the orifice is further closed by transverse ridges within.

From these two openings, the two terminal oviducts, lying beneath the outer integument of the posterio-dorsal surface of the operculum (*op.*), traverse the proximal part of the operculum, and, proceeding forward, upward and outward, enter the cephalothorax. Here each duct soon divides into two large branches, one of which takes a peripheral direction, while the other takes a course toward the central axis of the body, where it anastomoses with the corresponding branch of the opposite side, in the median line directly above the alimentary canal (*al.c.*). The point of divergence of the central and peripheral branch of each duct is entirely obliterated, giving the two branches the appearance of one continuous tube, in the middle of the ventral side of which is inserted the slightly larger terminal duct. Between the point of insertion of the terminal oviduct (*ov.d.*)

and the point of anastomosis of the right and left branch, each of these branches gives off a large secondary branch (*ov.t.*), which passes backward on the right and left side of the alimentary tract to the anus (*an.*). These are not simple tubes, however, as has been affirmed by Owen ; but rather a network of anastomosing tubes which surrounds the alimentary tract, nearly concealing it, except immediately over that part of the operculum where the external openings are situated (*g.o.*). The lateral tubes of this system when filled with eggs are larger than the tertiary branches above and below.

After anastomosing in the median line the two secondary branches previously described again separate, and, retaining approximately their original size, pass forward on each side of the alimentary tract (*al.c.*), along the adductor muscles (*m.*), in the meantime sending smaller anastomosing branches over the alimentary tract, and large branches between the muscles. Finally they unite again in the anterior part of the cephalothorax immediately over the oesophagus.

The right and left peripheral branches of the divided oviduct, retaining for some distance the original dimensions, proceed outward, slightly backward and downward, giving off, close to the adductor muscles, a large branch. This passes along the muscles to the right and left of these till they become united in front of the adductor muscles to the tubes previously described. They again give off branches passing between the adductor muscles toward the central axis of the body; and these unite with the corresponding tertiary branches proceeding from those running parallel with the intestine previously described.

This system of large tubes lying over the alimentary canal and surrounding the adductor muscles has been described by Owen ('73) as the ovary of Limulus.

In the further course of the peripheral branch of each of the secondary branches numerous tertiary branches are given off. The whole finally resolves itself into a number of small tubes that anastomose with their neighbors and with tertiary branches given off from the large system of tubes surrounding the adductor muscles. Thus the whole ovary becomes a network of tubes covering the whole ventral and dorsal surface of the animal, and, uniting at the extreme borders of the cephalothorax, enclose the massive liver and the other internal organs.

The ovary, whose double nature is evident only from the two terminal oviducts, has a bilateral symmetry; but this is incomplete, because of the somewhat irregular anastomoses over the median line.

The secondary branches, surrounding the adductor muscles and running parallel with the alimentary tract, are comparatively large. Owing to their relatively thick walls, they maintain more or less uniform dimensions, even when filled with eggs. These being branches of the oviduct, they serve, like the latter, as reservoirs and channels of transmission for the vast number of eggs that are discharged into them by the numerous tertiary branches. This network of tertiary branches (*ov.*), covering the entire animal outside of the adductor muscles, is the real egg-producing portion of the ovary. A portion of one of these tubes is represented in Pl. XIII, Fig. 16, drawn with a camera from living material, taken from a young animal thirteen inches long, including the tail.

In the adult animal these tubes are usually filled with eggs. Owing to the feeble resistance offered by their thin walls, the eggs in them are not evenly distributed along the lumen, but are often massed together into large masses, causing irregular swellings that obliterate the meshes between the tubes. This gives the ovary a very irregular appearance, as if it were nothing else than a huge mass of eggs covered by a thin membrane. Over and between these large masses of eggs the various stages of new generations of eggs can be seen.

After the discharge of the eggs this chaotic appearance of the ovary, for the most part, disappears, and most of the irregular sac-like swellings resume the normal dimensions of the ovarian tubes. These now become conspicuous, not only from the shining aspect of their walls, but from the fringes of the various generations of new eggs that dot their surface. In the adult animals, except such as have recently moulted, the tubes (*ov.t.*) are never completely collapsed, but contain a larger or smaller number of eggs. These, when not very numerous, are arranged in rows along the lumen, like so many intersecting bead strings. The arrangement and appearance of the completely empty tubes can best be observed in a half-grown animal, where no eggs have yet been discharged into the tubes.

This network of tubes is suspended between the carapace and the liver in a subcutaneous alveolar tissue, to be described more minutely later. In the turgid condition of the ovary this tissue becomes greatly flattened. The entire space between the liver and carapace is occupied by the enormous mass of eggs, the peritoneal tissue being reduced to a thin film. This does not entirely obscure the eggs when the carapace (*ca.*) is removed.

2. *Muscle coats.* — The terminal portion of the oviduct is characterized by the firmness of its wall. This is due to a highly developed muscle coat (Pl. XIII, Fig. 15, *m.c.*). This coat consists of an outer tunic, underneath which is a thick coat of muscle fibers woven together, apparently without much order, into larger bundles that intersect and cross each other, leaving vacant meshes between. These meshes, however, are small as compared with the muscle bundles themselves. In cross section the cut end of these muscle fibers and bundles show various outlines, corresponding to the various degrees of obliquity in which they have been cut ; but no trace of a differentiation into a longitudinal and circular zone is to be recognized. Sections of the larger branches of this duct, however, show a distinct, rather thin inner, circular muscle coat, outside of which is a thicker zone of intersecting and dividing muscle fibers, with connective tissue, blood lacunae, blood vessels, and capillaries. The outermost coat consists, for the most part, of longitudinal muscle fibers, the whole forming a muscular wall considerably less in thickness than that of the terminal duct.

This same muscle coat is continued over the ovarian tubes proper, but in a very loose and attenuated form (Pl. XIII, Fig. 15, *m.c.*). In both transverse and longitudinal sections of the ovarian tubes the same features present themselves. This is true chiefly in the mature ovary where follicles have already been formed. The distinctive feature is that the fibers, both in longitudinal and in transverse section of the tube, are cut transversely or obliquely, longitudinal sections of the fibers rarely appearing. The cause of this becomes plain when the tube is slit open, spread out on a slide, and stained *in toto.* Those large sac-like swellings previously mentioned have been selected for this purpose. Killed in picro-sulphuric, and hardened in alcohol, the wall of the greatly expanded tube remains sufficiently tough to allow its removal from the mass of contained eggs; and its elasticity is sufficiently destroyed to prevent its return to its normal contracted state after removal of the eggs. Such a preparation, stained with the Biondi-Ehrlich mixture, shows the following arrangement of the fibers. Very symmetrically arranged oval areas are observed where no fibers are present. Around these areas the fibers run parallel to one another, constituting a sort of striated rim or border around the area. Between these areas with the encircling muscle fibers the fibers cross and intercross, some becoming continuous with the encircling fibers of one area, others passing around another area, and so on. The beginning and the end of these fibers could not be made out. They seem to branch freely and have nuclei imbedded in their substance.

The oval areas are due to the characteristic arrangement of the muscle fibers; and this arrangement is the expression of the regularly arranged follicles in the adult ovary of Limulus. The oval areas themselves are the follicles which have become obliterated through the stretching of the walls of the ovarian tubes. That the muscle fibers retain this characteristic arrangement in the wall of the tube, when greatly extended, is perhaps sufficient evidence that these are permanent features of the muscle coat, and not, as might be supposed, transient features due to displacement by developing eggs and likely to occur at any point where an egg might chance to develop. In the adult ovary it is this arrangement of the muscle fibers which determines the position and makes possible the characteristic follicles of Limulus. Something more concerning the origin of this arrangement and the part which the growing egg may have in its production is to be considered in connection with the development of the ovary.

3. *The peritoneal coat.* — As will appear more clearly in the account of the developing ovary, the muscle coat of the ovarian tube is surrounded by a loose coat or mantle, belonging to the honeycombed peritoneal tissue. This has been mistaken for the tunica propria by Kingsley ('92). It serves to suspend the ovary between the carapace and the liver. This tissue is seen to be laminated, and consists of greatly flattened cells, joined edge to edge like the peritoneal linings of lymph spaces generally. The meshes of this laminated tissue appear to serve as lymph sinuses, and are filled with granules and corpuscles of various kinds. It is between two such lymph spaces that the ovarian tube lies. The tube is organically connected with this tissue along one of its sides, so as to hang suspended in a loose tube, the walls of which constitute the walls of neighboring lymph spaces. This peritoneal mantle or peritoneal coat of the ovarian tube is comparatively loose; and it is the looseness of this coat which permits the eggs, as they develop, to push out through the follicular fenestrae of the muscle coat, and to occupy the space between the ovarian tube and encircling peritoneal coat. Because of its looseness, also, the ovarian tube is enabled to greatly enlarge when the eggs are discharged into the tube. In dissecting out the ovarian tubes, the connection of the tube with this peritoneal mantle is usually severed and the mantle does not appear in connection with the ovarian tube. In one sense, it belongs rather to the system of lymph sinuses than to the ovarian tube; but its relation to the ovarian tube is such that it serves a double purpose. The nuclei in the cells of this coat are conspicuous. The corpuscles and granules, so conspicuous in the lymph spaces, are not found within the periovarian cavity bounded by the peritoneal coat.

4. *The lining epithelium.* — In the adult ovary, in its empty and contracted state, the tunica propria becomes folded between the follicles, owing to the tonicity of the muscle coat.

The folding is especially prominent around the borders of the follicles, where rachis-like projections appear to extend into the lumen of the follicle.

This folding causes a considerable lateral pressure on the epithelial cells, which thus become greatly elongated and compressed.

On the expansion of the ovarian tube, this lateral pressure is relieved and the cells assume a more spherical form.

The size of the epithelial cells is subject to considerable variation, and the thickness of the epithelium also varies within considerable limits. This is due, in part at least, to the varying lateral pressure on the cells, and on the amount of folding of the tunica propria; for, where the tube is distended, the epithelium may become flattened into what appears to be a mere thin protoplasmic layer with scattered nuclei. In other portions of the same tube, where the folding and lateral pressure still exists, the epithelium appears to have considerable thickness. In all cases, however, it consists of a single layer of cells.

This epithelium can always be distinguished from all other tissues by its glassy transparency, except at certain stages, when the cell protoplasm becomes filled with secretion granules. The minute structure of the protoplasm assumes different appearances, as the cells are seen to be compressed or expanded. The distinctness of cell boundaries also varies with the amount of lateral pressure. Where the tube is expanded, and the cells are more than usually flattened, cell boundaries are difficult to make out. The cytoreticulum of one cell area seems to be continuous with the cytoreticulum of neighboring cell areas, the nuclei in each affording landmarks. Careful examination of this protoplasm seems to indicate that the various nuclei are connected by means of this system of cytoplasmic fibrils. These fibrils can be seen to be massed into strands or bundles. The bundles, however, can be analyzed into finer fibrils connecting larger microsomes. An increase of the magnifying power shows that the meshes of these fibrils are again traversed by still finer fibrils. At each node there is always a stainable, spherical enlargement, which usually diminishes with the fibril under consideration. The whole has thus the appearance of a network within a network, through a considerable series of gradations in size, both of the fibrils and their nodal enlargements. There appears to be no limit, except that which the microscope imposes, to this continued decrease in the size of the fibrils, the size of the stainable nodes, and the size of the enclosed meshes.

The terms fiber and fibrils have been here used as descriptive terms for appearances as they present themselves under the microscope; not in any way to designate the real character of the appearance.

In the relaxed state of the ovarian tube, and in the folded condition of the basement membrane, this appearance can no longer be discerned. With the increase of lateral pressure the epithelium becomes thicker, and faint indications of distinct cell outlines present themselves. The fibrous network described above is either very obscure or else not visible at all. In the latter case the cell contents appear homogeneous, with granules scattered throughout the protoplasm, and a distinct apparently vesicular nucleus situated nearer the base of the cell. But this is not a permanent condition. Cell outlines become more and more distinct. The granules become larger, clearly defined, uniform in size, and stain with some difficulty, except in such powerful stains as acid fuchsin. They often have a more or less bead-like arrangement. As the granules increase, however, they form large aggregations, occupying nearly the entire cell, and often obscuring the nucleus. At this stage the cell can often be seen to be distinctly striated. The fibers to which this striation is due run parallel with each other, and with the long axis of the cell, perpendicular to the basement membrane. The cells increase greatly in size, and, varying with the lateral pressure, their long axis may be many times that of the short or transverse axis. The form and apparent size of the cell vary with the position which it occupies, and the consequent variation in pressure. Its short or transverse axis may be equal throughout, or the cell may be greatly narrowed at the base and expanded at the free end.

At this stage the free end of the cells has a regular, clean-cut outline, and the fibers of the cell body appear to extend to the very surface of the cell. This, however, is not a permanent condition of the cell; for the free border gradually becomes ragged; the fibers appear to be continued into the lumen of the tube, in which a fibrous, deeply staining substance appears to have been secreted. With this substance the cell becomes apparently more and more continuous. The free border of the epithelium, previously distinctly and uniformly outlined, now becomes obscure and ill-defined; the fibers of the cell body become more distinct and fewer in number; cell boundaries seem to become very indistinct, and large, open, non-stainable spaces appear to occupy the larger portion of the former cell. The remaining fibers appear packed along one side of the former cell, and in this remnant of protoplasm the somewhat obscured nucleus lies imbedded. The whole epithelium in section now has a very irregular, ragged outline.

Owing to these changes in the epithelial cells, the same method of preservation has different effects, not only on the cytoplasm of the cell body, but also on the nuclei. In well-preserved conditions of the cell, previous to the considerable accumulation of granules, the chromatin consists of deeply stainable spheres arranged in a circle around the periphery of the spherical, vesicular nucleus, forming in haematoxylin stains a dark beaded ring around a clear central area. At times, but not always, a dark body more minute than the chromatin granules can be seen to occupy the center of this clear central area.

After the accumulation of the granules in the cytoplasm, however, this expanded spherical condition of the nucleus and the regular peripheral arrangement of the chromatin is not to be observed. The nucleus seems to collapse, become oval, and the distribution of the chromatin becomes irregular. The chromatin bodies themselves, losing their uniformity in size and their regular spherical form, appear as if broken up. They may also become aggregated into a more or less homogeneous mass. This latter condition of the nuclei is often to be observed in those cells of the egg stalk which show evidence of gradual disintegration. Since the other forms of the nucleus are found in their immediate vicinity, this peculiarity cannot be attributed to the reagents used. Certain methods of killing, however, as, for example, Ehrlich's bichromate, when allowed to act for several days, give to the chromatin of all the nuclei an appearance resembling this latter form.

5. *Formation of follicles.* — The pressure caused by the tonicity of the muscle fibers is greatest between the follicular

fenestrae. The epithelium over the area of the fenestrae, being relieved of this pressure, protrudes through these as evaginated pouches (Pl. XIII, Fig. 15).

The cells lining these follicles are spherical, as compared with the greatly elongated, compressed cells between the follicles. The cell outlines are not so distinct as in the elongated cells, and the epithelial lining of the follicle often appears more like a layer of protoplasm containing nuclei than like a well-marked epithelium of columnar cells.

In very many cases these follicles are filled with a secretion resembling yolk. This secretion is seen to arise in the cells of the epithelium often in the form of granules, and frequently in the form of a non-stainable mucus-like substance, which is accumulated in large masses, occupying nearly the whole cell. The secretion which is poured into the lumen of the follicle may be discharged through the communication of the follicle with the lumen of the ovarian tube into the latter, the follicle in such a case serving the purpose of an ordinary gland. There is no reason for supposing that these lining cells of the follicle differ in any essential respect from those lining the ovarian tube proper, since their difference in form can be accounted for by their freedom from pressure. The epithelium lining the follicles and that lining the tube proper are, in fact, a continuous layer of similar secreting cells, the peculiar arrangement which they have being due solely to the fenestrated nature of the muscle coat.

It frequently happens that, instead of being an open space as it is often found to be, or else a space filled with secretion, the lumen of the follicle is filled with large masses of protoplasm in the form of large, ill-defined, irregular cells (Pl. XIV, Fig. 23). These cells, occupying the entire follicle, are also seen to be secreting, inasmuch as they become filled with yolk-like granules (*s.g.*), often to such an extent as to entirely obliterate cell boundaries. In such cases the granular secretion appears to arise and accumulate around the nucleus of each cell to such an extent as to obscure the nucleus, and often to render its detection difficult. The chromatin granules of the nuclei are seen between the secretion granules, often apparently imbedded in their substance. The secretion takes a dark coloration in osmic acid, and, like the secretion of the ordinary epithelial cells of the ovarian tube, stains with difficulty, except with such powerful stains as acid fuchsin. Occasionally such a follicle is seen to have been transformed into a mass of non-stainable, homogeneous substance, showing irregular dividing lines, perhaps the original cell boundaries. These lines, probably the last remnants of the protoplasm of the cells originally present in the follicle, stain in haematoxylin. At their intersections are observed deeper staining patches that might suggest the presence of nuclei.

The large accumulation of secretion here described occurs only in those follicles where an egg is absent.

Development of the ovary. — In a young animal of one inch or less there are indications of a subcutaneous alveolar tissue between the liver and the carapace. At this stage numerous deeply staining nuclei can be observed lying between the carapace and the forming liver. The body of these cells can be seen to be flattened, and the various cells bear a certain fixed relation to one another. The meshes between the cells increase, owing perhaps to accumulation of liquids. It then appears that the cells, originally closely packed, now form the thin walls of vesicles or cavities. The nuclei are still prominent. Each of these vesicles has a wall of its own, composed of flattened cells set edge to edge, very much as in the case of lymph spaces in general. As these spaces increase in size the cells of their walls become flatter and the nucleus less distinct. Where two of these spaces are contiguous the wall appears double, as if the two walls had become closely applied.

In sections of the young animal at this stage, and earlier, there can sometimes be seen between these contiguous walls isolated cells with a distinct, deeply staining nucleus, and a well-defined cell body of protoplasm, having that characteristic clearness which is peculiar to the germ cells during the period of division, previous to the period of growth. The position of these cells corresponds to the position of the ovarian tubes, when they can first be definitely recognized as such.

In a series of transverse sections the cells are not to be seen in all sections, but, in favorable cases of tangential-longitudinal sections, they appear to be arranged in a chain between the lymph spaces. Sometimes it appears as if every other cell belonged to opposite walls of the contiguous lymph spaces; and, being separated considerably, they alternate in such a way as to appear to be a single chain of cells. It seems highly probable that these cells give rise to the future ovarian tubes.

I have not been able to trace the origin of the tubes, but it seems to be somewhat as follows: by division these cells give rise to chains of cells; which chains, consisting of perhaps four cells in cross section (possibly enclosing a cavity or lumen), take the direction of the lymph sinuses between whose walls they lie. But the walls of these lymph spaces intersect in various ways and at nearly all angles. The chains or tubes, therefore, also intersect in the same way, and thus a network is established. The cells continue to proliferate, and the number of cells in cross section increases. Such minute ovarian tubes, from four to six cells in cross section, can be seen in young animals from four to five inches.

With the increase in cross section the peritoneal walls between which the tubes lie become more and more separated; and these walls, originally closely applied as the boundaries of lymph spaces, become the peritoneal mantle, or loose peritoneal coat of the ovarian tubes of the adult animal.

The cells of the rods early show an arrangement as far from the common center of the long axis of the tube as space will permit; and thus a lumen early makes its appearance. Cells can be seen to continue dividing by karyokinesis, and, becoming pushed into the lumen, temporarily obliterate it. In animals from five to seven inches the lumen of the tube is already well established. The cross section of the tubes does not increase equally in all tubes, and their development takes place more rapidly on the dorsal than on the ventral side.

When the cells are sufficiently numerous in cross section to constitute a tube, they are seen to be surrounded by a second membrane, the tunica propria. At this early stage the tunica propria is closely applied to the original peritoneal coat, although they are easily seen to be entirely distinct, the peritoneal coat being conspicuous by the distinct nuclei of its cells, while in the second coat, or tunica propria, no nuclei can be seen (Pl. XIV, Figs. 33–41, *t.p.*).

Comparing the tube with its tunica propria, with the walls of the enclosing lymph spaces, it is seen to differ from these in this, that while in the latter the body of the cells is greatly flattened (Pl. XIV, Figs. 37–41, *p.c.*), so that the nucleus finally seems imbedded in the outer lamella, in the former the body of the cell remains conspicuous, and the nuclei are accordingly considerably removed from the common investing membrane, the tunica propria. In the peritoneal cells the intercellular or cementing substance is formed between the cells, which thus become adherent at their circumference; while in the germ cells this intercellular substance appears at the outer pole of the cell, which thus retains more of its spherical form.

In some of the tubes of an embryo seven inches long, traces of muscle cells can be seen between these two coats; and this is, of course, the future muscle coat of the ovarian tube. As the animal increases in size the muscle coat becomes more and more pronounced.

Even in an embryo five inches long it can be seen that some of the germ cells have passed the period of multiplication, and have entered on the period of growth (Pl. XIV, Fig. 38). As they grow they push their way out, causing a separation of the peritoneal membrane and the tunica propria on either side (Pl. XIV, Figs. 40, 41, *p.c.*, *t.p.*), and this circumstance seems to determine the position of the follicular fenestrae of the muscle coat (Pl. XIII, Fig. 15, *m.c.*, *t.p.*). It might be asked whether the increased pressure thus produced between the egg and the peritoneal membrane, and the resulting diminution of pressure on the other side of the egg, has anything to do with the peculiar arrangement of the muscle fibers. It will be seen later that these oöcytes make their appearance at regular intervals as the animal grows; and that this is intimately connected with the increasing diameter of the ovarian tube. But the further account relating to this will be introduced with the history of the growing egg.

The Egg.

The germ cells lining the ovarian tubes of the young animal when the lumen has appeared are spherical in form (Pl. XIV, Fig. 38). The cytoplasm of the body is relatively abundant in the resting state, and is comparatively free from granules. The nucleus occupies approximately the center of the cell. It is not relatively large, but it contains considerable chromatin, which stains deeply.

As these cells prepare for division the nucleus becomes greatly enlarged, and the chromatin assumes a distinct and highly characteristic network (Pl. XIV, Fig. 33). Dark, straight fibers are seen to intersect other straight fibers, at various angles; and these, again, can be seen to unite with similar fibers at the periphery of the nucleus. Here dark, bead-like chains of chromatin granules appear to constitute the only boundary of the nucleus. These bead-like bodies are not always evenly distributed, but considerable spaces sometimes appear between them. By adjusting the focus, however, similar granules appear in these spaces, indicating that we have here to do with a network which lies at the boundary of the nucleus. At the points of intersection of the chromatin rods within the nucleus there is always a considerable enlargement. The karyolymph is hyaline, and the chromatin element does not appear to be so abundant as to make the nucleus as conspicuous as it becomes in the next stage (Fig. 35). Now the nucleus, retaining its former size or even slightly increasing, is seen to be filled with a deeply stainable thread which appears to have the form of a coil and which fills the nuclear space. It could not be determined whether this is a single thread or several threads. Appearances seem to show that there are more than one thread; at any rate, several apparently free ends could be seen at all times. Careful examination of this thread shows distinctly that it consists of spherical bodies arranged in single rows closely applied so that in places the rod seems continuous; but the granules are often slightly separated one from the other. This, in all probability, may be taken to be the preliminary phase, spireme, of karyokinesis, which follows in the ordinary way (Pl. XIV, Figs. 36, 37).

Unfortunately, notwithstanding the comparatively large size of the nuclei in the earlier stages of this division process, the spindles are small; and the hyaline nature of the cytoplasm renders it difficult to determine the conditions at the poles of the spindle. The difficulty is further increased by the rather peculiar phenomena of several contiguous cells seeming to divide at the same time. Occasionally, single cells in the various stages can be seen; but, as a rule, a group of from four to eight contiguous cells are in precisely the same phases of division. The spindles seem to lie in all planes; and the closeness of the elements renders it difficult to observe an entire spindle with its two poles. Sections of the equators of the spindles showing the chromosomes are numerous; but the confusion arising from the crowded condition of the elements is such that any attempt at counting the chromosomes belonging to a given spindle would not lead to reliable results. A distinct centrosome imbedded in the accumulation of protoplasm at one pole, however, has occasionally been observed.

The stage with the greatly enlarged nucleus and the characteristic chromatin thread is the most conspicuous one in the process. These have been observed in most of the ovarian tubes of young animals from five to eight inches, and in material preserved in the nitro-picro-sulphuric mixture (see methods), as well as in material preserved in Merkel's fluid. In the latter material the thread is most beautifully preserved; while in the former the archoplasm and cytoplasm of the cell are excellently preserved.

As stated above, these cells usually occur in groups; but single cells, in this phase, can also be observed. The most natural conclusion to be drawn in regard to the cause of these cells occurring together in this way is perhaps that they are the sister cells of an original mother cell; and, being of equal ages, they pass through the same cycle of changes at the same time.

In these groups of cells the large nuclei, occupying nearly the whole cell, seem to lie closely applied to one another (Pl.

XIV, Figs. 33, 35), and on first observing them one might easily receive the impression that they are fusing. The distinctness of the outlines of the nuclei, however, renders it easy to make out almost equally distinct cell boundaries. The protoplasm of each cell is reduced to a thin rim, in which can be seen the few fibers of the cytoreticulum, often not exceeding three or four in number. Owing to the pressure the cell body becomes pentagonal, each side being straight and closely applied to a corresponding surface of a neighboring cell. Yet the cell boundaries are distinct.

These groups of cells can be seen in both transverse and longitudinal sections of the tube. In either case the general appearance of the group is the same. In transverse sections of the tubes only one group can be seen; while in longitudinal sections several groups can be seen at regular intervals along the tube.

In such sections the epithelium on the side opposite the group has only about one-fourth of the thickness of what it has on the side where the groups are situated. This same relation holds also in transverse sections, and it is due to the fact that each group pushes into the lumen of the tube, so as to obliterate it at that point (Pl. XIV, Fig. 35). But in the larger larvae, where the epithelial cells have become considerably elongated in the direction of the lumen, these groups of cells are more frequently enclosed by the protoplasm of neighboring cells as if they had originated beneath them, and pushed their way partly up between them. The group, therefore, does not project freely into the lumen.

In the larva these cells, when they assume the resting stage, acquire the general appearance of the neighboring epithelial cells. They, however, remain grouped together for some time.

After a certain number of divisions (the exact number cannot be made out), one of these cells, usually one lying close to the basement membrane, increases in size more rapidly than the others. Without assuming the characteristic chromatin network, and chromatin coil of the preceding stage, the nucleus enlarges, the chromatin becomes divided into irregular granules that become distributed in an irregular fashion along a system of netted fibers, but yet more abundant around the periphery of the nucleus. Here some of the chromatin granules become aggregated into a homogeneous mass, which is often closely applied to the periphery of the nucleus, and flattened on that side, but otherwise spherical. This being, no doubt, the first appearance of the nucleolus, the nucleus has, at this early stage, the main features of the germinal vesicle of later stages. The cytoplasm is still rather limited in amount, but a distinct cytoreticulum can be made out. This possesses prominent cytomicrosomes that stain as readily in chromatin stains, especially at the nodes, as the chromatin within the nucleus.

In the cytoplasm, close to the nucleus, the archoplasm with a distinct centrosome can be seen (Pl. XIV, Figs. 42, 43).

8. *Relation of the egg to the ovary.* — On the first appearance of the growing oöcyte, in animals from five to six inches, the ovarian tubes are still round in section (Pl. XIV, Figs. 34, 37, 38), and no diverticula have appeared. As the oöcyte increases in size, however, it sinks more and more below the neighboring epithelial cells, as stated by Kingsley (Pl. XIV, Figs. 34, 38, 39). These, as has been observed, are the sister cells of the same group, and for some time partly enclose the growing oöcyte as temporary follicle cells.

The basement membrane becomes gradually pushed out as the egg grows, until it forms an investing membrane of the egg, which, remaining organically connected with the follicle cells only by a narrow isthmus, appears to lie wholly outside of the tube, between the germinal epithelium and the peritoneal coat (Pl. XIV, Fig. 40). Being still invested with the tunica propria, however, it is still within the ovarian tube, and in fact never leaves it (Pl. XIV, Fig. 19, *t.p.*). As the oöcyte moves outward the sister cells belonging to the group assume more and more the appearance of epithelial cells (Pl. XIV, Fig. 34). New oöcytes within the tube begin a similar career of growth. It thus happens that in an animal seven inches long where some of the tubes have acquired a considerable lumen, two or three stages of these young oöcytes may be observed (Pl. XIV, Fig. 40). Only one diverticulum, in cross section, has yet been fully formed, and this is, as yet, to be observed only on the tubes of the dorsal side of the animal.

In an animal eight inches long two diverticula in cross section are fully formed.

In an animal thirteen inches long, being about half grown, six diverticula are observed in a cross section of a tube.

Here also can be seen the relation of the ovarian tube to the enclosing peritoneal coat or mantle (Pl. XIV, Fig. 41; Pl. XIII, Fig. 15, *p.c.*). The germinal epithelium, with its basement membrane and enclosing muscle coat, is in organic connection with the peritoneal coat only along one of its sides (Fig. 41). Here the various tissue elements become intimately blended, and here, also, blood capillaries and blood vessels are to be seen. At this point the tube increases in size, and it is here that the earliest stages of the forming eggs are to be seen. The epithelial cells are considerably elongated radially. At the base of these cells at this point groups of closely packed, deeply staining nuclei can be seen. Gradually a large nucleus appears surrounded by a definite cell body, which, unlike the cytoplasm of the hyaline epithelial cells, is granular, and stains deeply in the carmine and haematoxylin stains. No evagination of the basement membrane at this point has yet appeared, but the cells lying above the young egg cell seem often to be bounded at their base by a definite membrane, which partly encloses the space in which the young egg lies.

On either side of this point of attachment of the ovarian tube where the first stage of the egg appears, the more advanced stages, in regularly increasing series, are to be seen. Passing around the tube the diverticula increase with the increasing size of the egg, to that point of the tube opposite the point of attachment, where the largest egg in the series is to be seen. There is no connection between the peritoneal coat and the ovarian tube except at the one point of attachment of the latter (Pl. XIV, Fig. 41). The ovarian tube with its diverticula hangs suspended from the inner wall of the enclosing peritoneal coat, along one of its sides. In sections the peritoneal coat is seen only when the ovary is sectioned *in situ*, which is the more convenient in the young forms.

In the adult the relation of the egg to the ovary and of the various parts of the ovary to one another is essentially the same as in the younger forms (Pl. XIII, Fig. 15). As compared with the younger forms the ovarian tubes, in cross section, are greatly enlarged, and the number of diverticula, in cross section, are proportionately increased. But this is not necessarily true of the number of eggs that may appear in cross section, for after the discharge of the first set of eggs into the ovarian tubes a number of empty pouches containing no eggs may be seen (Pl. XIII, Fig. 15). These occupy the same position which the discharged egg previously occupied. Originally they arise, as has been seen, in the young animal by the pushing out of the tunica propria through the fenestrae of the muscle coat as the egg grows. The sister cells of the egg after division of the oögonia become the lining cells of the stalk of the egg. The stalk of the egg, however, exists only during the empty state of the tube, for when the tube becomes stretched by mature eggs that have been discharged into it, the stalk of the egg disappears and its epithelium constitutes the lining epithelium of the ovarian tube.

While it is true that throughout the various stages of growth of the animal the number of eggs in cross section of a tube regularly increases, there appears to be a period beyond which no new eggs are formed. In an animal eighteen inches long eight eggs appear in a cross section of a tube. Several animals, among them a soft-shelled one, equaling in size some of the females that were observed ovipositing at Woods Holl, had discharged no eggs from the follicles, and yet the number of eggs in cross sections had not increased.

Notwithstanding a most careful examination of ovaries from a large number of adult females, collected both at Woods Holl and at New Haven, and showing empty follicles, having evidently reached the period of sexual maturity, I have in no case been able to observe the first stages of the forming egg as it is so easily done in the earlier stages of the growing animal. I infer, therefore, that new eggs are not formed after a certain period, and that this period is either earlier than the period of the first discharge of the oldest egg into the ovarian tube or else coincides with it. So far as I have been able to determine from an examination of many specimens throughout the growing series, the number of diverticula that appear in a cross section of a tube is equal to, and does not exceed the number of oögonia in the cross section of a tube previous to the formation of an oöcyte.

Furthermore, in the adult animal having empty follicles, the number of eggs in cross section of a tube decreases in proportion as the empty follicles increase; and the size of the smallest eggs is proportional to the number of empty follicles, and inversely proportional to the number of eggs in cross section.

In higher animals it is known that a period exists when eggs for the first time are discharged. It is also known that a period exists after which reproduction does not take place. It is also known that in many higher animals it is impossible to find the earliest stages of the egg in the adult animal, and it frequently has been assumed, on this account, that not only the origin, but the history of the egg in the adult differs radically from the history of the egg of the same animal in its early stages. Thus Balfour ('78), in Elasmobranchs, describes two methods by which the egg may arise: first, by a fusion of a number of cells, which he thinks is the normal process; and, second, by a gradual transformation of a primitive ovum into a permanent ovum.

To enumerate, briefly, the observations: In the young animal, up to five inches, the germ cells form the lining of the ovarian tube. At this period growing oöcytes make their appearance as diverticula, and continue to be formed up to the period of sexual maturity. After this period no new oöcytes are formed; but those already existing continue to grow as the animal grows, until the period of sexual maturity, when the eggs in the follicles first formed are discharged into the ovarian tube. The first oviposition takes place considerably later, and continues at intervals till all the eggs have been matured, which may cover a period of at least eight years. With an intermission of more than one year between the periods of oviposition, as seems probable from the observations recorded in the chapter on natural history, this period may of course be greatly extended. It is seen from those observations, also, that after the period of sexual maturity, which may be reckoned from the first discharge of eggs into the ovarian tubes, the phenomena of moulting, if not entirely suspended, become at least less frequent. It may be supposed that from the period of sexual maturity the animal does not increase so rapidly in size. That it does increase in size after the period of sexual maturity seems probable from the fact that females that were observed ovipositing differed considerably in size.

The original germ cells (oögonia), up to the time when the embryo measures five inches, including the tail, multiply by an equal division. At this stage they number about eight in cross section. This marks the end of the *period of multiplication.*

At about the sixth-inch stage of the animal a new period in their history begins — the *period of growth.* This is immediately preceded by a multiplication process differing from the former in that the products of division are dissimilar. The karyokinetic processes by which this takes place have previously been described. The result of this process is the formation of a group of cells, one of which becomes the growing oöcyte, while the others belonging to the same group become temporarily the follicle which ultimately forms the permanent epithelium of the ovarian tube. In this way the original germ cell has acquired a new environment, inasmuch as it is henceforth destined to be removed farther from the lumen of the ovarian tube, and is guarded by its daughter cells, which, as follicle and epithelial cells, serve to nourish and protect it.

This transformation of the original oögonia into the protected, specially nourished, and consequently growing oöcyte does not take place simultaneously in all the original oögonia, but it is first accomplished in that one farthest removed from the point of attachment of the ovarian tube (Pl. XIV, Fig. 41). From now on, this first-formed oöcyte continues to grow as the animal grows, and is the first to arrive at that stage of maturity which marks its discharge into the ovarian tube when the period of sexual maturity of the animal is reached.

At the seven-inch stage of the animal this first oöcyte has formed a complete diverticulum (Pl. XIV, Fig. 40). Longitudinal sections of an ovarian tube, when it passes directly in the plane connecting the point of attachment of the ovarian tube and the diametrically opposite side, show a series of these first oöcytes all practically equal in size.

In transverse sections of the tube, in this stage, it is seen that the immediate neighbor on the right is passing through the same process (Pl. XIV, Fig. 34); and this being formed, two diverticula of the ovarian tube may be seen in an animal about eight inches long. Now a third on the left is forming a follicle in the same way. Thus the forming oöcytes with their follicles and diverticula appear alternately on either side of the one first formed. In the thirteen-inch animal five have formed and a sixth is forming; while in the eighteen-inch stage eight diverticula have been formed, the smallest being close to the point of attachment of the ovarian tube.

As these oöcytes increase in size uniformly from the time of their first formation, the one first formed continues to be the largest, the others on either side of this being smaller and smaller, corresponding to the time of their appearance, as the point of attachment of the tube is approached.

The regular sequence in which the oöcytes make their appearance gives to each a definite amount of space, which relieves it from pressure during growth and preserves its spherical form. It is readily seen, also, that this sequence affords a compensation in the economizing of space in the periovarian cavity; for when the first oöcyte attains to a definite size it is discharged; and thus the amount of space by successive discharges, as each in its turn grows, remains practically the same throughout. A portion of an ovarian tube, taken from the living animal thirteen inches long, and examined under the microscope, presents the appearance of an elongated cluster of grapes (Pl. XIII, Fig. 16). In this way, also, it can be seen that the eggs decrease or increase uniformly in size as the tube is rotated on its longitudinal axis.

Up to this point it can be said that there exists a correlation of growth between the parent organism, the ovary, and the eggs, after they enter on the period of growth.

It is known from the observations of Lockwood, which I can fully confirm, that the young animal moults more frequently in its earlier than in its later stages. This seems probable from the observations related in the chapter on the natural history, where it was stated that the apparently grown soft-shelled specimens were found, on examination of the ovary, not to have any mature eggs in the ovarian tubes; and that animals of an equal size, having moulted earlier, were in a similar condition. Histological examination of the ovaries of these animals showed that no egg had been discharged from the follicles, this being an easy matter to determine.

It was also stated that animals having mature eggs in the ovarian tubes always had hard shells, which was also true of all those females observed at Woods Holl during the spawning season.

These observations seem to show that the moulting is not a phenomenon in any way connected with the season of the year, but that it is intimately connected with the phenomena of growth.

Now, it having been shown that the young of Limulus moult much more frequently than the adult animals, and that with each moult the young animal increases greatly in size, it is extremely probable that the animal increases in size much more rapidly in the earlier than in the later periods of existence.

From the comparative size of the first-formed diverticulum, and its contained egg in the seven-inch animal and the later stages, the same retardation of growth from earlier to later periods, observed in the animal, seems to hold good also in regard to the growth of the ovary and the eggs contained in it.

This may explain the apparent contradiction in the correlation of growth, which at first sight seems to present itself in the case of those eggs that are still growing after the period of sexual maturity is reached. For while the oöcyte first formed is discharged from the follicle at the first period of sexual maturity, the one which is just formed in an animal eighteen inches long may not be discharged for many years thereafter, even though the difference between the two in point of time of first appearance may be much less.

It is known that in many higher animals, especially in the human subject, precocious growth is often accompanied with precocious sexual maturity, and that this marks an important epoch in the life of the individual. It is known also that this period is evidence of maturity of the sexual organs. In Limulus it seems extremely probable that the discharge of the first egg into the ovarian tube marks the period after which no new eggs are formed. Those already formed continue to grow at the decreasing rate at which the animal increases in size, after the period of sexual maturity. They are discharged from the follicle when they attain to the size which is normal to them; and, continuing thus to be discharged and no new eggs being formed, the time of sterility finally arrives.

On the discharge of the egg from the follicle into the ovarian tube, it is severed from its organic connection with the parent organism and acquires a new environment. Here the egg increases to double its former size within a very short period of time. As will appear later, this change in environment and in the rate of growth is accompanied by marked internal changes in the constitution of the yolk. The important fact to note here is that with the severance of the egg from its organic connection with the parent organism the correlation in growth no longer exists ; and that the egg, having acquired an individual existence, grows at a rate entirely out of proportion to the rate of growth of the animal.

The egg now is surrounded on all sides by the secretion of the epithelial cells; it no doubt utilizes this secretion as nutriment. In studying the structure of the egg, it appears that the egg membrane is radially striated, and that these radial striae are due to protoplasmic fibers that extend out to the investing tunica propria and are in some way connected with it. When the egg is discharged it becomes separated from the tunica propria. This remains behind as the only wall at that point of the tube, and later becomes lined with a new epithelium, perhaps regenerated from the surrounding epithelial cells. The cavity in which the egg lay becomes practically obliterated by the stretching of the walls of the ovarian tube to accommodate the eggs within it, and only later bulges out as an empty follicle, after the tension within is relieved on the discharge of the eggs in oviposition.

Concerning the rôle which the radial protoplasmic fibers of the chorion may have in the transfer of nourishing material from without, I have nothing on which to base any positive statements. Neither do I know whether these fibers are retracted within the egg, thus leaving pores after the discharge of the egg from the follicle. It may be supposed, perhaps, that they serve somewhat as delicate pseudopodia in the transfer of nutriment. Among others, Eimer ('72) has ascribed such a function to them in the egg of reptiles.

However that may be, the fact remains that the eggs increase greatly in size and become unfavorable for sectioning, a feature that does not exist up to this time.

STAGES OF GROWTH.

The period of growth extends from the last division of the oögonia to form follicles to a somewhat indefinite period after the egg has entered the ovarian tube and has attained its full size. By regularly recurring internal phenomena this period divides itself into four stages. First, a stage extending from the beginning of growth to the formation of the first layer of the egg membrane. Second, a stage extending from the end of the first to the time when the germinal vesicle begins to move towards the periphery. Third, a stage beginning with the gradual approach of the germinal vesicle to the periphery of the egg and terminating with the discharge of the egg into the ovarian tube. Fourth, a stage extending from the time of entrance into the ovarian tube to the time of oviposition.

Each stage may be first briefly described, after which the history of each part of the egg will be considered separately.

Stage I. — The most striking peculiarity of the growing egg at the time when it can first be recognized as such is the deeply stainable granular cytoplasm which, previous to growth, is characterized by a peculiar glassy translucency. The germinal vesicle also, at first a nucleus not differing perceptibly from the neighboring nuclei of the follicle and epithelial cells, increases in size and becomes more conspicuous by the increase of stainable substance. Part of this becomes condensed, or separated off and collected into a nucleolus, which previous to this time could not be observed. At this time, also, the archoplasm, centrosome, or vitelline-body, is more conspicuous in the cytoplasm.

Perhaps the most conspicuous feature of the egg, as a whole, in this early stage is the strong affinity of the cytoplasm and germinal vesicle alike for carmine and haematoxylin stains. This peculiarity becomes gradually lost after the first stage is passed. Unlike the nuclei of the follicle and germinal epithelial cells, as well as the nuclei of other tissue cells of the ovary, the germinal vesicle cannot be made to show the green stain of the Biondi-Ehrlich mixture. The loss of this property appears to take place about the time when the nucleolus makes its appearance. The germinal vesicle and cytoplasm stain deeply in haematoxylin and carmine stains up to the time when the first layer of the egg membrane is formed.

In the cytoplasm, during this stage, there is an area, usually close to the germinal vesicle, which does not show this affinity for carmine and haematoxylin stains, but which, on the other hand, has a peculiar affinity for Lyon's blue, picric acid, eosin, acid fuchsin, and erythrosin. At this stage the germinal vesicle is regularly spherical, and its position is usually slightly, but at times very excentric. The proportion between its size and the amount of cytoplasm is perceptibly greater than it is found to be in later stages.

Stage II. — In this stage the amount of cytoplasm, as compared with the size of the germinal vesicle, has increased. The cytoplasm is surrounded by a thin layer of dense substance immediately under the investing membrane. The germinal vesicle, instead of being spherical as before, now shows sac-like diverticula that appear like buds on its surface. The nucleolus has increased proportionately in size, and shows changes that are not to be observed in the previous stage. This stage as contrasted with the previous stage is marked by the considerable loss, by the cytoplasm, of that affinity for chromatin stains, and by the greater size and clearness of the centrosphere. The yolk granules are more abundant and many of them seem to have increased perceptibly in size.

Stage III. — In this stage the germinal vesicle is relatively more excentric in position, and subject to great variations in form and size. Compared with the amount of cytoplasm and yolk it is perceptibly smaller. The nucleolus is often very large relatively, and shows many irregularities in form and structure. Numerous "Nebennucleoli" exist. The chorion has increased greatly in thickness by the addition of new layers. The cytoplasm is conspicuously marked by a polar differentiation, one pole being rich in yolk granules and the opposite pole comparatively free from these granules.

At the end of this period the germinal vesicle lies close to the periphery, partly surrounded by a spongy, hyaline protoplasm that does not stain readily. The egg having attained about half of its normal size, but as yet showing no true yolk spheres, is at the end of this period discharged into the ovarian tube. The manner in which this appears to be accomplished has been described above. The egg has now entered on its fourth and last stage.

Stage IV. — This stage is marked by a modification of the cytoplasm that renders sectioning in paraffine extremely difficult. This appears to be due to marked changes in the yolk granules. These assume regular spherical forms, and increase very rapidly in size. Owing to the rapid increase of the yolk spheres the egg increases proportionately in size, and this increase in size appears to be a very rapid one. In the first periods after its discharge from the follicle the egg can still be sectioned in paraffine, but the yolk bodies can be seen to have become vesicular and regular in outline, though still comparatively small. The yolk bodies, even now, adhere less firmly to the slide, so that passing the slide through different grades of alcohol or even dissolving the paraffine is liable to wash many of them away. This was not the case in the previous stages. All transition stages from these first definite yolk spheres to the fully grown yolk spheres can be observed, not in the same egg, but in a series of eggs, according to the time which has elapsed since their discharge.

In this stage the nucleolus has disappeared, and the greatly increased yolk spheres often render it difficult to find any trace of the germinal vesicle, except in the first part of the period, when it still can be seen immediately under the egg membrane or comparatively close to it.

Degenerative processes. — In the third stage it sometimes happens that the egg, instead of being discharged into the ovarian tube, undergoes degeneration. This has been observed occasionally in material collected both at Woods Holl and at New Haven; but it was most pronounced in the ovaries of those animals which were obtained from the aquaria of the United States Fish Commission at the World's Columbian Exposition in Chicago.

These animals had been kept in confinement for at least six or seven months. It is probable that they had suffered from lack of nourishment, as well as from other disturbing influences incident to a long confinement.

The ovarian tubes of these animals were filled with mature eggs, and oviposition had probably been prevented by their captivity. Many of the larger follicular eggs show the regressive metamorphosis referred to.

The metamorphic process appears to take place in two ways: first, by the gradual absorption of the egg without the invasion of cells; second, by the appearance, within the egg, of innumerable nuclei (Pl. XIV, Fig. 30).

In the latter case the germinal vesicle, so far as observed, is in all cases absent. On their first appearance the nuclei are found at the proximal pole, where, in this stage of the egg, the germinal vesicle is normally found. With the increase of these nuclei they spread throughout the central part of the egg; and, without at first producing any abnormal appearances of the yolk, gradually fill the entire egg (Pl. XIV, Fig. 30). Simultaneously with this, one or several layers of well-defined, polygonal cells surround the egg, between the outer tunic and the egg membrane, in many cases giving the appearance of a true follicle epithelium. At times this layer of cells may not extend to the distal pole; and in still other cases several layers may appear at various points.

These enveloping cells appear to be continuous with the epithelial cells of the stalk, but their boundaries are more sharply defined. The nuclei of these cells resemble the nuclei of the germinative epithelium, but their cytoplasm is always packed with stainable granules resembling yolk granules. The nuclei within the egg present every similarity to the nuclei of these surrounding cells; and, like the latter, in advanced stages of metamorphosis of the egg they are surrounded by deeply staining granular areas of protoplasm, indicating cell outlines. Often, however, the nuclei are seen imbedded in interwoven strands of protoplasm, where no cell boundaries are visible. This may occur in different portions of the same egg. On their first appearance the nuclei are often uniformly distributed throughout the yolk, in which cases the yolk may be normal, or else slightly broken up into comparatively large masses, giving a vague suggestion of cleavage.

In stages farther advanced the nuclei, which at first showed no indication of cell boundaries, become more or less grouped into patches. The yolk granules, previously evenly distributed throughout the egg, evidently disappear in patches at different times, till one pole of the egg may be nearly devoid of yolk granules. It then shows only the strands of protoplasm with scattered granules, and nuclei imbedded in them; while the other pole may still have the normal appearance, with the exception of here and there an isolated nucleus.

In section, except in the earliest phases of metamorphosis, the outlines of these eggs become irregular (Pl. XIV, Fig. 31). The egg membrane becomes indented, folded, and perforated in various ways. The perforations may pass transversely or obliquely, and in these perforations cells resembling the granular cells surrounding the egg are often observed. These perforations often communicate with spaces between the outer tunic and the infolded egg membrane, which spaces may be filled with granular cells resembling those observed in the perforations.

At the proximal pole the egg membrane is often partially or completely destroyed; and a nucleated mass of protoplasm within the egg appears directly continuous with the protoplasm of the cells lining the stalk (Pl. XIV, Fig. 31).

That these bodies in the egg are real nuclei there is no reason to doubt. They differentiate very excellently with the ordinary nuclear stains. Diluted Delafield's haematoxylin, slightly acidulated, makes them prominent; and they show the differential green stain of the Biondi-Ehrlich triple mixture. From material collected at New Haven, where the animals were in their normal habitat, preparations showing these nuclei were obtained by means of the double stain of Lyon's blue and lithium-carmine, the nuclei alone taking the carmine stain.

The final result of this process of absorption, both where nuclei are present and where these are not to be observed, seems to be the removal of the entire substance of the egg. The last traces that are to be observed are those of the egg membrane, which appears to persist for some time after its contents have been absorbed.

The lymph spaces adjoining the ovary containing such eggs are often seen to be crowded with granular cells resembling very much the granular cells surrounding the egg.

Strahl ('92) found that, in the mature follicles of Lacerta agilis, when the animals are kept in confinement and separated from the males, an atrophy takes place in the mature ovarian egg. The first evidence of this is the disappearance of the nucleus; second, the segmentation of the yolk as in cleavage, and finally the entrance of leucocytes. These at first appear aggregated around the point where the nucleus was situated, but later they distribute themselves throughout the egg.

The segmentation of the egg of the domestic fowl in an unfertilized state has frequently been affirmed, among others, by Oellacher ('72). In these, as well as in the unfertilized eggs of bony fishes, according to him, a division of the nucleus and a real cleavage takes place. The same has been described in the egg of the dove by Motta and Mayo.

Born claims to have observed a cleavage of the unfertilized egg of the frog. He was unable to state whether this was accompanied by a division of the nucleus.

Balbiani ('93) found that the ovarian eggs of spiders also degenerate ; and he figures follicles filled with cells.

I am unable to make any positive statements in regard to the immediate causes of metamorphosis. I believe, however, that the following statement can be made : the cause of the disturbance lies in the egg itself. In the present case there is no true follicle epithelium surrounding the egg. The cells of the stalk, which correspond to the follicle epithelium in other eggs, and which appear to have a similar relation to the egg so far as the function of nutrition is concerned, seem perfectly normal. They often appear to be unusually active and evidently enter the egg at the proximal pole (Pl. XIV, Fig. 31).

The conditions which make this possible, as it seems to me, lie in the egg itself, and not in an abnormal condition of the follicle cells, as has been supposed by Flemming ('95) in the case of other eggs.

THE GERMINAL VESICLE.

A network can be distinguished quite early in the germinal vesicle, and the stainable substance, losing more and more its definite form, becomes distributed in irregular granules over this network, and also between the meshes, being especially abundant at the nodes. The stainable substance tends to become massed at the periphery, and especially at one point, where the nucleolus early makes its appearance. As the nucleolus increases in size, the remainder of the germinal vesicle loses more and more its power of staining deeply in carmine and haematoxylin, and is no longer capable of being differentiated, as ordinary nuclei are, by means of the green of the Biondi-Ehrlich triple stain.

During the first stage of the egg the germinal vesicle is spherical and occupies a slightly excentric position when viewed in the plane passing through the centrosome and sphere. In a plane at right angles to this, its position is about central (Pl. XIV, Figs. 34, 38–41).

In the next stage the germinal vesicle shows a tendency to become irregular, owing to the appearance on its surface of numerous diverticula or pouches (Pl. XVI, Fig. 105; Pl. XIII, Figs. 1, 6–8; Pl. XIV, Fig. 24). These are often of considerable size. They are, for the most part, spherical and remain connected with the germinal vesicle by means of a narrow neck or isthmus. The network and stainable granules of the germinal vesicle extend into these, and they are frequently observed to contain pale "Nebennucleoli" (Pl. XIII, Figs. 7, 8).

Very frequently there is an accumulation of stainable granules at one point near the periphery of the germinal vesicle, and this is at times so prominent that it might be mistaken for a second "Hauptnucleolus." It, however, lacks the definite form of the "Hauptnucleolus," and consists of irregular bodies of very different sizes that stain deeply. When this is formed in the central part of the germinal vesicle, the strands of the nuclear network appear to radiate from it as a center (Pl. XIII, Fig. 10). Occasionally this is so marked that it assumes the appearance of an aster. In some cases the granules are less pronounced; and it can then be seen to have all the features of a centrosome and sphere — a deeply stainable central body, surrounded by a clear zone, which in turn is again surrounded by an outer ring, from which the larger strands of the nuclear network radiate. A somewhat similar arrangement of the nuclear network around the nucleolus is sometimes seen (Pl. XIII, Figs. 4, 8, 11). It is especially pronounced in material hardened in Flemming's fluid (Pl. XIII, Figs. 4, 8), but the appearances are by no means confined to such material. The chromatin network seems often to have a centralized arrangement, and the center of radiation may coincide with the nucleolus or be independent of it. When it is found near the periphery of the nucleus, the wall of the latter often shows an indentation in the form of an acute re-entrant angle at that point (Pl. XIII, Fig. 15). In such cases, which are of frequent occurrence, the principal strands of the network can be seen to radiate from this point in a fan-shaped manner. It can be seen that this point is connected with fibers proceeding directly from the centrosome and sphere in the cytoplasm. It often recalls very forcibly the observations of Auerbach ('96), Leydig ('83, '88), and Rabl ('89).

The nuclear network can also be distinctly seen in the living egg, without the use of reagents, by causing the contents of the egg to flow out. In such a preparation the nuclear network is very distinct, and presents all of the principal features seen in well-preserved material. It is clearer and better defined, owing to the comparative absence of granules which in preserved material obscure it. A germinal vesicle, removed in this way, remains surrounded on its exterior by a delicate network of fibers enclosing yolk granules. These seem to be intimately connected with the germinal vesicle, and render it impossible to obtain the latter entirely free from them. One might ask whether the peculiarly close adherence of these fibers is not due to a direct continuation with the nuclear network.

Everything seems to point to the conclusion that this stage of the germinal vesicle is a period of great activity.

The germinal vesicle, containing a "Hauptnucleolus" and many "Nebennucleoli," and having attained its maximum size, now begins to approach the periphery (Pl. XVI, Figs. 104, 114). It varies much in form and size. At times long pseudopodia-like processes extend radially far out into the body of the egg, giving the germinal vesicle the appearance of a very irregular amoeba. There may be one or several pseudopodia, and they may thin out to such an extent that it is difficult to trace them. The body of the germinal vesicle, in such a case, may be reduced to a small central area, in which the often very large nucleolus may be seen (Pl. XIII, Fig. 5). It may also be greatly extended in one direction, so as to become flattened out into the form of a fish or an arrowhead.

In all such cases the hyaline karyolymph appears to be wanting, or nearly so. The chromatin granules lie closely packed, and the peculiarly distorted body thus takes the stain with avidity. The "Hauptnucleolus" is always present. There is often a strong temptation to regard these peculiar forms of the germinal vesicle as shrunken conditions due to reagents. As they occur, however, in the best preserved material, it is not easy to regard them as artifacts. Careful study of the living egg reveals none of those movements of the germinal vesicle and nucleolus frequently spoken of in other eggs as amoeboid. The stage under consideration seems rather to be a period of suspended activity on the part of the germinal vesicle, and the processes extending out into the cytoplasm appear rather as the expression of pressure to which the germinal vesicle is being exposed owing to the increasing mass of yolk.

This period is followed later by one of renewed activity, in which the germinal vesicle again becomes filled with the usual hyaline karyolymph, and assumes a more definite spherical form (Pl. XIII, Fig. 14). Having reached the periphery of the egg, it is often comparatively large and is surrounded on all sides, except that immediately in contact with the yolk, by a hyaline, finely spongy protoplasm, which is comparatively free from yolk granules (Pl. XIII, Figs. 11, 14). The contents of the germinal vesicle in such cases show, especially around the periphery, a finely spongy protoplasm, in every respect resembling that surrounding it. It is still surrounded by an apparently well-defined membrane, and contains still a large, deeply staining nucleolus. This alone shows the characteristic stain of chromatin.

The ultimate fate of the germinal vesicle appears to be that its membrane disappears, the larger portion of its contents becomes diffused through the spongy protoplasm. This may be seen as a cap, covering perhaps half of the egg (Pl. XIII, Figs. 11, 14). It persists as such for a considerable period, until the yolk spheres, now increasing very rapidly, occupy practically all the space within the egg. They gradually encroach on the protoplasmic cap till it is reduced to a thin protoplasmic layer immediately under the egg membrane (Pl. XIII, Fig. 17).

The nucleolus having disappeared as such, the last remnant of the germinal vesicle can be seen as a deeply stainable, irregular, amoeboid body, lying in the yolk some little distance below the egg membrane (Pl. XIII, Fig. 12). The yolk surrounding this has a distinct radial arrangement, and this radial arrangement can be traced as parallel striae to the periphery of the egg, where they are continuous with a small mass of hyaline protoplasm. In one or two cases, stainable bodies suggesting chromosomes have been seen in the midst of this radial striation.

In one case, a mass of hyaline protoplasm, free from yolk and having the form of a spindle, was observed imbedded in the yolk some little distance below the egg membrane (Pl. XIII, Fig. 17). The latter showed a perforation running radially through it at this point, suggesting a micropyle. As this is the only trace of such a structure that has been observed, it cannot be definitely stated to be a micropyle. The lumen of the perforation was occupied by a number of small yolk granules.

In the case of other eggs, various causes have been assigned for the movement of the germinal vesicle towards the periphery.

The yolk accumulating at one pole continues to gradually increase until that pole in which yolk does not accumulate and with which the germinal vesicle is connected, becomes more and more flattened out (Pl. XIII, Figs. 11, 14; Pl. XVI, Fig. 104), the hyaline spongy protoplasm, of which it is composed, being forced more and more over the surface of the egg as the vegetative pole increases. This process continuing, the germinal vesicle soon comes to lie under the cap previously described. Owing to the growth of the yolk at one pole, the germinal vesicle and its surrounding hyaline protoplasm, originally near the center of the egg (Pl. XIV, Figs. 20, 24), becomes more and more displaced, the internal portion becoming turned out, so to speak. It might perhaps be designated as an evagination, somewhat like the finger of a glove when straightened out after being turned in on itself.

The nucleolus. — In the stages of the oögonia preceding the final division, resulting in the formation of a follicle, no trace of a nucleolus can be discerned (Pl. XIV, Figs. 33, 35, 37). It is first seen in the oöcyte, at the time when the latter has commenced to increase in size. As we have seen, the chromatin at this time loses many of its previous characteristics, both with regard to chemical reactions and general appearance. The chromatin bodies, so far as they retain their regular form, become imbedded in a more or less viscid (or granular), stainable substance. In this substance the nucleolus makes its appearance, usually close to the periphery of the nucleus (Pl. XIV, Fig. 39). At first it is often flattened on the side next to the wall of the nucleus, but elsewhere spherical, though at times irregular in outline. In this early stage it often appears to consist of granules. How much of this granular substance may be due to the reagents cannot be definitely ascertained. It soon becomes homogeneous and spherical, and takes up little by little a more central position (Pl. XIV). At a very early period in its history it can be seen to be differentiated into an outer and an inner zone (Pl. XIV, Figs. 34, 44). The outer zone seems more dense, and at first it seems like a comparatively thick investing coat of the internal central body. Occasionally two nucleoli of essentially similar appearance can be seen in this stage (Pl. XIV, Fig. 26). In both the investing, homogeneous layer can be seen to be thinned off at one point, so that the internal, central spherical body partly protrudes through the homogeneous covering.

One of these nucleoli usually increases more rapidly in size, and later becomes the only one visible. The growing nucleolus becomes the future "Hauptnucleolus," of which there is usually only one, but in some cases two.

As the nucleolus grows, it retains for a considerable time its spherical form; and throughout the first period usually remains more or less homogeneous, with now and then spherical vacuoles in its substance (Pl. XIV, Fig. 44; Pl. XV, Figs. 83, 87; Pl. XVI, Figs. 99, 100). These vacuoles are not always mere cavities or fluid particles; they may contain solid bodies that stain a deep black in Heidenhain's iron-haematoxylin.

In the second stage of the egg the nucleolus, although it often seems homogeneous, and filled with vacuoles of different sizes, is seen to possess, in a great many cases, a dense outer layer enclosing a central, spherical mass (Pl. XV, Fig. 68; Pl. XIII, Figs. 8, 9; Pl. XVI, Figs. 108, 115, 117, 118. The central mass often has an excentric position, so that the outer homogeneous part, in optical section, has the form of a crescent. This can be distinctly seen in the living egg (Pl. XVI, Figs. 106, 115, 117, 118). It is one of the most pronounced characteristics of the nucleolus at this stage. In the nucleolus of the living egg the outer crescent-shaped zone appears to be studded with spherical bodies imbedded in it (Pl. XVI, Fig. 108). Whether these are mere fluid vacuoles or solid bodies, cannot be made out. The central body often appears like a vacuole, but more frequently it is granular. The granules vary in size, not only in the same nucleolus, but in different nucleoli (Pl. XVI, Figs. 108, 115, 117).

In preserved material this central body is seen to be solid or composed of granules, as was the case in the living egg. The central body may at times be greatly enlarged. The outer crescent-shaped body then appears as a cap at one pole of the central body. The horns of the crescent, in sections, becoming greatly thinned out, extend along the sides of the central body. At other times the central body is not so large, the outer zone being larger in proportion. The central body may then be elongated into a cylinder-like body with rounded ends, the outer end projecting through an opening in the outer zone at the point where this is thinnest (Pl. XVI, Figs. 111, 113). This reminds me strongly of the observations of Aimé Schneider and Balbiani ('83). The inner end of the projecting body may be simply rounded, or it may be somewhat enlarged. The whole body may be spherical in form (Fig. 112).

In these cases the outer zone stains more deeply than the inner body, except in Heidenhain's iron-haematoxylin, in which the central body takes a dark stain.

The body can often be seen to have been extruded (Pl. XVI, Fig. 110; Pl. XV, Fig. 79). In such cases a cavity, which communicates with the exterior by means of a circular opening, exists in the nucleolus. The extruded body can be seen in all stages of extrusion. When this has occurred, it is sometimes seen lying close to the opening (Pl. XVI, Fig. 110; Pl. XV, Fig. 79).

The extruded body assumes a spherical form, and, except in Heidenhain's iron-haematoxylin, loses more and more its power of staining. Finally, it resembles an ordinary yolk sphere of the last stage of the egg.

I see no reason why this may not be regarded as a so-called "Nebennucleolus." The difficulty with which these bodies stain seems to correspond to the condition of bodies described under that name by various authors.

Besides this single body extruded in this way, it can sometimes be seen that the central cavity of the "Hauptnucleolus" is filled with a number of comparatively large spherical bodies that behave toward stains similarly to the one just described (Pl. XIII, Figs. 4, 8). In one case an opening in the thinnest part of the "Hauptnucleolus" was observed; and some of these internal bodies appeared to be on the point of being extruded (Pl. XIII, Fig. 4). One was lying at the opening outside the "Hauptnucleolus," and another just inside; the rest of the internal cavity was occupied by several of these bodies. They were surrounded by a finely granular substance which was strongly contrasted with the outer zone, this being very thin, but staining deeply.

I cannot say that all "Nebennucleoli" originate in this way. Occasionally one may be seen partly imbedded in the outer zone of the "Hauptnucleolus," and this may occur at any point where the outer zone is thickest.

Similar bodies are found distributed throughout the germinal vesicle (Pl. XIII, Fig. 15). In the living egg they appear as shining vesicles, often occupying diverticula of the germinal vesicle. They can also occasionally be observed in the cytoplasm of the living egg (Pl. XIII, Fig. 3; Pl. XVI, Fig. 112). As I have never seen them actually pass out from the germinal vesicle, I cannot say that they do so.

If the living egg is ruptured, and the contents made to flow out, they can be seen still within the germinal vesicle, and also in its neighborhood.

On a closer examination they are seen to be vesicles, consisting of a delicate membrane, within which are a number of granules, apparently suspended in a liquid. This can be made to flow out when the membrane is ruptured.

The "Hauptnucleolus" increases as the egg increases in size, and, in the third stage of the egg, may often reach gigantic proportions (Pl. XIII, Fig. 5; Pl. XVI, Figs. 104, 114). It is extremely variable. As a rule, it is spherical; but it may be perforated with holes and cavities. The center is often finely granular (Pl. XIII, Fig. 7). These granules may constitute the entire nucleolus, except a thin outer homogeneous membrane (Pl. XIII, Fig. 6).

Then again the center may be occupied by a relatively small, spherical, strongly refractive body, the outer zone being relatively uniform in thickness. Occasionally, this outer zone, surrounding the central body, is seen to be radially striated. The striae appear to be continuous with the network of the germinal vesicle.

Instead of a central body, there may be a central cavity in which nothing stainable appears to exist (Pl. XIII, Fig. 5). More frequently, however, the central cavity is filled with a network resembling the network of the germinal vesicle, excepting that the meshes are finer (Pl. XIII, Fig. 1; Pl. XVI, Fig. 107). As in the latter case, the fibers of the network are more or less covered with stainable granules, and the meshes between these fibers remain unstained. This caving in of the interior, so to speak, appears at times to continue till the nucleolus is nothing but a thin hollow shell (Pl. XIII, Fig. 6). This shell may be so large as to occupy nearly one-half of the germinal vesicle. Such cases, however, are not frequent. The interior of such a nucleolus is occupied by a chromatin network which in every way resembles the chromatin network of the germinal vesicle.

More frequently, in this stage of the egg, the nucleolus may be seen to have preserved a solid constitution even to the time when the germinal vesicle has reached the periphery. In most cases it is comparatively large, and stains more intensely than the rest of the germinal vesicle. Yet it is often completely honeycombed with little vacuoles. It often appears as if these vacuoles enlarge and flow together. The large nucleolus then appears like a system of variously connected, stainable strands of nucleolar substance, in appearance not unlike a coarse sponge.

In rare cases such a large, degenerated nucleolus is accompanied by another very much smaller, which does not show the signs of degeneration so conspicuously.

In eggs that have been discharged from the follicle into the ovarian tube no trace of the nucleolus could be observed. The last phases described seem to be stages of final dissolution, and absorption of the nucleolus. It seems that the discharge of the egg from the follicle marks its end, as the entrance of the egg into the follicle marked its beginning. Its history coincides with that period of growth of the egg in which the latter remains in organic connection with the parent organism. This would seem to associate it with the phenomena of nutrition and growth of the egg.

There are cases also, in this period of growth, in which there is no nucleolus in the germinal vesicle. Such cases occur when the germinal vesicle is surrounded by a zone of deeply staining granules, which resemble chromatin granules in their behavior towards haematoxylin and carmine stains (Pl. XIV, Fig. 29). Whether this is an abnormal condition, I cannot say. The appearances will be discussed more fully in connection with the cytoplasm. We have seen, also, that at the beginning there may be two similar nucleoli (Pl. XIV, Fig. 26), while later one of these has disappeared. In the second stage of the egg two nucleoli are rarely observed. But we have seen that towards the end, when the nucleolus has greatly degenerated, there may be a second smaller one apparently having recently arisen.

In view of these facts, together with its great variability, it is safe to say that it is not a permanent organ.

The appearances described above seem to show that the nucleolus is not simple, but composite. It consists of a framework of linin similar to that of the germinal vesicle, and a more or less homogeneous, semi-solid, stainable mass, which, accumulating at the nodes of the linin network, flows together into a spherical body, enclosing portions of the linin fibers. Within this mass chemical changes appear to take place which ultimately result in a substance resembling the yolk of the mature egg, and which, like it, assume the form of spherical refractive bodies. These when formed are extruded and give rise to "Nebennucleoli." The chemical or other processes within appear to continue; and the nucleolus, losing substance from within appears to receive additions from without. Thus a comparatively large hollow shell arises (Pl. XIII, Fig. 6). It would appear almost as if the addition from without is in the form of a precipitate, which becomes deposited on the surface of the nucleolus.

Owing to its relation to the linin network, which is often to be observed within it, the nucleolus may be considered as having a fixed position. Its movements within the germinal vesicle must necessarily be regulated by the linin fibers which constitute its framework. The "Nebennucleoli" appear to lie more or less free in the meshes of the network.

The main feature of both the "Hauptnucleoli" and the "Nebennucleoli" can be seen in the living egg (Pl. XVI, Figs. 106, 108, 115–118).

a. *Summary on the nucleolus.* — 1. The nucleolus appears at the time when the egg begins to grow.

2. It arises as an irregular or spherical mass in an amorphous stainable substance, surrounding the chromatin elements at the time when the germinal vesicle assumes its specific characteristics.

3. There is usually only one, but occasionally there are two in this early stage.

4. As soon as it has assumed a definite spherical form, it is differentiated into an outer zone enclosing a central body.

5. At first the entire nucleolus stains as readily as the chromatin.

6. The outer zone retains this power of staining, but the inner body gradually loses it.

7. The central body (endonucleolus or nucleololus) stains very intensely in Heidenhain's iron-haematoxylin. With double staining of the latter stain, combined with eosin, the entire nucleolus can be seen as a red outer zone and a black or blue central sphere.

8. This central body may become elongated, so as to protrude through the outer zone.

9. It is extruded from the nucleolus, which then appears as a hollow sphere with an opening at one pole.

10. Other similar bodies may form within, and these likewise are extruded.

11. These extruded bodies are the so-called "Nebennucleoli."

12. In carmine and Delafield's haematoxylin they stain feebly.

13. In the living egg they appear as shining vesicles, composed of a delicate membrane enclosing a fluid in which granules are suspended.

14. They have the appearance of yolk spheres; but as they arise at a time long before the yolk spheres are formed in the cytoplasm, they are not yolk spheres.

15. Similar bodies are seen in the cytoplasm at this stage, but they are not permanent.

16. The part remaining after this extrusion retains its power of staining in carmine stains, and may be designated the nucleolus.

17. This often has the form of a crescent.

18. The interior of this, in rare cases, contains no stainable substance, and appears as if it might be a fluid vacuole.

19. More frequently the interior is occupied by a linin network like that of the germinal vesicle, and, like it, having stainable granules imbedded in it or attached to it.

20. The crescent-shaped or circular nucleolus appears to lose substance from within, and to receive substance from without.

21. It may thus become a large hollow shell of stainable substance enclosing a reticulum.

22. The entire nucleolus may often appear as a spherical mass of granules enclosed by a homogeneous membrane.

23. These granules may sometimes be scattered, and lie imbedded in a homogeneous mass resembling the outer membrane. The granules, when they become refractive and lose their power of staining, may be mistaken for vacuoles. These, however, can be stained intensely in Heidenhain's iron-haematoxylin, so that a dark body appears to lie in an unstained vacuole.

24. When only one of these granules exists, it may occupy the center of the nucleolus.

25. Occasionally the outer part can be seen to be radially striated, so that when the central body is present the nucleolus has the main features of a centrosome and a sphere.

26. Such a structure is sometimes to be observed in addition to a second homogeneous nucleolus.

27. When the central body exists, there may be a network surrounding it, which in turn is enclosed by the outer layer of the nucleolus.

28. In such cases the nucleolus has all the features of a germinal vesicle, with nucleolus network and nuclear membrane.

29. The nucleolus can be seen as long as the egg remains in the follicle, but not after its discharge.

30. In the later stages it is often very large and stains deeply.

31. It may, however, become honeycombed with large openings.

32. In such a condition it may be accompanied by a very much smaller one, apparently more perfect, and, like it, staining deeply. This distinguishes it from the somewhat numerous "Nebennucleoli" that are spread throughout the germinal vesicle, and in carmine stains have a yellow coloration.

33. The nucleolus may, therefore, consist of three different constituents: (*a*) linin framework; (*b*) substance resembling chromatin; (*c*) substance resembling mature yolk globules.

34. Movements of the nucleolus are regulated by the linin framework which permeates it.

35. The nucleolus disappears as such when the egg is discharged from the follicle, and when, as we shall see, an entirely different process of growth of the egg takes place.

36. The history of the nucleolus coincides with the period of growth of the egg, *i.e.*, while it remains in organic connection with the parent organism.

b. *Literature.* — An extensive literature on the nucleolus exists, from which many similar observations could be cited. We are reminded at once of the observations of Balbiani ('83) and Aimé Schneider ('75).

I cannot accept Rhumbler's ('93) mechanical explanation of the radial feature of the nucleolus, nor his equally mechanical explanation of the endonucleoli.

Balbiani's explanation of an extruded body in eggs of Geophilus, as being a tube, would lose much of its incredible features if the term tube had not been applied to it. The observations themselves are no doubt correct, but his figures are as diagrammatic as his language is colored by a vivid imagination.

Space will not permit an extended consideration of the many problems concerning the nature and function of the nucleolus. Most of them are well known, and the bearings of these observations will be readily perceived. I would refer the reader to the following authors: Cramer ('48), Ludwig ('74), v. Wittich ('49), Leuckart ('53), Pflüger ('63), Will ('86), Rhumbler ('93), Balbiani ('83), Aimé Schneider ('75), Valentin Häcker ('95), Gegenbaur ('61), Stuhlmann ('86), Bumpus ('91), Waldeyer ('88), Leydig ('55), Brandt ('78), Korschelt ('89), La Valette St. George ('66), Gustav Mann ('93), McFarlane ('92), Goette ('75), Balfour ('78), Henking ('82), O. Hertwig ('77, '92), Flemming ('75), Auerbach ('74), R. Zacharias ('87), Scharff ('88), Mertens ('93), Klein ('78), Holl ('93), Jordan ('93), G. R. Wagener ('79), Platner ('86), Wielowiejski ('85).

Connection of the Egg with the Ovarian Tube.

Before the formation of the egg membrane the cytoplasm of the egg is continuous with the cytoplasm of the epithelium of the ovarian tube. At times the neck of the egg, by which it is joined to the epithelium, is comparatively large, so that the epithelium appears to lie in direct contact with the egg over a considerable area; and its cytoplasm appears continuous with several of the epithelial cells. In most cases the neck of the egg is constricted to a narrow bridge of fibrous protoplasm, proceeding from the epithelial cells and continuing into the egg body as a polar mitosome. This polar mitosome can often be seen to be continuous with a modified layer of protoplasm surrounding the egg immediately under the investing tunic; and it can also be seen to spread out in a fan-shaped manner in that part of the egg adjoining the stalk. In a few cases the parallel fibers of which the mitosome is composed have been seen to be connected with a body lying close to the germinal vesicle, the nature of which will be considered later. The polar mitosome is perhaps a remnant of the spindle of the last division of the oögonia, comparable to a similar structure observed by Platner ('86) in the sperm cells of Helix, and by Bolles Lee ('95), or to the Zellkoppel of Zimmermann ('91).

At the junction of the egg with the epithelial cells these fibers are firmly bound together by a body which is prominent in the younger eggs especially, and which stains deeply in acid fuchsin and eosin. In Heidenhain's iron-haematoxylin it stains very deeply, somewhat like the peripheral bodies, which are to be considered presently. If haematoxylin be followed with eosin, it appears as a doubly convex, bright-red body. In many cases sections through its center show the form of a ring. In the younger eggs it is large and conspicuous. It resembles very closely the so-called "Zwischenkörper" of Flemming ('91), which by him was homologized with the cell plate in plants. A similar body was observed by van Beneden in the egg of Ascaris, by Hertwig in Spirochona, by Carnoy ('85) in the spermatocytes of Arthropods, and by Henking ('91) in the spermatocytes of insects. This body does not perhaps differ materially from the numerous peripheral bodies which later make their appearance, and which, as we shall see, give rise to the first layer of the egg membrane. As the latter seem to be aggregations of little spherical bodies, which are the first indications of a forming egg membrane, so this polar body appears likewise to be a concentration of such granules.

The cytoplasm. — The cytoplasm consists of at least two distinct elements — a living formed element and a non-living amorphous element (Watasé). The former has the form of a reticulum of variously interwoven fibers, which show a centralized arrangement at the center of the egg. This will be discussed more fully in connection with the attraction sphere and centrosome. The living substance appears to have many of the characteristics of a sponge, in the lacunae, vacuoles, and meshes of which the various amorphous elements are lodged.

The yolk. — The yolk lies either massed together at different points, or else uniformly distributed throughout the egg.

It occupies the meshes of the cytoreticulum, and appears to be movable from one point to another, according to the condition of the controlling living substance. It is, therefore, subject to changes in mass, giving considerable variation to the appearance of the cytoplasm. It must be pointed out here that the unequal distribution of the yolk, as well as the variable condensation of the living substance in different parts of the egg, which is frequently to be observed, does not alter the spherical form of the egg. It is hardly probable, therefore, that the spherical form is due to surface tension, which implies an equilibrium of similar molecules in all radii.

In the younger eggs the amount of yolk varies considerably. It may be so abundant as to obscure the cytoreticulum, or it may be very limited in amount. It has been shown that the earliest formed eggs grow more rapidly than those formed in the period preceding sexual maturity. In the latter the yolk is sometimes relatively scarce, and the cytoreticulum is very distinct. In such cases the nutriment of the egg is presumably so limited that the surplus food material is used up in the growth of the living substance. At any rate, it is certain that the egg increases in size by the growth of the living substance, and by mechanical expansion due to the accumulation of yolk. The growth of the cytoreticulum predominates in the earlier stages, while the accumulation of yolk is the chief cause of increase in size after the eggs are discharged from the follicle.

It has been maintained that the yolk originates in all cases within the egg, and it appears to be with considerable reluctance that many, even now, admit the origin of yolk in any other way. This reluctance seems to date back to the early controversy regarding the cell nature of the egg. On the one side it was claimed that the yolk spheres represent real cells; on the other, that the yolk originates within the egg.

An external origin of the yolk has frequently been maintained. I need only mention Ayers ('84), confirmed by L. Will ('84), in the egg of Oecanthus niveus.

There are reasons for believing that in Limulus a substance having the essential characteristics of yolk is produced in the epithelial cells; and that this, in the form of granules, suspended in a fluid, enters the vacuoles and meshes of the living substance of the egg.

At the time when the first layer of the egg membrane is formed it is sometimes seen that part of the yolk is not included within the membrane, but is cut off and remains outside in that portion next to the stalk of the egg (Pl. XIV, Fig. 22, *y.s.*). The yolk lying outside of the egg in such cases is often considerable in amount, and resembles in every particular the yolk inside of the egg. Two explanations of this appearance suggest themselves, which, although appearing different, may be essentially the same. In the first place, it is safe to assume that the first layer of the egg membrane arises at the extreme limits of the formed, living substance of the egg. Now it may be suggested that in such cases as those under consideration the amorphous elements of the egg extend beyond the outer limits of the living protoplasm, and thus become cut off when the membrane arises. Or it may be that the yolk granules from the epithelial cells, being prevented from entering by the membrane, accumulate outside, later perhaps becoming dissolved and serving as food. It is suggestive that at this stage in the history of the egg, when the yolk granules from the outside are no longer capable of entering as solid bodies, the cytoplasm of the egg undergoes that peculiar change from an alkaline to an acid state of reaction.

The yolk spheres appear in their vesicular, clearly defined form only after the egg has been discharged from the follicle. Previous to this event several of the epithelial cells of the egg stalk appear to degenerate and break up into granules that have all the appearances of the yolk granules of the ovarian egg. On the first appearance of the definite yolk bodies they are small. Those first formed increase in size, and thus in somewhat later stages the yolk bodies may show many different sizes. Ultimately, however, they all attain to a considerable size and fill the egg completely. There can be no doubt that these yolk spheres originate within the egg. Their formation appears to be in some way associated with the new mode of nutrition of the egg after its arrival in the ovarian tube. As previously stated, it is here bathed in the secretion of the cells lining the ovarian tubes. These contain considerable quantities of such secretions.

The disintegration of cells above referred to recalls forcibly the condition in milk glands, as related by Foster ('93), and also the account given by Nissen ('86).

Polarity of the egg.—Adopting the terminology of Auerbach ('96), we have also here a "Kernpol" and a "Gegenpol." The archoplasm and centrosome determine the position of the "Gegenpol." Apparently this is the vegetative pole, for in later stages it becomes especially granular.

At the nuclear pole a hyaline area appears usually in the third stage of the egg (Pl. XVI, Fig. 104). This is often irregular, at times crescent shaped, but not sharply defined from the rest of the cytoplasm. It may partly enclose the germinal vesicle, the horns of the crescent gradually merging into the compact area at the vegetative pole. It often presents a striking similarity to the hyaline area figured by Andrews ('91) in the egg of Diopatra, and recalls the polar rings in the eggs of Clepsine and of Allolobophora observed by Professor Whitman ('78) and Miss Foot ('96), respectively. I do not know how far it could be compared to the polar differentiation observed by Mark ('90) in the ovarian egg of Lepidosteus, which would seem more closely related to the observations of Stauffacher ('93) in the ovarian eggs of Cyclas. Possibly these bodies are more closely related to the "Zwischenkörper" described by me in connection with the egg membrane.

For reasons which are discussed in connection with the cytoplasmic zones and yolk-nucleus, I consider this area due to an infiltration of substance derived from the germinal vesicle. Its position is evidently determined by the relative positions of the germinal vesicle and the sphere. It always appears opposite the vegetative pole. A line might be drawn through the vegetative pole, the germinal vesicle, and the nuclear-pole area (Pl. XVI, Fig. 104). This line, however, would not always pass through the point of attachment of the egg where the "Zwischenkörper" is formed. (See Plates.) The above statements hold true of all stages of the egg from the beginning of growth. (See Pl. XIV.)

As the cytoplasm increases at the vegetative pole, the nuclear pole becomes more and more crowded, till it, with the germinal vesicle, spreads out over the surface of the vegetative pole as previously described (Pl. XVI, Fig. 104; Pl. XIII, Figs. 11, 14).

It is to be remembered that no true yolk spheres exist yet, for these appear only after the discharge of the egg into the ovarian tube (Pl. XIII, Figs. 12, 17).

This process, it seems to me, has some of the features of gastrulation by invagination. The animal portion, less laden with food material, finally comes to lie externally to the vegetative portion. The gastrulation, therefore, might be said to take place previous to fertilization, or even to yolk formation, and the cleavage by delamination, described by Kingsley and also suggested by Brooks ('85) and Bruce ('85), might be regarded as only a continuation of these early conditions. The appearance after the discharge of the egg of a large accumulation of yolk spheres obscures these relations. Yet it is difficult to escape the conviction that a relation of some sort exists between these later developmental processes and the conditions that are found to exist even at the beginning of the period of growth of the egg (Pl. XIV, Figs. 42–47). According to both Kingsley and Brooks, the development of the fertilized egg of Limulus is peculiar in that the first evidence of cleavage appears only on the surface. From what I am able to gather from the accounts of these writers, this division into cells is a secondary matter, the whole egg ultimately being converted into an embryo.

The polarity of the ovarian egg of Limulus is not a matter of chance. It is not acquired during the growth of the egg, but it dates from the beginning. (See Pll. XIV–XVI.) The germinal vesicle alone does not constitute this polarity; for, as has been shown, the centrosome with its cytoplasm exists from the very beginning.

In the history of the germinal vesicle, more particularly the chromatin, I find nothing on which to base the assumption that it is the ovigenic element, and that it is this which presides over all the formative processes. I find no evidence that the chromatin is the basis of the structure that underlies these polar differentiations. As a matter of fact, the chromatin of the germinal vesicle seems to vanish when the metabolic processes, concerned with the elaboration of food, are at an end.

The peculiar arrangement of the chromatin in the spireme stage, and the various phases of karyokinesis (Pl. XIV, Figs. 33–37) seen in the dividing oögonia do not appear to me to be evidence of an organization existing in the chromatin, but rather an orderly arrangement of an inert mass, brought about by a structural basis which is common to the cytoplasm and nucleus alike.

The division of the oögonia is manifestly a division of the structural basis of both the nucleus and the cytoplasm, and the orderly separation of the chromosomes is due to an orderly separation of the spindle fibers. These spindle fibers I can regard as nothing else than the reticulum of the cytoplasm and the reticular basis of the nuclear network combined. The vital manifestations reveal themselves, not in the passive chromosomes, but in the centrosome, and in the network of which it is a part. The entire history of the chromatin offers nothing on which to base the assumption that it is the controlling element.

The uniaxial feature, which is so prominent in the spindle stage of the dividing oögonia (Pl. XIV, Figs. 36–38), continues to exist throughout the history of the ovarian egg, and can be accounted for only by the assumption of a continuity of structure. It is inherent in the living matter of the egg. Dr. Eycleshymer ('95) has reviewed the literature on this subject, and has tested the relation of the polarity in the amphibian egg to cleavage and to the orientation of the embryo.

Peripheral bodies and yolk-nuclei. — On the same slide can be seen the wide contrast between the eggs that are still in the first period and those that have entered upon the second period. In haematoxylin the former are dark blue, while the latter are a very light blue. If the slide be dipped into picric acid, previous to mounting in balsam, the former are not affected, while the latter have yielded their former stain for the new. The same result is obtained by means of borax-carmine followed with picric acid. With the double stain of erythrosin and cyanin the same difference can be observed, the eggs in the first stage taking the blue, those in the second taking the red stain. Lithium-carmine and Lyon's blue show the same peculiarity; the eggs in the first stage, in this case, taking the red carmine stain, those in the second stage taking the blue.

While this change in the cytoplasm is in progress, there is a period when portions have undergone the change, while other portions remain in the former condition. In such cases it frequently happens that in haematoxylin stains, while most of the cytoplasm takes a light-blue stain, round, dark-blue bodies resembling nuclei are found scattered through the cytoplasm (Pl. XIV, Fig. 27, *y.n.*). These may easily be taken for nuclei. They, however, disappear as soon as the critical line dividing the first and second period is reached.

I call these yolk-nuclei.

Another class of bodies, which I shall call peripheral bodies, are scarcely less puzzling when first observed. In many cases during the first period in the history of the egg, deeply stainable bodies resembling nuclei are found along the extreme border of the egg (Pl. XIV, Figs. 25, 28; Pl. XV, Figs. 66, 67, 77). They are often regularly arranged at equal distances from each other, and always immediately under the surrounding tunica propria or follicular membrane. They stain deeply in Ehrlich's, Delafield's, and Heidenhain's haematoxylin, as well as in carmine and safranin. They do not, except in some rare cases, have the clear outlines of nuclei, but seem rather diffuse (Pl. XIV, Fig. 28). A comparison of a tangential and transverse section shows them to be round discs with one flat side and one convex side, turned inward (Pl. XV, Figs. 79, 90). They are studded with regularly arranged shining dots (Pl. XIV, Fig. 28). At the end of this period the first layer of the chorion is formed. The nature of these bodies becomes evident when the formation of the chorion is observed (Pl. XIV, Fig. 25).

The egg membrane. — During the first stage of the egg, its only covering is that formed by the tunica propria, which, as has been shown, is probably a product of the epithelial cells, being a secretion of the protoplasm of the basal end of these cells (Pl. XIV, Fig. 19, *t.p.*).

The larger ovarian eggs offer excellent opportunities for the study of this tunica propria. When removed from the egg, and viewed in optical section under the microscope, it is seen to be a homogeneous membrane, without cell boundaries and without nuclei. It is, however, studded with closely-set shining dots, as if perforated with closely-set pin holes (Pl. XIII, Fig. 18, *p.c.*). The nature of these becomes sufficiently evident when the formation of the egg membrane is examined.

There being no true follicle epithelium surrounding the egg, the coverings, which in later stages make their appearance, arise from the egg itself. On account of the considerable development of this covering, I shall follow Packard and Kingsley and call it the chorion, being aware that, according to the nomenclature adopted by Ludwig and van Beneden, we should be obliged to call it the vitelline membrane.

The chorion is a product of the egg. In the living egg it has a semi-solid consistency and offers considerable resistance to pressure. It may be ruptured by inserting a needle and severing it in that way. In so doing, it may be drawn out to a sharp point somewhat like india-rubber; but, unlike rubber, it does not return to its former position. Fresh eggs examined in glycerine or normal salt solution often show the formation of extraovates, without the rupture of the membrane.

Examined in the living state, the chorion is seen to consist of one or several concentric layers according to the size of the egg (Pl. XIII, Figs. 12, 13, 17, 18). Each layer is uniform in thickness; but considerable variation may exist between the different layers. The layers appear to consist of a dense substance, between every two layers of which there is a thinner lamella of lighter, apparently less dense substance. These layers of darker and lighter substance are not clearly separated, but grade into each other. Preserved in most hardening reagents, the chorion becomes hard and brittle, offering in the mature egg considerable resistance to the entrance of paraffine or celloidin. In such preparations the lamellae may also be seen;

but they are now separated by clear-cut lines, the less dense, intermediate, lighter layer having apparently been converted into very narrow crevices. In all cases the outer layer, which is the first to originate, differs from the other layers (Pl. XIII, Fig. 18).

Both in the living egg and in the preserved material studied in sections the chorion is seen to be traversed by radial striations (Pl. XIII, Figs. 12, 17). These are closely set and perfectly parallel. In the different concentric layers the radial striations coincide as if continuous one with the other. The striations extend even into the outer or first layer of the chorion (Pl. XIII, Fig. 18).

The first layer of the chorion arises at the end of the first period, and seems to mark an important epoch in the history of the egg, inasmuch as it is at this time that the cytoplasm loses its affinity for haematoxylin, borax-carmine, and other chromatin stains.

It arises in the form of peripheral bodies, which are scattered at regular intervals over the surface of the egg, immediately under the investing tunic (Pl. XV, Figs. 66, 67, 77, 79, 90). These bodies appear at first as minute dots which increase in size, and stain deeply in chromatin stains. They are often so regularly arranged as to be easily mistaken for nuclei. As they grow, two or three may coalesce, forming a conspicuous body at the periphery of the egg (Pl. XV, Fig. 67). As they increase in size by coalescence, they gradually lose their affinity for chromatin stains, and eventually all blend into the first layer of the chorion which, when formed, does not stain readily (Pl. XIV, Fig. 25, *ch.*).

This first layer of the chorion sometimes appears to arise as a continuous layer instead of in patches, as described above (Pl. XIV, Fig. 20). The first indications of its appearance in such cases are not the peripheral bodies, but a layer of deeply staining dots just under the primary tunic. The granules are not concentrated into larger isolated bodies, but are spread out uniformly.

The history of the origin of the subsequent layers of the chorion is a different one, although the process in itself may be essentially the same. Immediately beneath the first layer of the chorion a homogeneous layer of protoplasm appears (Pl. XIII, Fig. 13), which does not stain so deeply as the more granular protoplasm which it surrounds. In favorable preparations this layer appears to be composed of fibers which resemble those of the cytoplasm except in the absence of the conspicuous cyto-microsomes. The fibers are at first arranged more or less perpendicularly to the surface of the egg (Pl. XIII, Figs. 13, 18). At this stage they present the appearance of regularly arranged cilia, covering the surface of the egg, and they are imbedded in a transparent substance which solidifies into the inner layer of the chorion.

The radial striations so conspicuous in the chorion of Limulus eggs are therefore due to protoplasmic fibers. Originally, at least, the radial striations are not due to radial pores, as is so frequently asserted of other eggs. The process appears to be practically similar to the formation of chiton and other cuticular substances by the fusion of cilia. The shining pores previously mentioned in connection with the primary egg covering and the peripheral bodies (Pl. XIII, Fig. 18; Pl. XIV, Fig. 28) are either transverse sections of these fibers or their points of insertion. As we have seen, the outer primordial covering, — tunica propria, — which is the original basement membrane of the germinal epithelium, arises in essentially the same way as a cuticular hardening of the outer ends of the epithelial cells.

The peripheral bodies, which in their earlier stages resemble nuclei, call to mind the so-called "Binnen" epithelium of Eimer ('72), which figured so prominently in the discussions concerning the cell nature of meroblastic eggs, and may possibly explain the much disputed observations of Clark ('57) in the ovarian egg of the turtle. They may possibly be compared to the bodies observed by O. Schultze ('87) and Goette ('75) in the peripheral layer of amphibian eggs, and I believe they serve to explain the observation of Schütz ('82) in the egg of spiders. So far as I am aware, he is the only student of those eggs who has claimed the existence of a follicular epithelium surrounding them. Bruce ('85) and Brooks ('86) have shown that the "inner egg membrane" mentioned by Packard ('71) is the protoderm, the "rudely hexagonal cells" of Packard being the casts of the ends of the blastoderm cells.

ZONES AND YOLK-NUCLEUS.

The cytoplasm is often divided into two distinct zones — an outer and an inner zone (Pl. XIV, Figs. 19, 21, 24, 29). These two zones are often separated by a distinct line suggesting the presence of a membrane between them (Pl. XIII, Fig. 16). In other cases the zones are separated by a line of large microsomes (Pl. XIV, Fig. 21). This line may run at a uniform distance from the germinal vesicle (Pl. XIV, Fig. 24; Pl. XIII, Fig. 16), or it may be extended at the proximal pole towards the stalk (Pl. XIII, Fig. 16). Instead of this line of large microsomes, the zones may be separated by parallel fibers resembling those of the polar mitosome. In this line the vitelline-body or sphere may sometimes be observed (Pl. XIV, Fig. 21).

The inner zone at times appears less granular than the outer zone, *i.e.*, the granules appear smaller, making the inner zone less stainable than the outer zone (Pl. XIV, Fig. 24). In other cases this inner zone consists of large, irregular, closely packed granules that stain intensely in haematoxylin and other chromatin stains, the outer zone staining less deeply (Pl. XIV, Figs. 19, 29). This form is of frequent occurrence, and is found in the best preserved material, on the same slide with other eggs showing no trace of it.

In the living egg of a half-grown specimen these two zones can be distinctly recognized (Pl. XIII, Fig. 16). In this case the outer zone is translucent, with the exception of a few scattered granules. The inner zone, very sharply separated from the outer zone, is opaque. The germinal vesicle in this case contains a distinct nucleolus. The inner dark zone may form a regular circle around the germinal vesicle (Pl. XIII, Fig. 16, *b*), or it may be extended on the proximal side towards the stalk of the egg, where it seems to become continuous with the protoplasm of the epithelium of the stalk (Pl. XIII, Fig. 16, *c*).

As these eggs were taken from the ovary of the living animal, and examined at once in the fluids of the ovary, the effects of reagents cannot be considered responsible for these appearances.

An examination of the same eggs in a living condition shows also the vitelline-body, or sphere, and its intimate connection with this peculiar inner zone of the cytoplasm (Pl. XIII, Fig. 16, *a*). Pl. XIV, Fig. 21, shows a similar egg in section. The sphere is seen to bear a close relation to the dividing line between the two zones, on the one hand, and to the polar mitosome, on the other.

Eggs showing these zones were preserved and mounted entire. The main features are excellently preserved.

General considerations. — The division of the body of the egg into an outer and an inner zone has frequently been observed in other eggs. Among others by Pflüger ('63), cat; Cohn ('56), rotatorian; O. Schultze ('87), Bambeke ('75, '83), frog; Waldeyer ('70), bird; His ('73), fish; Will ('86), Korschelt ('89), insects; Holl ('90), Leuckart ('53), chick; Ludwig ('74), echinoderms; Lancaster ('75), molluscs; K. Schulin ('81), bat and human; Henneguy ('93), fish; Goette ('75), bombinator; J. V. Carus ('50), spiders; Leuckart ('53), Scharff ('88), Eimer ('72), Ransome ('67), fish.

The zones in Limulus eggs present all the essential characteristics of the figures given by the above observers, and may also be seen to undergo many of the modifications that have been observed in other eggs; for example, by Pflüger.

A so-called free space around the nucleus of ordinary cells has also been described; among others, by Leydig ('88) and Brass ('83). A zone around the nucleus of sperm cells has recently been observed by Auerbach ('96).

In the case of eggs the zones are usually considered as being connected with the phenomena of growth and nutrition; but the manner in which they arise is a disputed question.

Leydig and Auerbach take different views. Leydig interprets the inner zone as a free cavity in the cell, into which the nucleus has crowded by a process of budding from the cytoplasm, to which it remains intimately connected by means of a narrow bridge or stalk. Auerbach, on the other hand, regards it as the expression of a condensation of the cytoplasm, which takes place previous to the division of the cell, and which ultimately results in the formation of a spherical body, the sphere, or "Nebenkern."

Such an explanation of the zone around the germinal vesicle in the egg of Limulus cannot be offered, inasmuch as it appears during the period of growth of the egg, when it has ceased to divide, and often after it has attained to a considerable size. This appears to be true in the fish egg also, according to the observations of Scharff. He considers the outer zone as corresponding to the "Rindenschicht" of Eimer, which the latter believes to be identical with the "Zonoid schicht" of His.

One objection to applying Auerbach's interpretation to the inner zone of the egg is the sharp line which in certain stages separates the two zones (Pl. XIII, Fig. 16). This feature is peculiarly striking in the living egg of Limulus, and is especially emphasized by Pflüger as observed in the egg of the cat, and appears to have attracted the attention of Schulin in the egg of the bat and in the human ovum, and also of Will in the egg of *Colymbetes fuscus*. It is, of course, difficult to say what effect a condensation might have.

The changes of the inner zone from a hyaline to a granular condition, observed by Pflüger in the egg of the cat, and which is so evident in the egg of Limulus (Pl. XIV, Figs. 19, 24), appear to be evidence of chemical changes taking place in the interfilar amorphous substances immediately surrounding the germinal vesicle. This may, of course, be accompanied with an increased condensation of the reticulum; but it would seem that the latter would be more apparent than real, and due rather to the increase in the amorphous granules. The sharp limitation of the inner zone in certain phases of the contained granules, it seems to me, points to differences in composition of the amorphous or granular matrix in which the cytoreticulum lies (Pl. XIV, Fig. 48).

In endeavoring to account for the existence of this inner zone, there are four important elements which demand attention.

If we reject the explanation of Leydig, and also that of Auerbach, how shall we account for the first feature of this zone, *i.e.*, the hyaline stage? Certain features that I have noticed in connection with the germinal vesicle and nucleolus appear to throw light on this question. It was seen that the nucleolus extrudes bodies in the form of vesicles, consisting of a membrane within which is a fluid containing granules (Pl. XVI, Figs. 110–113). These vesicles, which I have called "Nebennucleoli," were seen to vary in number, and apparently to appear and to disappear. There is reason for believing that these vesicles lying in the meshes of the nuclear reticulum finally dissolve, and add their contents to the nuclear sap, or karyo-lymph. Where they happen to lie near the periphery of the nucleus, the discharge of their contents extends the area of the germinal vesicle in that direction, thus causing pouches of the nuclear wall (Pl. XVI, Fig. 105; Pl. XIII, Figs. 3, 6–8). The nucleus, to all appearances, is not bounded by a solid wall, but by a special arrangement of the cytoreticulum.

It has been seen that the germinal vesicle, having become greatly extended, more or less regular in outline, may in another phase of activity become amoeboid, greatly contracted, and apparently devoid of karyo-lymph (Pl. XIII, Fig. 5). In all such cases a hyaline area is found to exist outside the germinal vesicle, apparently caused by the entrance into the cytoplasm of the karyo-lymph, which now becomes either a hyaline zone around the germinal vesicle, or in later stages appears as the polar area referred to in another place (Pl. XIV, Fig. 24; Pl. XVI, Fig. 104).

It would appear that this hyaline zone may become lost in the cytoplasm, in which it becomes diffused throughout the interfilar spaces. We may now consider the question of the origin of the internal granular zone of the egg.

The definite yolk spheres appear only after the egg is discharged from the follicle into the ovarian tube. It is hardly necessary, therefore, to consider the explanation of an internal zone in the egg of the fish offered by His, as his well-known theory of migrating granular cells would not apply in this case, and, so far as I know, has never been seriously considered in recent years. The theory of Waldeyer also offered in explanation of two zones in the cytoplasm of the bird's egg, and extended also to the observation of Pflüger in the egg of the cat, namely, the direct apposition to the primitive egg cell of the outer zone, conceived to be derived from the follicle epithelium, does not apply in the present case, inasmuch as no true follicle epithelium can be said to be present. Equally inapplicable is the somewhat vague but decidedly radical theory advanced by Leuckart ('53) in regard to all eggs, and the somewhat similar though more metaphysical theory advanced by Balbiani ('83), according to which, in myriapods, the egg consists of a "partie germinative fondamental" and of a "partie nutritive," each of these parts being constituted "isolement et pour son propre compte." The objection to such an explanation would be that in the egg of Limulus the division of the cytoplasm into an outer and inner more or less granular zone is not a constant, but a periodical feature.

It remains to be considered how far the direct elimination of chromatin from the germinal vesicle into the surrounding cytoplasm can explain the existence of the internal granular zone. Such an explanation has been offered by Will in eggs of amphibians and insects, by Bambeke ('93) and by Calkins ('95) in the egg of Lumbricus. Calkins's observations receive increased interest and importance from the evident acceptance of his results by Professor Wilson ('96) in his new work on the cell, and the evident stress which the latter author places upon it in his theory of synthetic metabolism of the chromatin of the germinal vesicle. In addition to the doubt cast upon Calkins's results by the observations of Miss Foot ('96) on the eggs of a closely allied species, Allolobophora, from which she seems inclined to believe that Calkins's results were obtained from pathological material, the following considerations may be urged against the sufficiency of the theory of elimination of chromatin to explain the internal deeply staining zone: 1. It does not explain the existence of the hyaline zone, which appears to precede or to follow the granular condition. The existence of the hyaline zone proves that the zone is not due to the extruded chromatin granules. 2. The elimination of chromatin granules from the germinal vesicle cannot explain the extension of the inner granular zone towards the point of attachment of the egg, as it can be seen both in sections and in the living egg of Limulus (Pl. XIII, Fig. 16). 3. Contrary to the results of Calkins, the internal granular zone does not behave like ordinary nuclear chromatin towards the Biondi-Ehrlich triple stain. In the degenerating eggs described in another chapter, the chromatin that is found in considerable quantities in the cytoplasm, and having the form of nuclei, does take the green stain with the Biondi-Ehrlich mixture (Pl. XIV, Figs. 30, 31).

I can find no reason, therefore, for concluding that the deeply staining granules of the inner zone are eliminated chromatin granules.

On the other hand, it has been shown that the epithelial cells of the ovarian tube and of the egg stalk secrete a substance which, under the influence of reagents, becomes granular, and that this substance is seen at times to accumulate at the point of attachment of the egg (Pl. XIV, Fig. 22, *y.s.*). From a consideration of the foregoing objections and the facts presented by such appearances as are represented in Pl. XIII, Fig. 16, and Pl. XIV, Figs. 19, 20, 22, as well as from the figures of Korschelt ('89) in the case of insect eggs, I conclude that this secretion enters the egg and is carried along toward the germinal vesicle, where, acted upon by the hyaline karyolymph derived from the "Nebennucleoli," it becomes converted into a stainable substance which I will call *metaplasm*.

While I have called the extension of the inner zone towards the stalk of the egg a channel, I do not mean to imply by that term that it is a tube in the sense in which Balbiani ('83) used the term in the case of the egg of Geophilus. It appears to be rather the expression of the existence of an interfilar fluid or substance, which, for the time being, does not mix with the surrounding hyaline cytoplasmic matrix, and which is especially favorable for the entrance and chemical modification of the crude food material which serves the egg as nourishment. I would not therefore regard the inner hyaline zone as an open space in the sense of Leydig, nor as a cytoplasmic condensation in the sense of Auerbach, nor as a funnel-shaped tube in the sense of Balbiani, but rather as an interfilar digestive fluid in the sense of Scharff, without the bodily migration of nucleoli as held by him; the granular phases being due to the entrance of food material, as held by Korschelt in the insect egg, and being the result of a combination of a nucleolar product with the nutritive material.

The result of this combination is the metaplasm which later becomes distributed throughout the body of the egg or else collected around the centrosome and sphere.

It is evident, however, that the internal zone is closely related to the centrosome and sphere; and our next problem will be to consider what this relation may be. A relation of some kind has been pointed out by Balbiani, Bambeke, Oscar Schultze, Henneguy ('93), and Auerbach, and is evident from their figures as well as from Pl. XIII, Fig. 16; Pl. XIV, Figs. 19–22, 24, 48, in the egg of Limulus.

In the case of the sperm cell, Auerbach considers the spherical "Nebenkern" as a further condensation of the inner zone. He, however, can give no reason why such a condensation takes place at one place rather than at another; and if I understand him rightly, he does not insist on a structural relation of the cytoreticulum that might serve as a basis for such a condensation.

In the case of the other observers mentioned above, it would seem that they, in a somewhat similar manner, regard the body — vitelline-body of Balbiani — as a fortuitous aggregation of the granules of the internal zone, the granules being regarded either as extruded chromatin or as disintegrated migrating nucleoli.

Against such a fortuitous aggregation can be urged the observation of Balbiani himself, in the case of Geophilus, where a structural body in the form of a centrosome, sphere and aster, is clearly figured and described, in the midst of amorphous granules supposed to be derived from the germinal vesicle. Balbiani does not prove that this sphere and aster originate from the amorphous granules. Furthermore, Mertens ('93) has conclusively shown that a centrosome and sphere exist, apparently independently of the granules derived from migrating nucleoli.

In the egg of Limulus I have conclusive evidence of the existence, both in earlier and in later stages, of a centrosome and sphere in the midst of the granules of the inner zones (Pl. XIV, Figs. 21, 47, 48); and as will appear from the following considerations of that body, I believe that this is the structural basis around which the metaplasmic granules of the inner zone collect and give rise to the conspicuous body known as the vitelline-body of Balbiani. In that way I would account for the appearances in the living egg represented in Pl. XIII, Fig. 16, *a*.

The Centrosome and Sphere (Vitelline-Body).

As soon as the growing egg can be distinguished as such, there may be seen in the cytoplasm, in the immediate neighborhood of the germinal vesicle, a body which differs from all other parts of the egg in its staining reactions (Pl. XIV, Figs. 34, 38, 40). It is brought prominently into view by means of Heidenhain's iron-haematoxylin; by the Biondi-Ehrlich mixture; Weigert's picro-carmine; Delafield's haematoxylin, either alone or followed with picric acid; by means of borax-carmine and picric acid; Ehrlich's haematoxylin and acid fuchsin or eosin; by erythrosin, either alone or followed with cyanin; and finally by means of Lyon's blue and lithium-carmine or safranin.

When first observed, it has the form of a crescent closely applied to the germinal vesicle. In the latter double stain it may be made very conspicuous, being differentiated from all other parts of the egg, both germinal vesicle and cytoplasm. These stain red, while the crescent stains a bright blue. In the widest portion of the crescent is a clear area containing central granules (Fig. 42).

This central area, with its granules, appears to be the essential part of the body, inasmuch as it is this which continues to exist in various forms as the egg continues to grow. The horns of the crescent seem to disappear early (Figs. 42–48).

A high magnifying power shows the body to consist of the following parts: first, a round central body, surrounded at a slight distance by a circle of microsomes (Fig. 45). From this circle radial fibers pass out in all directions to another circle of rather large microsomes (Fig. 47). Outside of this again a dense layer exists, which can be seen to consist either of granules or of closely interwoven fibrils, which radiate out into the cytoplasm, and send larger or smaller strands out along the outer wall of the germinal vesicle. In Weigert's picro-carmine, in which the circles of microsomes, as well as the dense fibrous layer, are very distinct, the central granule is not visible. This, however, can be distinctly seen in the carmine and Lyon's blue (Figs. 42–44). After the disappearance of the horns of the crescent, the body appears essentially as before. In Lyon's blue it is a large blue mass of fibers, with a somewhat darker center, situated close to the germinal vesicle, and nearly equal to it in size (Pl. XIV, Fig. 40). It is flattened or slightly concave next to the germinal vesicle; and it is surrounded by a clearer zone, which is traversed by radial fibers that pass out into the cytoplasm, where they become lost in the red cytoplasmic network. Stained with haematoxylin, followed with picric acid, the cytoplasm and the contents of the germinal vesicle stain a deep blue; the body, on the other hand, appears yellowish (Pl. XIV, Figs. 45, 46). It is seen to consist of granules somewhat closely packed at the center, but less closely packed at the periphery, where the granules are seen to be connected by the same substance as that of the cytoreticulum, into which it passes by imperceptible gradations (Fig. 46). Eggs of the same size as this, however, may show the typical form described above. The indentation next to the germinal vesicle may, however, be so marked as to cause the central granule to lie close to the germinal vesicle (Fig. 45), but yet distinct from the latter, as is clearly seen by the differential stain of Lyon's blue and carmine. At a somewhat more advanced stage, however, the body may be seen about midway between the germinal vesicle and the periphery of the egg (Fig. 49). When stained in Lyon's blue and safranin, all parts of the egg, except this body, take the red safranin stain. The body, however, appears as a blue or green sphere of interwoven fibrils, in the center of which may be found one or several red granules arranged more or less in a circle (Fig. 49). By means of this double stain, the body can be seen in all stages up to the time of the formation of the egg membrane (Pl. XV, Fig. 79), when the changes in the cytoplasm render the carmine and safranin ineffectual. But even in this case it can often appear very distinctly on account of its greater affinity for the blue, and its consequent deeper stain. In most cases of this kind it appears as a definite, spherical, compact body, consisting of closely interwoven fibers that may at times show a concentric arrangement about a somewhat modified central body (Pl. XV, Fig. 75). There may be two of these central bodies (Pl. XIV, Fig. 58). They appear somewhat like nuclei, each being surrounded by a compact layer of the outer mass of fibers.

In comparatively small eggs stained with various stains, such as the Biondi-Ehrlich mixture or haematoxylin, either alone or followed with acid fuchsin, the fibers of the cytoreticulum can be seen to converge to a point near the center of the egg (Figs. 50, 51, 54, 55). At the point where the fibers meet, a highly refractive body staining deep red in acid fuchsin can be seen. At times when the cytoreticulum is particularly distinct, the body is comparatively small and regular (Figs. 50, 51, 54). When the cytoplasm is more granular, the body may be comparatively large, apparently composed of refractive granules closely packed, and either regular in outline or serrated by projecting processes comparable to the points of a star, the body being very conspicuous because of its greater affinity for the stain than the rest of the cytoplasm, the reticulum of which can be seen to have a somewhat indistinct radial arrangement with reference to it (Fig. 56).

In favorable cases, when the cytoreticulum is especially distinct, the body towards which the fibers of the cytoreticulum converge appears as a ring of dense fibers, the reticular nature of which can, however, be distinctly seen (Pl. XIV, Fig. 32). Within this ring is a delicate network of fibers with distinct microsomes at the nodes, and from the ring surrounded by the cytoreticulum, comparatively straight isolated fibers radiate into the cytoreticulum, where they can be traced for a considerable distance.

In cases where a distinct refractive body exists at the point of convergence of the cytoplasmic fibrils, this point often lies near the germinal vesicle; and being about in the center of the egg, the latter occupies a somewhat excentric position (Pl. XIV, Fig. 51). The fibers converging at this point, or, as we may say, radiating from this point, are independent of one another over a considerable area surrounding the point of convergence; but they ultimately become continuous with the more reticulated portions of the cytoplasm. An area thus exists near the germinal vesicle, where the fibers radiate; and being less dense, this area, at times at least, appears lighter (Pl. XIV, Figs. 50, 51, 54, 55). The area is usually round, about equal to the germinal vesicle in size, and often flattened or concave at that point where it is in contact with the germinal vesicle (Fig. 50). At times the radial fibers may be very distinct and numerous (Fig. 52). The radial fibers are sometimes definitely limited; but at other times they can be traced to the periphery of the egg, at that point opposite to the germinal vesicle (Fig. 57), the central body being at times inconspicuous (Fig. 52), or at other times apparently consisting of a relatively large granular body into which the radial fibers can be seen to extend at variable distances (Fig. 57). The system of radial fibers is not always so close to the germinal vesicle as described above (Pl. XVI, Figs. 87, 92, 97). The condition of the radial fibers seems to vary. At times they appear as rows of microsomes (Pl. XIV, Fig. 57); while at other times they appear as comparatively homogeneous silken fibers, in which varicosities do not come prominently into view (Pl. XV, Fig. 87). Some of this difference in appearance can no doubt be ascribed in many cases to difference in the staining, but it appears clear that the difference is often due to the condition of the fibers themselves.

In somewhat larger eggs the body appears more complicated. Stained in Weigert's picro-carmine, it consists of a round, dark, homogeneous central body surrounded by a clear zone which again is surrounded by a somewhat lighter zone of granules (Pl.

XV, Fig. 89). This zone of granules is often not so definitely outlined as the central body, but its outer border is often serrated. It is, however, sharply differentiated from the surrounding cytoplasm. From this dark-brown body radial fibers extend into the yellow cytoplasm surrounding it. In Ehrlich's haematoxylin, followed with eosin, the body may show a blue center composed of granules. This is then surrounded by a red zone of concentrically wound fibers more or less interwoven; and from this again red fibers can be seen to radiate throughout the cytoplasm; and in some sections of the latter, where granules are less numerous, the radial fibers can be traced to the very periphery of the egg (Pl. XIV, Fig. 60; Pl. XV, Fig. 67). As the egg grows the body increases in size, and the form described above may thus become relatively large and conspicuous. Stained in erythrosin and cyanin, such a large body may be seen to consist of a large central, spherical mass of granules with a blue tint. This is again surrounded by a comparatively thick zone of bright-red fibers arranged concentrically around the central granules. And around this again can be seen a radial arrangement of the cytomicrosomes (Pl. XVI, Fig. 101).

In Ehrlich's haematoxylin and acid fuchsin the body may appear as a conspicuous deep red, rather small, definite, refractive body in the center of a zone of blue granules (Pl. XV, Figs. 68, 82). The cytoplasm having a less intense red coloration, this form of the body is often a conspicuous and beautiful preparation. From this as a center, also, radial fibers can often be distinctly traced to the periphery of the egg. Many of the red radial fibers can be seen to penetrate the blue zone of granules, and to proceed directly from the red central body (Pl. XV, Fig. 68). At other times the blue granules of the zone are so abundant that the radial fibers cannot be observed in it, and they consequently appear to end at the periphery of the blue zone (Fig. 67). The round, red central body is occasionally seen to be surrounded by a clear space which again is bound by a definite red staining wall, around which the blue zone of granules is arranged (Figs. 67, 82). The central body may be very minute; and radial fibers, and, as it often appears, radially arranged granules, immediately surround the small central body. The radial structures are then often limited by a broader or thinner zone of concentrically arranged fibers — the whole being conspicuously differentiated from all the rest of the cytoplasm (Pl. XIV, Fig. 53; Pl. XVI, Fig. 100). The outer concentrically arranged fibers may be absent, and the center may be a mass of granules surrounded by short, stiff, radial rods that penetrate unequally into the central granular mass. In the Biondi-Ehrlich stain, the central granules are yellow, while the radial rods are conspicuously red, the whole being definitely limited and sharply contrasted from the rest of the cytoplasm (Pl. XV, Fig. 80). In this case three bodies, connected by dense cytoplasmic fibers, are seen in the neighborhood of the spherical body; and from this the cytoplasmic fibers, arranged radially, can be seen to extend to the periphery of the egg, where they become continuous with the peripheral layer of fibrous protoplasm, thus rendering this portion of the egg peculiarly different from other portions of the same section. Instead of the radial rods and the three neighboring bodies, a comparatively large vesicular body, consisting of granules surrounded by a very distinct thin wall or membrane, has been observed. In this case only one body existed in its vicinity; and, as in the case above, the cytoplasmic fibers had a similar arrangement.

Occasionally a number of refractive bodies are to be seen at the apex of a cone of fibrous protoplasm, whose base is continuous with the peripheral protoplasm (Pl. XV, Fig. 90). It may also take the form of an oval sphere of interwoven fibers, enclosing in its meshes refractive bodies, and joined to the peripheral zone of fibrous protoplasm by a narrow stalk of similar fibrous protoplasm (Pl. XV, Fig. 84). Occasionally two refractive bodies removed from each other, but connected by a band of fibrous protoplasm, can be seen. From each of these refractive bodies, bundles of radial fibers extend far out into the cytoplasm, which is sharply contrasted from it. The body, when stained in Ehrlich's haematoxylin and eosin, is also seen to consist of a central sphere of yellow granules, which is surrounded by a bright-red, homogeneous, fibrous, protoplasmic zone of considerable thickness, and having the form of a horseshoe (Pl. XV, Fig. 76). At the upper part of this fibrous protoplasm are imbedded several aggregations of blue granules; and, surrounding the whole, and slightly removed from it, in the red cytoplasm, is seen an irregular circle of these blue granules like a wreath, which, at the opening of the horseshoe-shaped protoplasm, forms an irregular mass of the blue granules. When stained in Ehrlich's haematoxylin alone, the body appears as an unstained spherical mass of fibrous protoplasm of considerable size, and definitely limited, around which are arranged, in a radial manner, numerous deep-blue granules apparently associated with a radial system of fibers proceeding from the body as a center (Pl. XVI, Fig. 96). Within such a body similar blue granules are found distributed, but most frequently aggregated into groups occupying vacuoles which with the blue granules appear like nuclei. Again it may appear as a conspicuous spherical body of the same fibrous protoplasm, which stains a deep red in erythrosin or acid fuchsin. It may enclose one or two nuclei-like bodies surrounded by a denser zone of the same fibrous protoplasm (Pl. XV, Figs. 64, 86). If the acid fuchsin be preceded with Ehrlich's haematoxylin, the central body (or bodies) is seen to contain the same blue granules resembling chromatin granules of nuclei (Figs. 86, 88). There may be several of these central bodies containing blue granules scattered irregularly between the fibers of a large felt-work of delicate protoplasmic fibers, as seen in Pl. XVI, Fig. 95. Occasionally the blue granules appear as highly refractive bodies lying between the fibers, that may have a comparatively regularly concentric arrangement (Pl. XIV, Fig. 61; Pl. XV, Fig. 72). Occasionally none of the granules can be observed (Fig. 69), but this may be due to the staining. Erythrosin, for instance, makes the whole body very conspicuous, but does not always differentiate the internal structures. The whole body may be relatively large, oval in form, and homogeneous in structure, having in this case, as in most cases, a system of surrounding radiations that extend far into the cytoplasm (Pl. XVI, Fig. 99). It may at times have the form of a spindle, with its longitudinal axis not exceeding the transverse axis, the spindle appearance being due to the rather definite arrangement of the fibers with reference to two poles (Pl. XV, Fig. 65). At one of these poles appears a structure consisting of concentrically arranged microsomes, from which radiate in a somewhat irregular manner protoplasmic fibers.

In many cases the conspicuous, definitely limited spherical body, enclosing a central structure or nucleus, is seen to be surrounded by a definitely limited zone of protoplasm and granules that differ somewhat from the rest of the cytoplasm (Pl. XV, Fig. 71). The investing, interwoven, or concentrically arranged fibrous protoplasm, which so frequently surrounds the central nucleus or body, and which makes the whole so conspicuous when properly stained, is often limited in amount, or even apparently absent. The body may then be a spherical granular mass enclosed by a thin definite membrane. There may be two of these situated close together. They are made conspicuous by means of acid fuchsin or eosin (Pl. XV, Fig. 66).

As the egg grows the cytoplasm becomes more and more granular, and the body is less easily traced. The peculiar fibrous protoplasm, which often renders it so conspicuous, becomes less and less apparent. It often appears as a spherical mass of granules surrounded by a zone, in which they are few or entirely absent, and around which is another zone of similar granules. These granules being larger than those of the cytoplasm, and reacting differently towards stains, the body is still conspicuous (Pl. XVI, Fig. 102). With continued growth of the egg the body often attains to considerable dimensions. It may be a definitely spherical body surrounded by a clear ring, which, in connection with its deeper stain, sets it off conspicuously from the rest of the cytoplasm (Pl. XVI, Fig. 109). Or it may be irregular in outline, rather star shaped, granular, and may contain within it a spherical central body which alone often comes prominently into view (Pl. XVI, Fig. 114). This may be situated in the center of the egg and near to the germinal vesicle (Figs. 103, 104, 114). It varies, however, considerably in size; and it is at this stage difficult to differentiate it from the rest of the cytoplasm. Repeated attempts with a variety of stains often bring it prominently into view even after all hopes of observing it have been abandoned.

Occasionally it may be more excentric. It is often very large, and consists of densely packed granules, which grade gradually into the surrounding protoplasm in which more conspicuous traces of the reticulated arrangement of the granules can be seen (Fig. 114). Here a zone of similar granules extends around the periphery of the egg, under the perivitelline layer of protoplasm. It is made conspicuous by means of the Biondi-Ehrlich stain, in which it takes a darker tinge than the rest of the cytoplasm. By means of Lyon's blue it also appears as a much darker body. By means of the double stain of erythrosin and cyanin it can be differentiated as a blue body, the rest of the cytoplasm being red (Fig. 104). The staining, however, must be applied with the greatest of care, and in a manner that can be learned only by repeated experiment. Having once acquired the necessary skill, however, it is a comparatively easy matter. From this it is not to be inferred that it is all a matter of staining, for the preliminary method of preserving is of even greater importance. Even with the best method of staining, it is to be observed only in the most perfectly preserved material. This is true not only of the later stages just described, but it is true also of the earlier stages.

In this description no attempt has been made to exhaust the subject, but merely to give the more prominent features of the body as it appears in the various phases of the growing egg.

All the figures in the plates, beginning with Fig. 48, Pl. XIV, are drawn with a Leitz camera, obj. 5, oc. 1-Leitz. Figs. 42–49 are drawn with a Leitz $\frac{1}{12}$ oil immersion.

Fig. 32 is one of the smallest eggs observed in the adult ovary, drawn with a camera, Leitz $\frac{1}{12}$ oil immersion.

Interpretation and Summary.

After the last division of the oögonia to form a follicle, the centrosome, with its surrounding structures, persists in the cytoplasm. It has been observed in this case, not only during karyokinesis, but after the last reconstruction of the nucleus, when the increased size of the cell and its nucleus shows it to be a growing egg (Pl. XIV, Figs. 34, 38–40). It consists, in this early stage, of two concentric circles of microsomes — a large outer circle and a smaller inner circle. In the center of the smaller inner circle is a granule, which at first hardly exceeds in size one of the microsomes of the surrounding circle. The microsomes of each circle appear to be connected by a less conspicuous fibrous substance; and from the microsomes of the inner circle to the microsomes of the outer circle radial fibers connecting these can be observed (Pl. XIV, Figs. 45–47). The minuteness of the inner circle and the body contained in it does not permit a determination, at this stage, of the presence or absence of radial fibers surrounding the central granule. The central granule, however, is present, though its minuteness often renders its detection difficult.

I do not hesitate to say that this is the centrosome of the dividing oögonia, and that the central granule, with its surrounding structure, corresponds very closely to that described by van Beneden in the dividing egg of Ascaris.

The centrosome and surrounding circles of microsomes, with their radial fibers, appear to be imbedded in a specially modified, more or less amorphous substance which, through the various effects of reagents and stains, renders the former obscure or even invisible. For the present I will adopt the term used by Boveri and call this imbedding substance archoplasm. This archoplasm is more conspicuous in some stains than in others; and for that reason the centrosome and surrounding structure may alone be distinctly visible; while with other stains the archoplasm appears prominent, often showing no internal structure. The former is true of such stains as picro-carmine (Fig. 47); the latter is often true of such stains as Lyon's blue and erythrosin and cyanin (Figs. 39, 40). A careful comparison of these different effects shows that at other stages both the radial system and circles of microsomes, as well as the archoplasm, are present.

Being a direct continuation of the centrosome of the dividing oögonia, it cannot be said to originate in the cytoplasm of the growing egg.

But is it derived from the young germinal vesicle? It exists before a nucleolus has made its appearance in the germinal vesicle. In this early stage the contents of the germinal vesicle and the granular cytoplasm stain a deep red in carmine or safranin. If one of these stains be properly associated with Lyon's blue, the centrosphere with its archoplasm stains a deep blue, the rest of the egg, both nucleus and cytoplasm, being bright red. The blue body is then observed as a conspicuous crescent-shaped structure partly enclosing the germinal vesicle. In the broadest portion of this crescent-shaped body the structure of the sphere and an enclosed centrosome, described above, can be seen (Figs. 42–47). The horns of the blue crescent appear to be due to an extension of the archoplasm of the sphere and an aggregation along the sides of the young germinal vesicle of the radial fibers belonging to the sphere. The blue crescent, although lying close to the germinal vesicle, is sharply differentiated from all parts of it, and is also at first sharply differentiated from the cytoplasm (Figs. 34, 38), although it later grades gradually into it. The red stain of the contents of the germinal vesicle and cytoplasm is due to the presence of granules of the nuclear network and the granules of the cytoplasm that are strongly affected by the carmine or safranin. The blue stain of the crescent-shaped sphere and archoplasm I would interpret as indicating an absence of the chromophilous granules. The nucleolus, when present, is also strongly affected by the carmine and safranin stains. When these stains alone are employed, the body remains obscure because of the absence of staining in that region; and one might easily, in such a case, be led to say that the body is not present. If, however, the carmine be followed with picric acid, the body comes into view as a yellowish body instead of the blue of the former double staining. There being present none of those granules which carmine so strongly affects, and which are the distinguishing features of chromatin of the nucleus, there is no ground on which to base the statement that this body originates from the germinal vesicle. Its form also will hardly admit the statement that it is a bud of the germinal vesicle. Furthermore, such a bud would necessarily contain chromatin granules or else some substance capable of fixing the Lyon's blue more strongly than the carmine. The latter substance is not to be observed in the germinal vesicle, for no part of it takes the blue stain when carmine or safranin is associated with the Lyon's blue.

From these considerations, and others that will appear in another connection, it may be said that *the vitelline-body does not arise in the cytoplasm of the growing egg; neither does it arise as a bud of the germinal vesicle; nor as extruded chromatin, nor as migrating nucleoli. It contains no nuclear chromatin.*

In this connection, a few further considerations concerning Lyon's blue as a stain may be added. This stain not only differentiates the body under consideration in its earliest stages; but, in material preserved in suitable hardening reagents, it differentiates it conspicuously as long as safranin or carmine can be associated with it. This, however, ceases when the first period of growth is passed, since, after that period, these stains do not affect the granules of the cytoplasm. Yet, even after this, the body is made conspicuous by means of Lyon's blue used alone, because of its greater affinity for the stain and consequently deeper blue coloration. It may thus profitably be employed even in larger eggs of the second and third stages.

Even in those cases where nothing corresponding to the archoplasm appears, where the fibers of the cytoreticulum converge to a point as previously described, thus forming either a real, conspicuous aster, or a more irregular area with radial fibers, the center of this system is made conspicuous by the Lyon's blue, all the other parts of the egg being stained red by means of safranin. Where the body assumes the form of a large compact sphere of interwoven or concentric fibers, also, it is made prominent as a blue or green sphere standing out conspicuously from all the rest of the egg.

Although this stain, therefore, has a decided affinity for this body, I cannot regard it as a specific stain, for it shows also a decided affinity for the egg membrane after it has acquired several layers. Its general nature as a stain is evident further in those eggs belonging to the third stage, where it has been pointed out that numerous nuclei are found within the egg.

In such cases the carmine or safranin stains the chromatin granules of the nuclei ; and when this is followed with Lyon's blue, everything in the cytoplasm, except these red nuclei, stains a deep blue, making indeed handsome preparations. I should certainly hesitate, therefore, to regard everything as archoplasm in the sense in which Boveri used that term, which stains blue with this when combined with carmine and safranin. An examination of the plates of Miss Foot ('96), where the effect of this stain is extensively represented, would tend to increase rather than diminish such a reluctance. When associated with carmine or safranin, these are the specific stains. The value of the Lyon's blue lies in this, that it brings prominently into view those areas containing no chromophilous granules ; and for this purpose it is very convenient. In the first period of growth of the egg of Limulus it has been pointed out that both germinal vesicle and cytoplasm contain these chromophilous granules, this body alone being devoid of such granules. When eggs of Limulus are properly preserved, there are none of those irregular areas in the cytoplasm which Miss Foot has found in the egg of Allolobophora by means of this stain.

The vitelline-body having been shown to possess, in its earliest stages, all of the features of the centrosome and sphere, and to be, in fact, the centrosome of the dividing oögonia, it remains to show that the body found in the cytoplasm in later stages is the same centrosome. For this purpose the plates will afford better evidence than a labored description. The figures being drawn with a camera with the same magnifying power, show the body in the various stages of the growing egg. It can be seen that even in advanced stages of the egg the body often presents the fundamental features seen in the earliest stage, and often very nearly the features of a typical centrosome and sphere (Figs. 42–48, 67, 68, 82, 89, 114). These are characterized by the presence of a strongly refractive spherical body often surrounded by a clear zone, which again is surrounded by a zone of metaplasm (archoplasm Boveri) and provided with a system of radial fibers which can be seen to traverse the metaplasmic zone and to extend far out into the cytoplasm. As these appearances have been described elsewhere, they need not be repeated here. The central refringent granule, staining deep red in acid fuchsin and surrounded by these radial fibers and metaplasmic zones containing the blue granules, is undoubtedly the centrosome; and it, with the surrounding structures, constitutes a real sphere. According to van Beneden ('87), the sphere consists of a central body (centrosome) surrounded by a clear zone (medullary zone), which again is surrounded by a granular zone (cortical zone). All of these conditions can be seen in the vitelline-body, in the egg of Limulus. According to Boveri ('89, '95), the centrosome is surrounded by a zone of archoplasm, which in some way grows out into the cytoplasm in the form of astral rays, which gradually replace the cytoreticulum. The vitelline-body presents the features of the sphere as defined by Boveri, and also the characteristics of a real aster (Figs. 43–46, 50–52, 54, 55, 57, 60).

But it is the unusual features which this body assumes that offer the greatest difficulties. Some of these are its excentric position, its large size, and the fantastic appearance which it often presents. The more common of these is the great increase in size of the central body (Pl. XV, Figs. 71, 77; Pl. XVI, Fig. 101), or the apparent absence of a definite central structure; the concentric arrangement of the fibers; their great increase or diminution; the often granular aspect of the body; the vesicular form which it sometimes assumes; and finally the combination in various ways of these different features. An attempt to account for these features will be made in the suggestions that are to be offered in the following chapters on some of the physiological problems of growth and metabolism.

These features are not foreign to the centrosome and sphere as these are now understood. 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 ('95) and Meves ('94, '95), and in nerve cells as figured by Lenhossek ('95). 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 the 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 oögonia, and continues to be the centrosome and attraction sphere of the growing ovarian egg.

That this centrosome and sphere may assume the form of the vitelline-body as originally described, seems evident from a comparison of Figs. 60–64, 70–72, 95 with the figures of Balbiani ('**64**), ('**79**), ('**82**), ('**83**), ('**93**); of v. Wittich ('**49**); Carus ('**50**); Schütz ('**82**); and Henking ('**87**); and becomes very evident when preparations of the ovary of Limulus and of the spider are directly compared.

The most important recent papers on the vitelline-body, Julin ('**93**), Mertens ('**93**), Balbiani ('**93**), and Henneguy ('**93**), also suggest strongly a probable relation of this body in other eggs to the centrosome and sphere.

Position of the sphere. — In the youngest eggs the sphere is always situated close to the germinal vesicle, as described above. It may remain in this position or it may become removed from the germinal vesicle so as to occupy a position midway between it and the periphery of the egg (Pl. XIV, Figs. 49, 56). At times it may even occupy a more excentric position (Fig. 70).

There appear to be three causes that can be assigned for this difference in position. First, a difference in the tension or contraction of the radial fibers; second, a difference in the local accumulation of the amorphous substances in the interfilar spaces; third, differences in actual growth of the cytoplasmic body.

The position of the metaplasm varies with reference to the central structure. It may spread out on either side of it, causing a density of the radial fibers lying close to the germinal vesicle, and thus causing the horns of the crescent (Pl. XIV, Fig. 47). From this position it may collect around the central structure, greatly obscuring the latter, and causing the whole body to appear very conspicuous as a homogeneous solid body (Fig. 40). On the other hand, it may spread out in a circle surrounding the germinal vesicle (Fig. 48), and even become extended towards the point of attachment of the egg (Pl. XIII, Fig. 16). In that way it appears to form a channel by which food material is conveyed into the egg. In the vicinity of the germinal vesicle the food material is acted upon, or at least comes in contact with a clear fluid, perhaps karyolymph or nuclear sap, and becomes converted into conspicuous stainable granules. The metaplasm then, instead of collecting around the central structure, may move out into the cytoplasm, causing the fibers of the crescent to expand into the general cytoplasm; and the food granules surrounding the germinal vesicle may likewise be variously distributed, causing the inner zone surrounding the germinal vesicle either to entirely disappear or else to become hyaline and devoid of granules (Pl. XIV, Figs. 19, 24).

The variable disposition of these three elements — the cytolymph, as it may be called, the food granules, and the metaplasm — appears to be responsible for many of the variations, not only in the position, but also in the form of the vitelline-body. The position of the metaplasm with reference to this body appears to determine the direction of growth. If the metaplasm surrounds the central structure uniformly, the cytoplasm increases uniformly, and the body thus becomes gradually removed from the germinal vesicle. On the other hand, if the central structure lies close to the germinal vesicle, and the metaplasm on the distal side, the cytoplasm appears to increase in the direction of greatest amount of metaplasm; and if the metaplasm is wholly absent from the structure, as often appears to be the case (Figs. 50, 51, 55), the structure most frequently is found, even in later stages of the egg, to occupy the position which it formerly had (Pl. XVI, Fig. 104). In the absence of the metaplasm from the vicinity of the central structure, this latter does not appear as a conspicuous massive body, but as a fibrous framework of radial fibers with its refractive body in the center (Pl. XIV, Fig. 54).

It has been pointed out that eggs showing this feature of the body most conspicuously are chiefly those arising in later stages of growth of the parent organism, and therefore growing more slowly. This appears to be owing to the absence of nutritive material, which reveals itself in the very pronounced appearance of the reticulum (Pl. XIV, Fig. 32), which in eggs of the same size, arising earlier in the history of the animal, is often greatly obscured by the presence of an abundance of amorphous granules.

Nature of the metaplasm.—The yolk-nucleus (Pl. XIV, Fig. 27), I believe, can be considered as an early stage of the yolk, through which all yolk material passes on its way to become definite yolk spheres. It is often associated with the vitelline-body. Such a case seems to present itself here in the form of the metaplasm surrounding the centrosome and sphere.

The metaplasm consists of at least two kinds of granules. Some of these granules possess an affinity for haematoxylin, which is evident from their retaining this stain even when followed with such a powerful stain as acid fuchsin. In picrocarmine these granules show a reddish coloration in marked contrast with other portions of the granular metaplasm.

Careful examination of a large quantity of material showing these granules in connection with the vitelline-body and centrosome appears to show conclusively that they issue as little drops from the living substance of the cytoplasmic fibers and remain closely adherent to them. This appears to take place most freely when the fibrils are relaxed and in a state of rest. It is evident that where the fibers converge and are most densely packed, the number of these granules would be greatest. There is reason to suspect that these granules may be converted into yolk bodies, or be reabsorbed by the fiber so that no trace of them remains.

It has been shown that the granules in the cytoplasm of the first stages of the egg show an affinity for chromatin stains, and that later this affinity disappears as the definite yolk is formed. It has also been shown that these stainable granules are more abundant in those eggs which increase rapidly in size, and which may be supposed to be abundantly supplied with nutriment. It has also been shown that in those young eggs which grow more slowly, being formed in a later period of the development of the parent organism, when growth is considerably retarded, and when therefore it may be supposed to be scantily supplied with nutriment, these granules are often comparatively scarce, often almost absent (Pl. XIV, Fig. 32). This fact suggests that the granules may have been used as food for the living substance, and that this is analogous to the appearance and disappearance of those granules which later appear in connection with the vitelline-body.

Growth of the Cytoplasm.

The vitelline-body, as we have seen, is at first a central granule situated close to the germinal vesicle, surrounded by circles of large microsomes, probably connected by linin strands, and a system of fibers connecting the microsomes radially (Pl. XIV, Figs. 45, 46). In many cases this spherical structure is seen to become more granular, the granules being arranged concentrically, but yet closely packed. It may thus increase greatly in size (Pl. XIV, Fig. 56), while remaining refractive and apparently homogeneous, staining a bright red in acid fuchsin, and becoming surrounded by a zone of granules which retain the blue haematoxylin stain (Pl. XV, Fig. 78). In later stages this body may become still more enlarged, and consist of a large number of granules staining like the microsomes of the cytoreticulum and be surrounded by concentric or interwoven fibers, which again are surrounded by still another zone of granular substance (Pl. XV, Fig. 77). At the periphery of the central granular body the meshes between the granules may gradually increase so as to acquire the essential structure of the cytoplasm (Pl. XIV, Figs. 32, 46); or else a number of blue granules, like the chromatin of nuclei, are found within a felted mass of fibers which at the periphery passes gradually into the cytoreticulum (Pl. XV, Figs. 70, 76; Pl. XIV, Fig. 53).

In the absence of the blue granules the vitelline-body remains a compact mass consisting of closely packed microsomes or fibrillae (Pl. XV, Fig. 69). *On the appearance of these granules, vacuoles arise; and the microsomes expand into the cytoreticulum* (Pl. XIV, Figs. 46, 53; Pl. XV, Figs. 72, 86). The formation of vacuoles and the resulting expansion may continue until the entire vitelline-body is reduced to a network (Fig. 32), and may seem to have entirely disappeared. The disappearance, however, is manifestly an illusion (Pl. XIV, Figs. 50, 51, 54, 55).

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

The growth of the cytoplasm is greatest in the direction in which the blue granules are most abundant.

When the blue granules are unequally distributed around the central body, growth takes place unequally, leaving the body close to the germinal vesicle, or widely removed from it.

When the blue granules are equally distributed around the central body, growth takes place equally in all directions, and the body becomes the center of the cytoplasm.

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REFERENCE LETTERS.

m.	muscle.	*y.n.*	yolk-nucleus.
ov.t.	ovarian tube.	*ca.*	carapace.
an.	anus.	*t.p.*	tunica propria.
ov.	ova.	*p.b.*	peripheral bodies.
op.	operculum.	*al.c.*	alimentary canal.
ov.d.	oviduct.	*p.c.*	peritoneal coat.
g.o.	genital opening.	*p.*	follicular pouch.
fl.	follicle.	*mpl.*	metaplasm.
m.c.	muscle coat.	*c.*	centrosome.
e.s.	egg stalk.	*ast.*	aster.
g.v.	germinal vesicle.	*n.*	nucleus.
h.n.	"Hauptnucleolus."	*ch.*	chorion.
n.n.	"Nebennucleolus."	*ncl.*	nucleolus.
p.p.	polar protoplasm.	*arch.*	archoplasm.
y.z.	yolk zone.	*sph.*	sphere.
v.b.	vitelline-body.	*b.g.*	blue granules.
y.s.	yolk secretion.	*p.m.*	polar mitosome.
s.g.	secretion granules.	*i.z.*	inner zone.

EXPLANATION OF PLATE XIII.

FIG. 1. Germinal vesicle with a crescent-shaped nucleolus, containing within it a reticulum resembling that of the germinal vesicle.

FIG. 2. A female Limulus with dorsal carapace removed, showing the netted ovary, the ovarian tubes on the right, *ov.t.*, being filled with eggs. The left side represents the condition in younger animals before eggs have been discharged into the ovarian tubes; *ov.*, young growing eggs; *an.*, anus; *op.*, operculum; *g.o.*, genital openings; *ca.*, carapace; *ov.t.*, ovarian tubes extending along the alimentary canal, *al.c.*, to the anus; *ov.d.*, terminal oviducts.

FIG. 3. Germinal vesicle showing "Nebennucleoli," and a body resembling these within the "Hauptnucleolus."

FIG. 4. Germinal vesicle showing a nucleolus having numerous internal bodies, one of which is about to be extruded.

FIG. 5. An amoeboid germinal vesicle containing a large nucleolus, in which there is a large vacuole containing nothing stainable.

FIG. 6. Germinal vesicle, containing a large hollow nucleolus in the form of a deeply staining shell. Within the nucleolus there is a granular network resembling the network of the germinal vesicle.

FIG. 7. Germinal vesicle showing diverticula, and containing a ring-shaped nucleolus containing within it a network resembling that of the germinal vesicle.

FIG. 8. Germinal vesicle with diverticula, and containing a nucleolus having a radial striation, a central granular mass, in which is imbedded a deeply staining, homogeneous spherical body — the endonucleolus.

FIG. 9. Germinal vesicle; a nucleolus in form of a deeply staining crescent containing a finely granular substance surrounding a vacuolated endonucleolus.

FIG. 10. An egg with its germinal vesicle containing a nucleolus, and also a radial arrangement of the chromatin network about a center resembling a centrosome.

FIG. 11. The nuclear pole ("Kernpol") of an egg about to be discharged from the follicle, showing the position of the germinal vesicle, and the spongy polar protoplasm spreading out under the egg membrane.

FIG. 12. Mature egg of the ovarian tube, showing an amoeboid remnant of the germinal vesicle, and its connection with a peripheral mass of protoplasm.

FIG. 13. Portion of an egg showing the formation of the egg membrane after the first layer has been formed; the orderly radial arrangement of the protoplasmic fibers previous to the hardening of the interfilar substance, showing that the radial striae of the chorion are due originally to protoplasmic fibers.

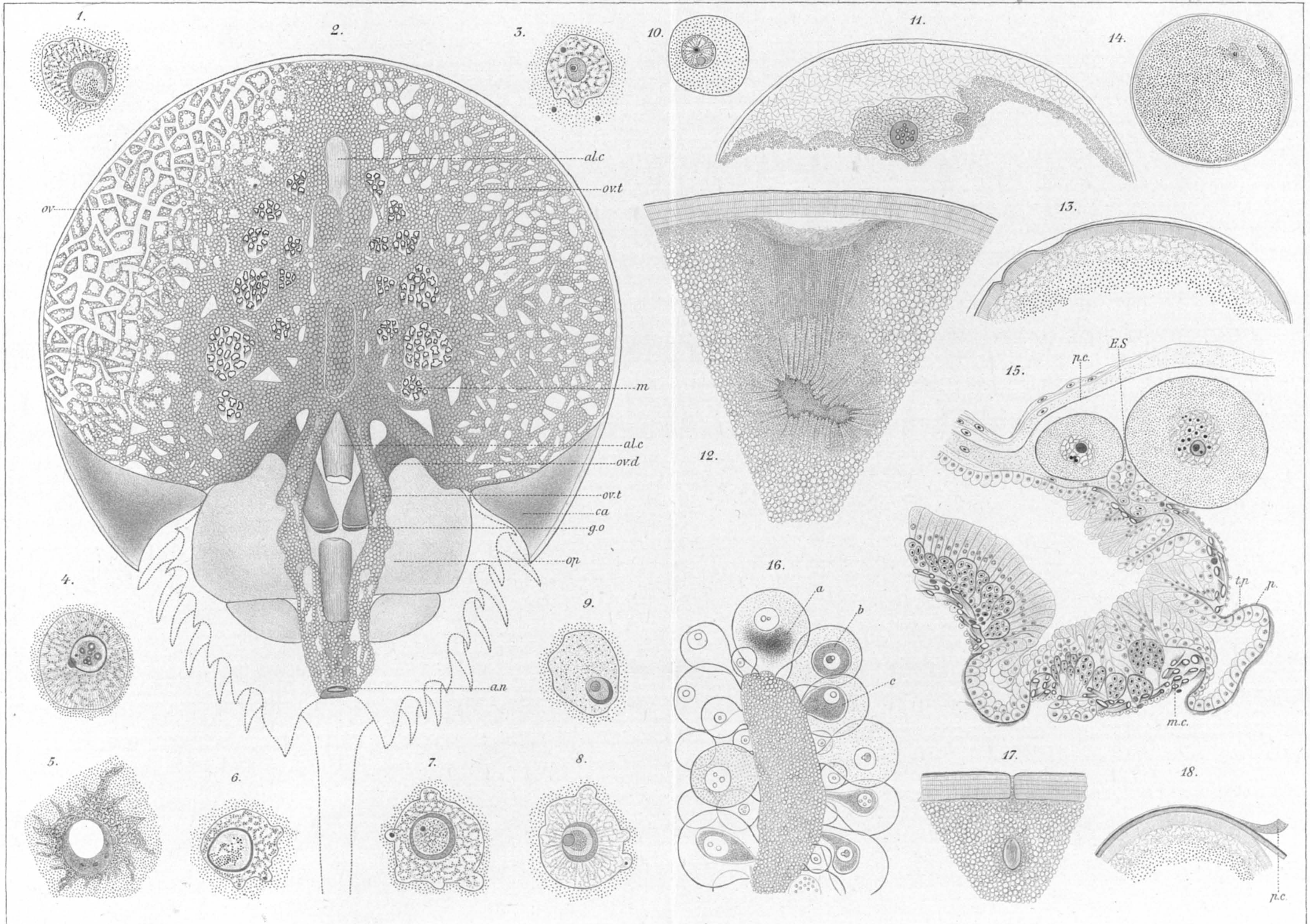
FIG. 14. Section of an egg about to be discharged into the ovarian tube, showing the movement of the germinal vesicle and its relation to the "Kernpol" area.

FIG. 15. Section of an adult ovarian tube, showing the folding of the germinal epithelium and with it the tunica propria, *t.p.*; the variable size and form of the epithelial cells; the formation of empty follicles, *p.*, by evagination through the fenestrae of the muscle coat, *m.c.*; the relation of the number and size of the eggs to the number of empty follicles; the position of the immature eggs with reference to the empty follicles and the point of attachment of the tube; the relation of this latter to the enclosing peritoneal coat, *p.c.*

FIG. 16. Portion of an ovarian tube taken from a living animal thirteen inches long, and examined in the normal fluids of the ovary, drawn with a camera; a sharply defined granular zone surrounding the germinal vesicle, as in *b.*, or extended on the proximal side to the point of attachment of the eggs, as in *c.*, or collected into a dense body in the cytoplasm, as in *a.*

FIG. 17. A portion of a mature egg in the ovarian tube, showing an opening in the chorion, and some distance below it the last remnant of the nucleus in the form of a spindle, the laminated structure of the chorion, and also its radial striations.

FIG. 18. Structure of the egg membranes; *p.t.*, primary tunic, corresponding to the tunica propria — the original egg membrane; the first egg membrane fully formed; the second having the form of radial protoplasmic fibers; the interfilar substance not yet hardened into the definite chorion. The primary tunic shows the bright dots — the original points of insertion of the radial fibers.



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EXPLANATION OF PLATE XIV.

FIG. 19. An egg showing an inner granular zone of the cytoplasm, and its connection with the stalk of the egg. The usual chromatin network of the germinal vesicle is wanting. No nucleolus is present. The tunica propria forming the basement membrane of the epithelium and the outer boundary of the stalk of the egg is continuous with the investing membrane of the egg; *m.c.*, muscle coat; *t.p.*, tunica propria; *y.z.*, internal yolk zone.

FIG. 20. An egg showing a hyaline inner yolk zone which is extended towards the point of attachment of the egg.

FIG. 21. An egg showing an inner yolk zone, *y.z.*, surrounding the germinal vesicle; in the dividing line between the inner and outer zone, the vitelline-body, *v.b.*, surrounded by a modified polar mitosome.

FIG. 22. The proximal portion of an egg showing the accumulation of yolk secretion, *y.s.*, at the base of the stalk of the egg, and the formation of the first layer of the egg membrane.

FIG. 23. An empty follicle serving as a yolk gland, showing the secretion products obscuring nuclei and cell boundaries; *s.g.*, secretion granules; *m.c.*, muscle coat.

FIG. 24. An egg showing an internal, hyaline protoplasmic zone surrounding the germinal vesicle as a uniform ring, *i.z.*; the germinal vesicle with diverticula and the nucleolus containing a vacuole.

FIG. 25. An egg showing the peripheral bodies, *p.b.*, and their gradual fusion into the first layer of the egg membrane or chorion, *ch.* Low magnifying power.

FIG. 26. Small egg with a germinal vesicle containing two "Hauptnucleoli," *h.n.*, each extruding a "Nebennucleolus," *n.n.*

FIG. 27. The proximal portion of an egg, showing deeply stainable bodies resembling nuclei — the yolk-nucleus, *y.n.* The basement membrane of the stalk of the egg is seen to be continuous with the primary egg-tunic, *t.p.*

FIG. 28. Tangential section of an egg, showing the form and distribution of the peripheral bodies, *p.b.*, and the round bright dots or pores with which they are pierced.

FIG. 29. Small egg showing the internal granular zone, *y.z.*, in the form of a ring surrounding the germinal vesicle, the latter containing no true chromatin reticulum and no nucleolus.

FIG. 30. An egg in the third stage, filled with nuclei, and partly surrounded by nucleated granular cells; no germinal vesicle.

FIG. 31. An egg (similar to Fig. 30) undergoing regressive metamorphosis; no germinal vesicle; proximal portion of the yolk filled with nuclei and a hyaline protoplasm; egg surrounded by a false follicle epithelium of granular cells, as in Fig. 30; chorion folded, distorted, and pierced in various ways.

FIG. 32. A small egg of an adult ovary (magnified $\frac{1}{12}$ oil immersion), showing a very distinct cytoreticulum, and a sphere in the form of a central dense network with very distinct radial fibers, many of which appear to be continuous with the general cytoreticulum; the cytoreticulum apparently continuous with the nuclear network; metaplasm (archoplasm) scant or absent.

FIG. 33. Section of an ovarian tube of a young Limulus (Leitz, oc. 1, obj. 7), showing a group of cells undergoing the preliminary phases of karyokinesis previous to the formation of a growing oöcyte.

FIG. 34. Young Limulus, six-inch ; transverse section of ovarian tube showing oögonia ; the spireme of karyokinesis, and a growing oöcyte. The young germinal vesicle contains a nucleolus ; in the cytoplasm, close to the germinal vesicle, the crescent-shaped archoplasm. Method : nitro-picro-sulph., lithium-carmine, Lyon's blue. Archoplasm alone a deep blue.

FIG. 35. Six-inch animal ; longitudinal section ovarian tube ; chromatin thread — spireme of karyokinesis. Method : Merkel's fluid, Heidenhain's iron-haematoxylin, oc. 1, obj. 7 ; *t.p.*, tunica propria ; *p.c.*, peritoneal coat.

FIG. 36. Six-inch animal ; transverse section ovarian tube, cut obliquely ; metaphase and anaphase of karyokinesis ; centrosome at the pole of one spindle.

FIG. 37. Seven-inch animal ; transverse section ovarian tube ; oögonia in karyokinesis ; spindles ; equatorial plate. Method : Merkel's fluid, Heidenhain's iron-haematoxylin.

FIG. 38. Seven-inch animal ; transverse section of ovarian tube ; oögonia ; growing oöcyte with blue archoplasm. Method (3) (see method), Lyon's blue, lithium-carmine.

FIG. 39. Seven-inch animal ; ovarian tube ; oögonia ; growing oöcyte forming a diverticulum, and surrounded by the tunica propria and by the nucleated peritoneal mantle. Blue archoplasm conspicuous.

FIG. 40. Seven-inch animal ; oblique section of ovarian tube, showing three growing oöcytes, one of which has formed a diverticulum, and remains attached only by a narrow stalk ; enclosed first by the tunica propria, and second by the peritoneal nucleated coat. Method : No. 3, Lyon's blue and lithium-carmine. Blue archoplasm very distinct.

FIG. 41. Thirteen-inch animal ; transverse section of ovarian tube, showing the relation of the ovarian tube, lined with an epithelium, which is bounded by the tunica propria, *t.p.*, to the investing mantle or peritoneal coat, *p.c.* The point of attachment of the tube is seen to be also the point of origin of the eggs. From this point the eggs are seen to increase in size, regularly, to the point opposite where the largest egg is found.

FIGS. 42–47. Growing oöcytes from ovary of animal seven inches. All drawn with Leitz camera, $\frac{1}{12}$ oil immersion, and showing the centrosome and archoplasm, and the relation of these to the cytoreticulum. Method (3).

FIG. 42. Eosin and nigrosin.

FIGS. 43, 44. Lithium-carmine and Lyon's blue ; archoplasm and centrosome, blue ; everything else, red.

FIGS. 45, 46. Delafield's haematoxylin and picric acid ; centrosome and sphere, yellow ; everything else, blue ; sphere, very distinct.

FIG. 47. Weigert's picro-carmine ; archoplasmic sphere ; cytoreticulum and central radial structure very distinct.

FIG. 48. One of the smallest eggs from an ovarian tube, like that shown in Fig. 41. A distinct centrosome in the center of a light area, in the widest portion of the metaplasmic zone surrounding the germinal vesicle. In the latter, a prominent nucleolus.

FIGS. 49–104, 109, 114. The entire series of figures from Fig. 49 is drawn on the same scale, Leitz camera, oc. 1, obj. 5.

FIG. 49. One of the smallest eggs observed in the adult animal, showing a germinal vesicle, *g.v.*, with a granular nucleolus ; a very distinct blue sphere, *sph.*, with bright-red central granules. Method : Merkel's fluid, safranin, and Lyon's blue. Sphere, *sph.*, alone bright blue ; everything else, red.

FIG. 50. One of the smallest eggs of an old animal having many empty follicles. Cytoreticulum and aster, *ast.*, very distinct. Metaplasmic granules or archoplasm, very scarce or absent. Method: Merkel's fluid, Biondi-Ehrlich stain.

FIG. 51. Similar to Fig. 50; egg a little larger; metaplasmic granules more abundant. Method same as Fig. 50.

FIG. 52. A very distinct, sharply limited aster, *ast.*; *g.v.*, germinal vesicle; *ncl.*, nucleolus, containing a central vacuole.

FIG. 53. Egg from adult animal, showing a blue sphere, *sph.*, consisting of a central body, centrosome, surrounded by radial astral rays that become lost in a zone of archoplasmic granules, which again is surrounded by compacted fibers that merge gradually into the cytoplasmic reticulum.

FIG. 54. Egg showing a distinct cytoreticulum, in which there is a conspicuous aster, *ast.* This is farther removed from the germinal vesicle, *g.v.*, than the similar structures seen in Figs. 50, 51.

FIG. 55. Section of an egg showing position of aster in a plane at right angles to the primary egg axis.

FIG. 56. Section of an egg preserved in Flemming's fluid and stained in acid fuchsin. A large, bright-red body, *c.*, in the cytoplasm, whose reticulum is arranged radially around the centrosome and sphere.

FIG. 57. Section of an egg showing a central body or centrosome, and a conspicuous aster, *ast.* Method: corrosive acetic, haematoxylin, and acid fuchsin.

FIG. 58. Section of an egg showing sphere, *sph.*, with two central structures surrounded by archoplasm. Method: safranin and Lyon's blue. The sphere alone, deep blue; the rest of the cytoplasm and the germinal vesicle, red.

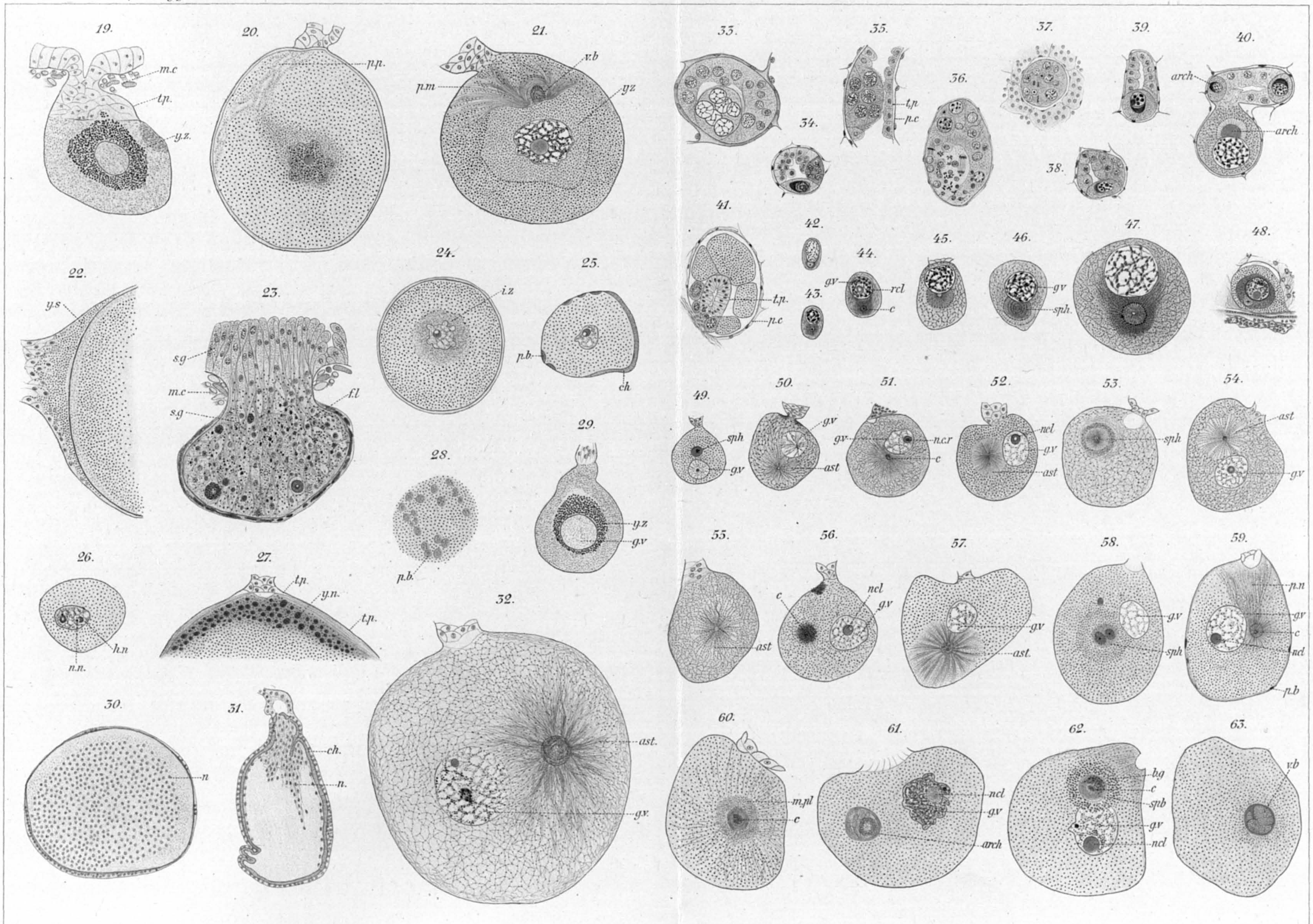
FIG. 59. Section of egg showing germinal vesicle, *g.v.*; nucleolus, *ncl.*; peripheral bodies, *p.b.*; and a centrosome and sphere, *c.*, connected with a fibrous polar protoplasm, or polar mitosome, *p.m.*

FIG. 60. Section of an egg showing a large granular central body, *c.*, a zone of fibrous protoplasm, and a zone of granular metaplasm (archoplasm), *m.pl.*, from which radiate many cytoplasmic fibers or astral rays.

FIG. 61. Section of egg showing archoplasm, *arch.*, containing many vacuoles and a central granular body, the centrosphere; *g.v.*, germinal vesicle; *ncl.*, nucleolus.

FIG. 62. Section of egg showing a conspicuous sphere, *sph.*, containing a central body, centrosome (*c.*), and surrounded by a zone of blue granules, *b.g.* The sphere, *sph.*, red. At the proximal pole, the modified polar mitosome.

FIG. 63. Section of egg showing a conspicuous vitelline-body, *v.b.* The peripheral protoplasmic layer is seen to extend into the central granular mass, and to thus divide the latter into three portions.



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EXPLANATION OF PLATE XV.

FIG. 64. Section of egg showing a conspicuous, deep-red, homogeneous vitelline-body or sphere, containing central granular bodies or centrioles.

FIG. 65. A deep-red vitelline-body, whose fibers are arranged with reference to two poles, at one of which there is a sphere with archoplasm and radial fibers. Method: Merkel's fluid, eosin.

FIG. 66. Section of egg showing peripheral bodies, a germinal vesicle with nucleolus; in the cytoplasm, archoplasmic sphere containing two large spherical central bodies.

FIG. 67. Section of egg showing peripheral bodies, a conspicuous red centrosome, surrounded by a clear zone, which again is surrounded by a zone of blue granules, and this again surrounded by archoplasm. Radial fibers proceeding from this can be traced throughout the entire cytoplasm. Method: Merkel's fluid, Ehrlich's haematoxylin and eosin.

FIG. 68. Section of egg showing germinal vesicle, containing a nucleolus, in which is seen a central spherical body, the endonucleolus. In the cytoplasm is a conspicuous deep-red centrosome, surrounded by a zone of blue granules, and this again by a system of red astral rays extending to the periphery of the egg. Method: Merkel's fluid, Ehrlich's haematoxylin, and acid fuchsin.

FIG. 69. Section of egg preserved in Hermann's fluid, showing a conspicuous homogeneous vitelline-body with indications of astral rays proceeding from it. Method: Hermann's fluid, acid fuchsin.

FIG. 70. Section of egg showing germinal vesicle and nucleolus; and in the cytoplasm a conspicuous vitelline-body containing granular vacuoles, a system of astral rays, and numerous blue granules. Method: Merkel's fluid, Ehrlich's haematoxylin, and acid fuchsin.

FIG. 71. Section of egg showing germinal vesicle containing a large "Hauptnucleolus" and small "Nebennucleolus." Close to the germinal vesicle, a sharply defined sphere with indistinct granular rays, and a large granular central body. Method: Merkel's fluid, eosin.

FIG. 72. Section of egg with germinal vesicle containing nucleolus; in the cytoplasm a deeply staining sphere containing numerous granular vacuoles.

FIG. 73. Section of egg with germinal vesicle, nucleolus, a prominent vitelline-body or sphere, consisting of a sharply defined granular body, partly surrounded by a zone of fibrous archoplasm. Method: Merkel's fluid, haematoxylin, and eosin.

FIG. 74. Section of egg with germinal vesicle, a "Hauptnucleolus," and a "Nebennucleolus." In the cytoplasm a conspicuous sphere with a central lighter granular body, surrounded by a broad zone of deeply staining archoplasm. Method: Kleinenberg's picro-sulphuric, haematoxylin, and acid fuchsin.

FIG. 75. Section showing the same as above. The sphere, deep blue, shows central body, centrosome, and a somewhat regular arrangement of radial fibers to a peripheral concentric protoplasmic zone. Method: Merkel's fluid, safranin, and Lyon's blue.

FIG. 76. Section with germinal vesicle, nucleolus; in the cytoplasm a vitelline-body, consisting of a central granular body which is partly surrounded by a horse-shoe-shaped archoplasmic zone. In the outer portion of this red archoplasm are

three vacuoles filled with blue granules. The entire body is surrounded by a zone of blue granules, which are more numerous at the opening of the horseshoe-shaped archoplasm. Method: Merkel's fluid, Ehrlich's haematoxylin, and eosin.

FIG. 77. Section showing peripheral bodies, a central sphere with astral rays. The center of the sphere consists of closely packed blue granules, and this is surrounded by a thick dense red limiting membrane. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 78. Section showing a proximal polar mitosome, a conspicuous vitelline-body, staining red, and surrounded by a zone of blue granules. Similar granules are seen also at the pole opposite the stalk of the egg.

FIG. 79. Section of egg showing germinal vesicle with a prominent "Haupt-nucleolus," and an extruded "Nebennucleolus"; peripheral bodies at the periphery of the egg; in the cytoplasm, an archoplasmic sphere, containing two central structures. Method: Merkel's fluid, safranin, and Lyon's blue. The sphere alone, blue or greenish; everything else, red. A deep-red circle at the proximal pole.

FIG. 80. Section showing a vitelline-body, consisting of a central, irregular, granular mass, from which radiate straight fibers, which at a certain distance from the central body are again limited by the granules of the cytoplasm. A peculiarly modified fibrous protoplasm exists in the neighborhood of the body, and this is connected with three small refractive bodies imbedded in a strand of protoplasm. Method: Merkel's fluid, Biondi-Ehrlich.

FIG. 81. Section showing germinal vesicle with vacuolated nucleolus; a vitelline-body or sphere, consisting of fibrous protoplasm, containing two central granular bodies. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 82. Section showing germinal vesicle, nucleolus; in the cytoplasm a large, sharply defined, deep-red centrosome, surrounded by two zones of archoplasm and astral rays, the latter on one side being modified into a conspicuous polar mitosome. Method: Merkel's fluid, haematoxylin, and acid fuchsin.

FIG. 83. Section showing germinal vesicle with vacuolated nucleolus; in the cytoplasm a large, homogeneous sphere containing vacuoles with blue granules, partly surrounded by groups of blue granules resembling nuclei. Method: Merkel's fluid, Ehrlich's haematoxylin. The homogeneous part of sphere, unstained.

FIG. 84. Section showing germinal vesicle, a vacuolated nucleolus; in the cytoplasm a large oval fibrous vitelline-body, containing granules, and connected with the periphery of the egg by a modified fibrous protoplasm resembling that of the vitelline-body. Method: Merkel's fluid, Biondi-Ehrlich stain.

FIG. 85. Section showing germinal vesicle with diverticula; a large vesicular nucleolus, containing granules, and in the cytoplasm a large, finely granular sphere, which is partly surrounded by a dense zone of granular archoplasm. Method: corrosive-acetic, haematoxylin, and picric acid.

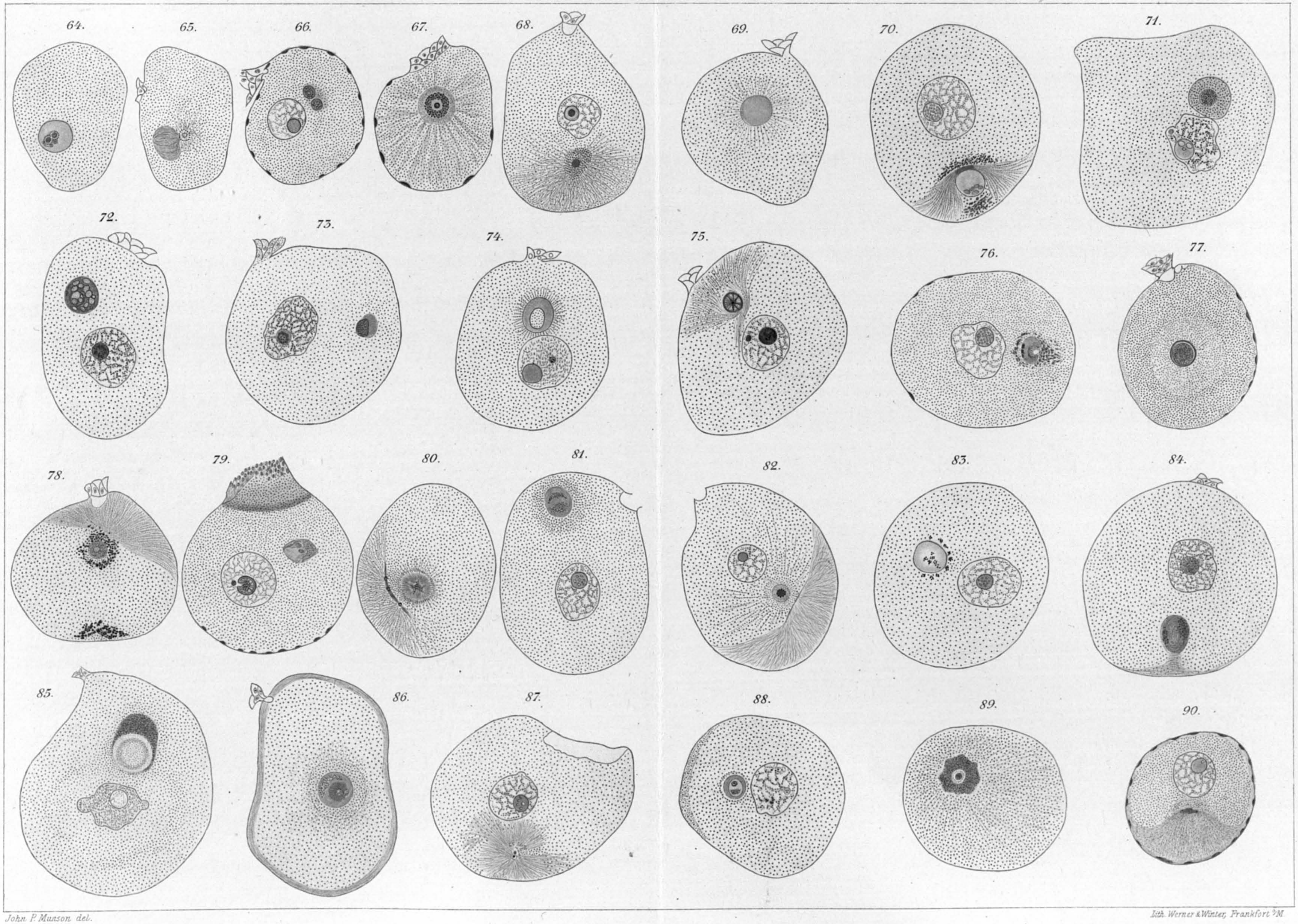
FIG. 86. Section showing a conspicuous, deep-red, fibrous vitelline-body (sphere), containing two central vacuoles with blue granules, and numerous peripheral vacuoles with similar blue granules, the whole being surrounded by a zone of larger metaplasmic granules and astral radiations. Method: Merkel's fluid, Ehrlich's haematoxylin, acid fuchsin.

FIG. 87. Section showing germinal vesicle, a vacuolated nucleolus, and in the cytoplasm one or two centrosomes surrounded by conspicuous silken astral rays that are not sharply limited. Method: corrosive-acetic, haematoxylin.

FIG. 88. Section showing germinal vesicle, a pale "Nebennucleolus"; in the cytoplasm a sharply defined, deep-red, fibrous sphere, containing two centrioles. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 89. Section showing sphere with a distinct centrosome in a lighter space, which is surrounded by a broad zone of archoplasm and astral rays extending throughout the egg. Method: Merkel's fluid, Weigert's picro-carmine.

FIG. 90. Section showing germinal vesicle, nucleolus; in the cytoplasm a granular elongated body at the apex of a fibrous cone of protoplasm. Around the periphery are numerous peripheral bodies. Method: Merkel's fluid, Biondi-Ehrlich.



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EXPLANATION OF PLATE XVI.

FIG. 91. Section showing germinal vesicle, nucleolus; in the cytoplasm a granular yolk-nucleus at the proximal pole; connected with it, a proximal polar mitosome. Method: Merkel's fluid, Biondi-Ehrlich.

FIG. 92. Section showing germinal vesicle; in the cytoplasm a conspicuous aster with a large, deeply staining granular centrosome. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 93. Section showing germinal vesicle with nucleolus; peripheral bodies at the boundary of the egg; a peculiar vitelline-body consisting of a spherical mass of fibrous protoplasm, connected by a stalk with a finely striated protoplasm at the pole opposite the stalk of the egg; a few refractive granules, near the stalk of the spherical body.

FIG. 94. Section showing germinal vesicle with a large vacuolated nucleolus; in the cytoplasm a large homogeneous fibrous vitelline-body, having a mass of granules at one pole.

FIG. 95. Section showing germinal vesicle with diverticulum, and a conspicuous nucleolus; a large vitelline-body, having numerous vacuoles, containing the blue granules and a central structure, probably the centrosome. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 96. Section showing vitelline-body with astral rays, and surrounded by groups of blue granules arranged radially; within the body, also, a group of blue granules. The fibrous portion unstained. Method: Merkel's fluid, Ehrlich's haematoxylin.

FIG. 97. Section showing vitelline-body as a large granular central body, surrounded by a zone of archoplasm and a conspicuous system of radial fibers, which extend to the periphery of the egg, and is especially pronounced at the pole opposite the point of attachment of the egg. Method as above.

FIG. 98. Section showing vitelline-body with several granular central areas. Method as above.

FIG. 99. Section showing germinal vesicle with a large vacuolated nucleolus; in the cytoplasm a large, homogeneous sphere staining deeply, and surrounded by radial fibers. Method: Merkel's fluid, erythrosin, and cyanin.

FIG. 100. Section showing germinal vesicle, containing vacuolated nucleolus; in the cytoplasm a distinct sphere, containing a central granule, centrosome, with radial fibers and granules, which is again bounded by layers of fibrous protoplasm concentrically arranged; the body, surrounded by a zone of large, stainable granules; on the proximal side a modified polar mitosome. Method: Merkel's fluid, Biondi-Ehrlich.

FIG. 101. Section showing central sphere, consisting of a large, spherical, central body staining blue, and composed of blue granules, and another zone of concentrically arranged fibrous protoplasm; indications of astral radiations. Method: Merkel's fluid, Ehrlich's haematoxylin, and acid fuchsin.

FIG. 102. Section showing germinal vesicle with large central nucleolus; in the cytoplasm a conspicuous sphere, consisting of large granules, a central granular body being separated from an outer granular zone by a light ring nearly free from granules. Method: Merkel's fluid and Biondi-Ehrlich stain.

FIG. 103. Section showing germinal vesicle with a large nucleolus. At one pole of the germinal vesicle in the cytoplasm there is an area which shows a faint

radial striation and also a faint concentric striation. It is less granular than the rest of the cytoplasm, and is no doubt the sphere. It resembles the condition seen in Fig. 104, but is less conspicuous.

FIG. 104. Section drawn with a camera with the same magnifying power as the preceding figures, and showing the sphere in connection with the germinal vesicle. The latter contains a large nucleolus. The hyaline protoplasm, constituting the "Kernpol" area, is seen near the point of attachment of the egg. Method: platinum chloride, erythrosin, and cyanin.

FIG. 105. Germinal vesicle with diverticula containing the nuclear network and "Nebennucleoli"; also a large "Hauptnucleolus," showing an opening into the central cavity.

FIGS. 106, 108, 115-118. Nucleoli taken from the living egg of an animal thirteen inches long. In Figs. 115, 116 two "Hauptnucleoli," one large and one small, are present. In Fig. 118 the two are closely united.

FIG. 107. Germinal vesicle with a nucleolus containing within it a network resembling that of the germinal vesicle.

FIG. 109. Section showing a condition of the sphere similar to Fig. 104, cut at right angles to the principal egg axis. Method: same as above.

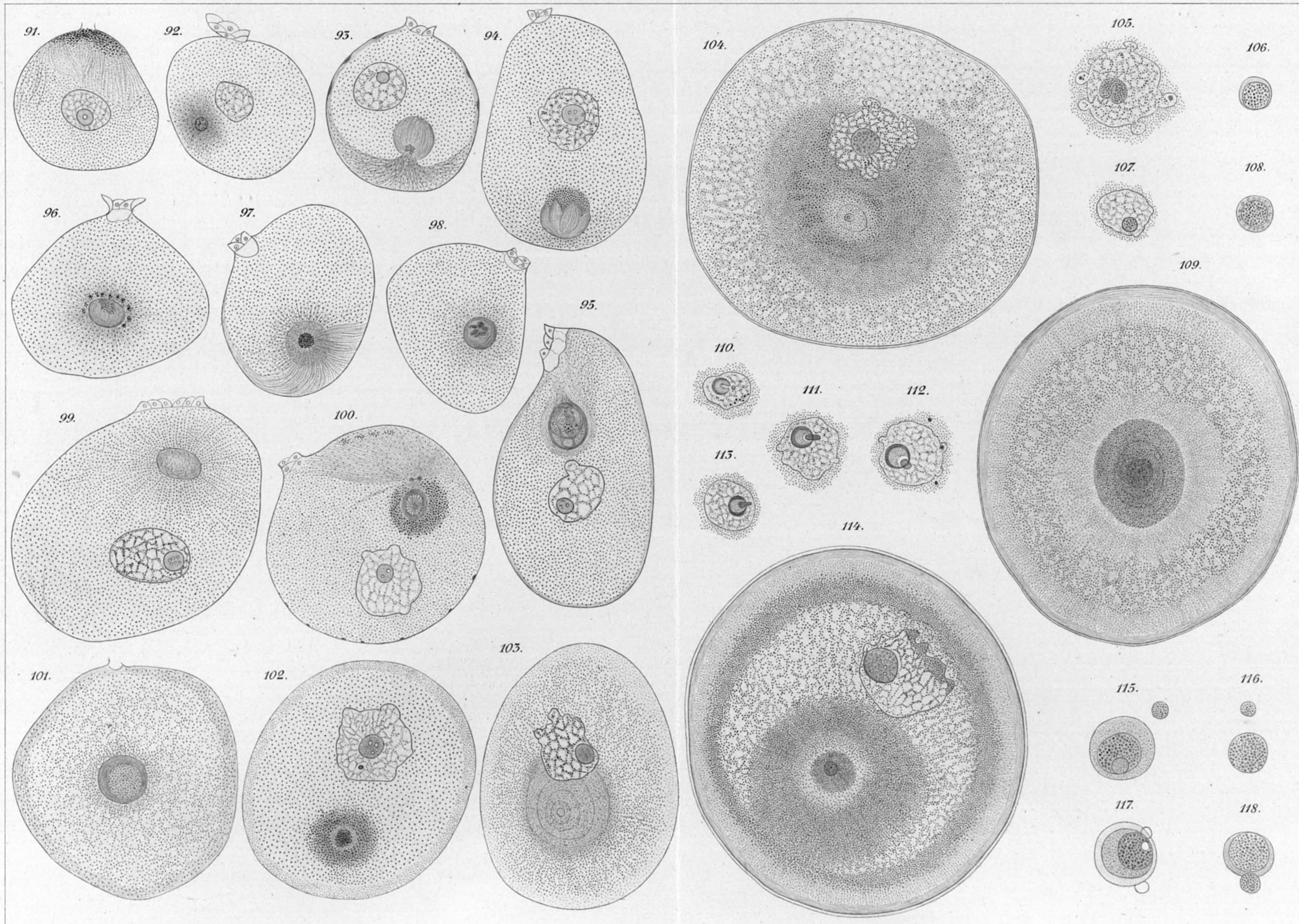
FIG. 110. Germinal vesicle containing a "Hauptnucleolus," from which a "Nebennucleolus" has been extruded.

FIG. 111. Germinal vesicle containing a nucleolus with the "Nebennucleolus" partly extruded.

FIG. 112. Germinal vesicle with a "Nebennucleolus" partly extruded. In one diverticulum of the germinal vesicle is seen a pale "Nebennucleolus," and in the cytoplasm near by are two similar bodies.

FIG. 113. Germinal vesicle with a "Hauptnucleolus," from which a "Nebennucleolus" is partly extruded.

FIG. 114. Section showing the germinal vesicle containing a large nucleolus; and in the cytoplasm a large sphere, in which a radial striation can be made out. The center contains a large clear area, in which a centrosome is difficult to find, when the other portions of the sphere are made prominent. In the present figure the central body with its centrosome and archoplasmic zone was taken from another section of an egg of exactly the same size as the present one, as in that case the central portion was better preserved than the peripheral portion. Method: platinum chloride and Biondi-Ehrlich stain. Excellently preserved.



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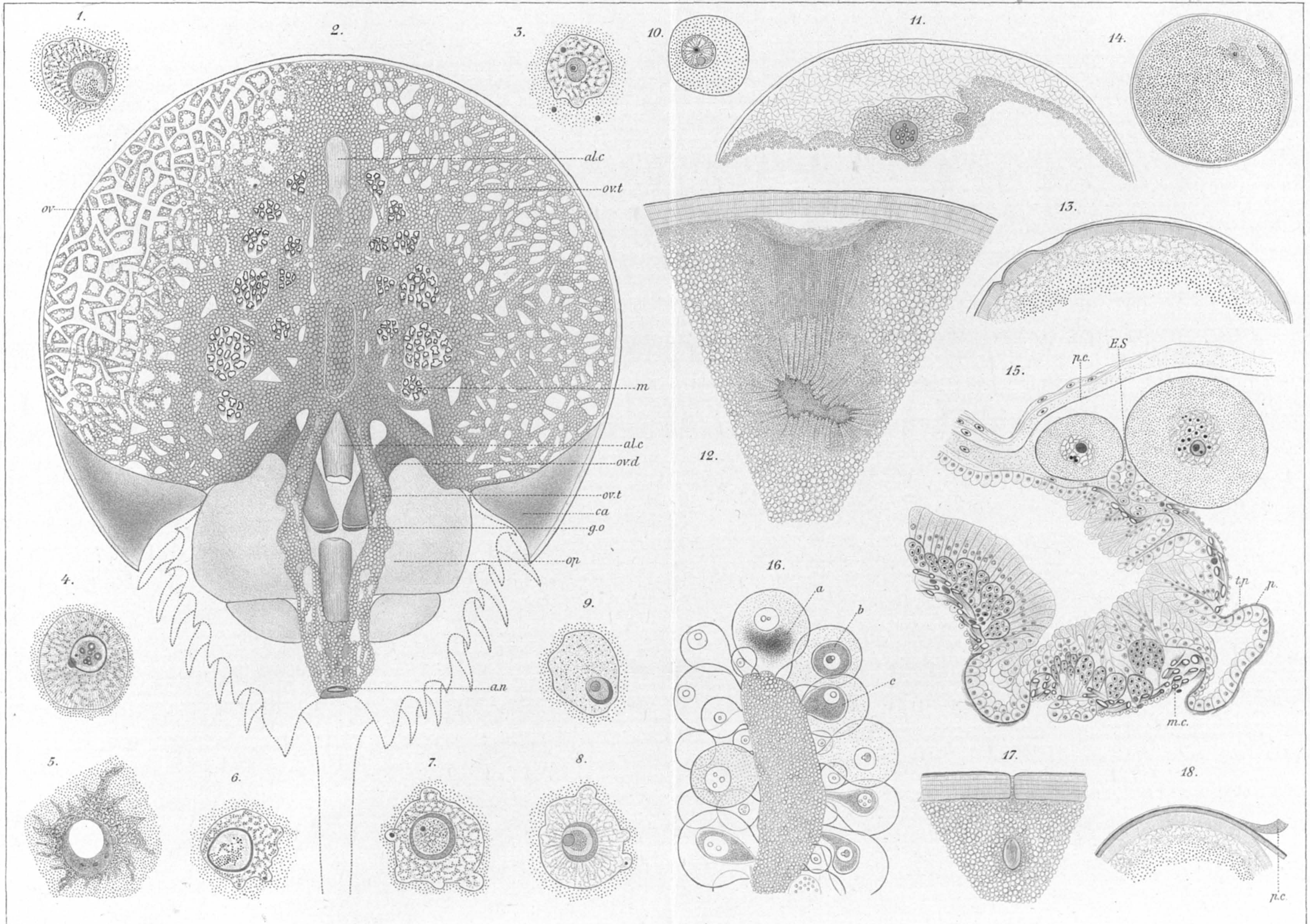

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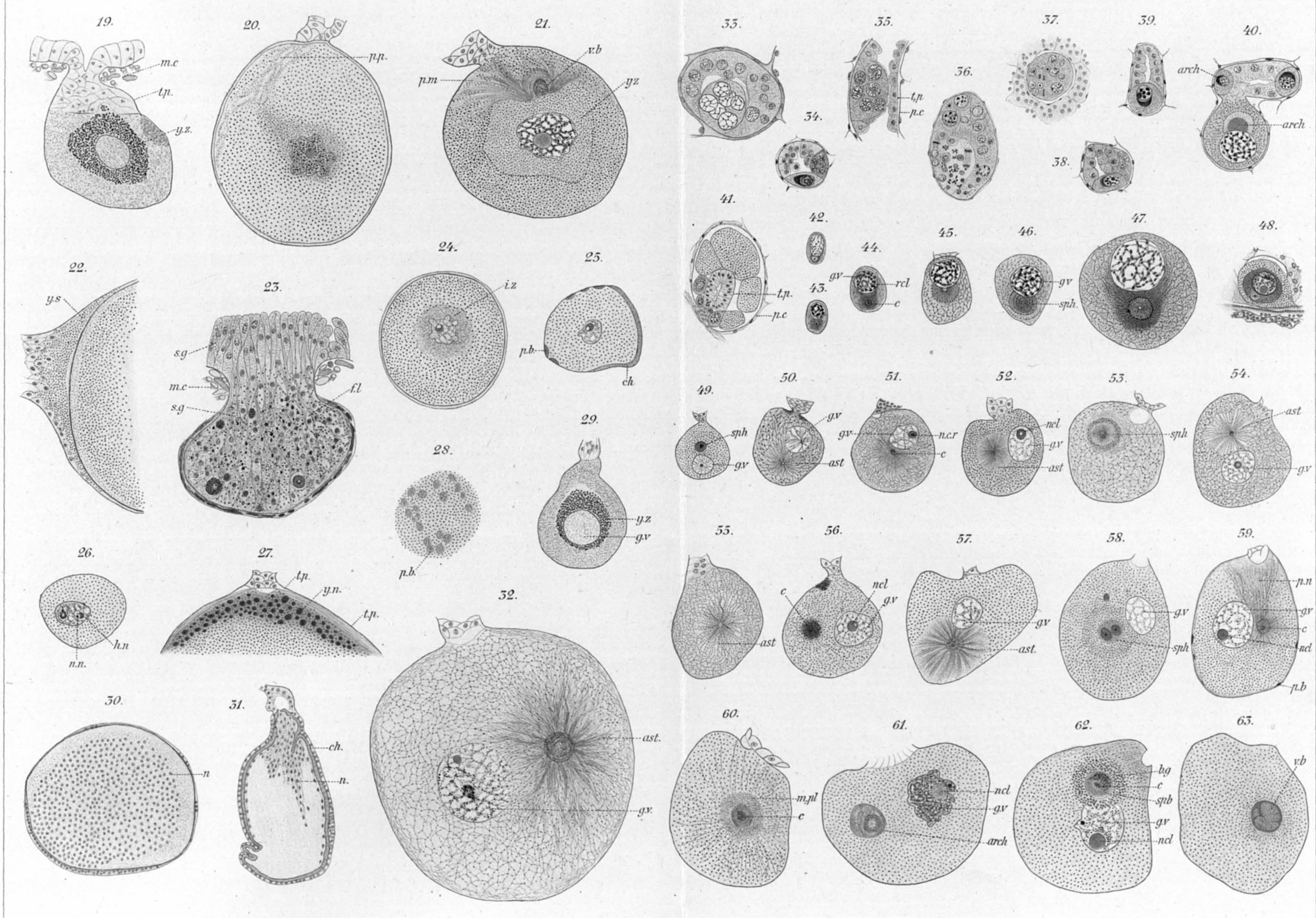
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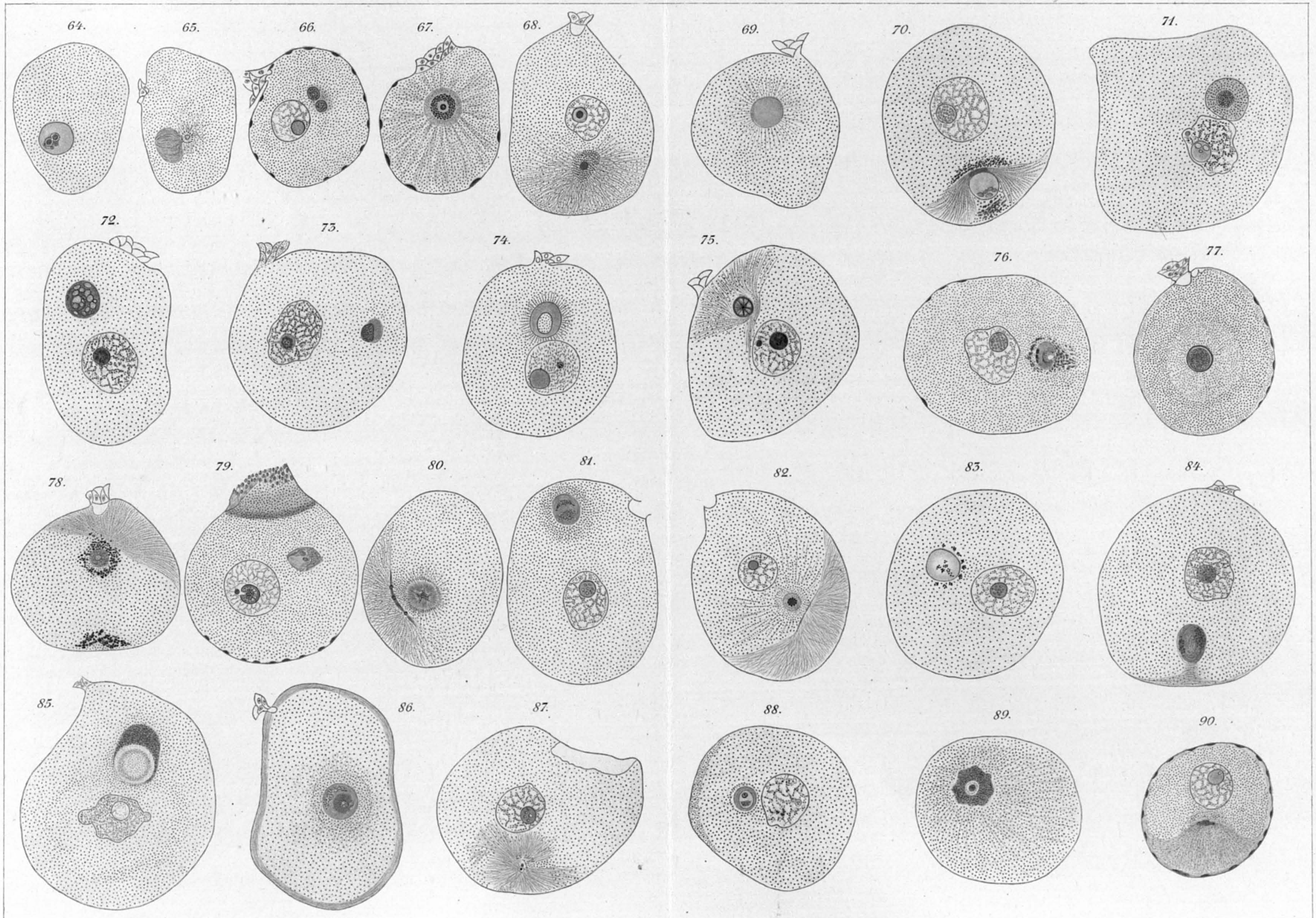

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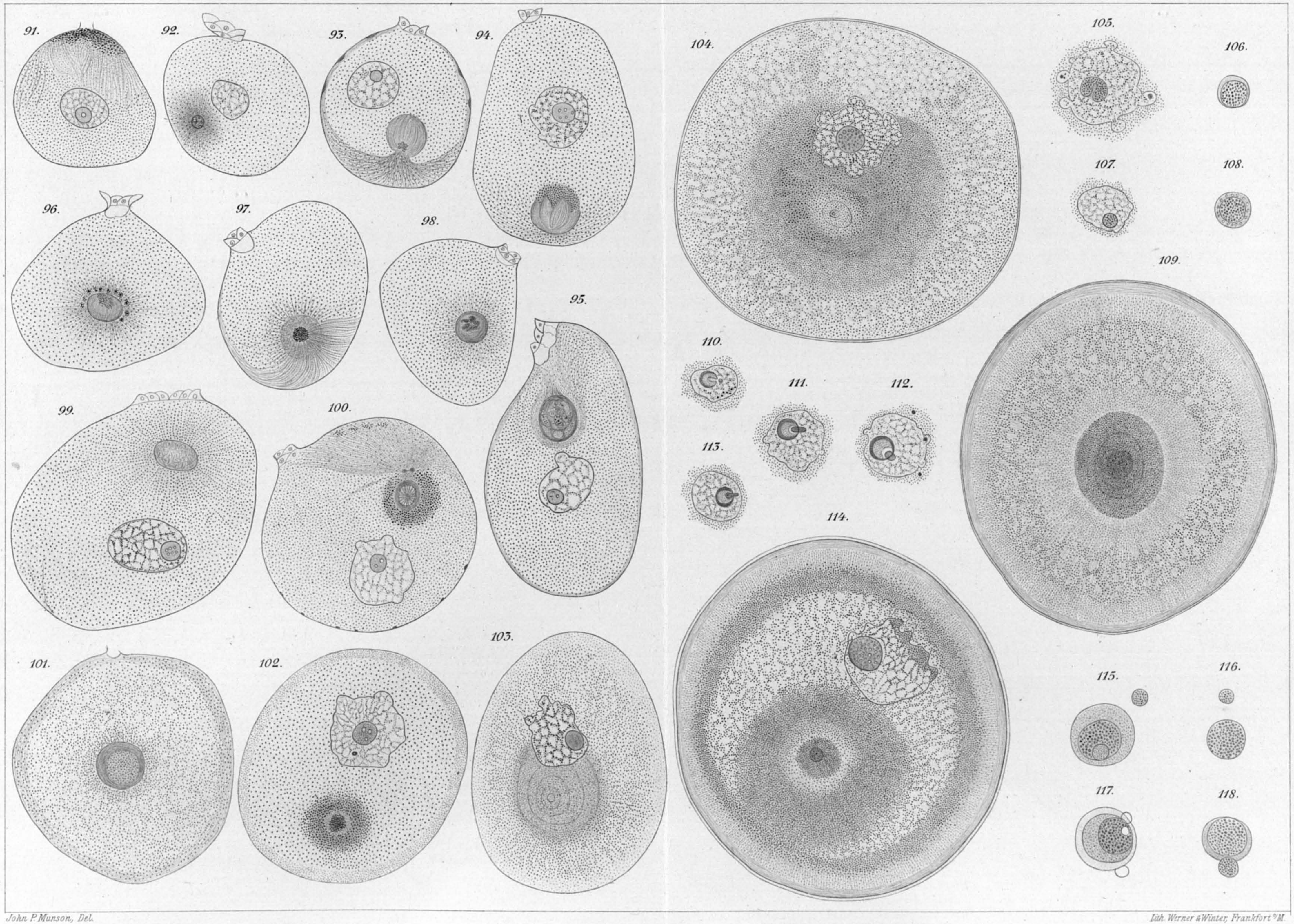

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