RESEARCHES ON THE OOGENESIS OF THE TORTOISE, *CLEMMYS MARMORATA*.

BY

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WITH 7 PLATES.

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Cytological problems are so numerous and yet so prominent in the minds of investigators that it may seem unnecessary to call attention to them again here. Oscar Hertwig, E. B. Wilson, Whitman and others have stated these problems so well that I cannot do better than to refer to these writers, and call attention to the following suggestive quotation from Wilson, **84**: "On the one hand, it has been suggested by Flemming and Van Beneden, and urged especially by Whitman, that the cytoplasm of the ovum possesses a definite primordial organization, which exists from the beginning of its existence even though invisible, and is revealed to observation through polar differentiation, bilateral symmetry, and other obvious characters in the unsegmented egg. On the other hand, it has been maintained by Pflüger, Mark, Oscar Hertwig, Driesch, Watase, and the writer (Wilson) that all the promorphological features of the ovum are of secondary origin; that the egg-cytoplasm is at the beginning isotropous, *i. e.,* indifferent or homaxial, and gradually acquires its promorphological features during its preembryonic history.[1]

In the work of the writers cited above, the literature on the subject up to the present time is extensively reviewed, especially by Wilson, **84.** I may perhaps be justified, therefore, in confining myself in the present paper to a concise statement of my own observations. To show their relation to the work of other observers, and the bearing of my conclusions on present-day theories, would make my paper undesirably lengthy and involved. I hope in the near future to consider this phase of the subject in connection with observations that I have made on the ovarian egg of the crayfish.

My present observations have been made on the ovarian egg of the Tortoise—*Clemmys marmorata.* I have found it a favorable egg to work with, and have been gratified to find so many of my conclusions regarding the history and organization of the egg of Limulus, beautifully confirmed.

Regarding my paper on that subject, I take pleasure in expressing here my appreciation of the favorable mention which it has received; and I desire especially to express my thanks to Prof. C. F. Hodge of Clark University, to Prof. Dr. R. Fick of Leipsic, and to Dr. Fritjof Nansen of Christiania for very kind courtesies and favors.

DESCRIPTION OF THE TORTOISE.

Clemmys marmorata is a tortoise inhabiting the western part of North America. I have had no opportunity to study its distribution. But as I am not aware that it exists east of the Rocky Mountains, and as it is not mentioned in Jordan's Manual of the Vertebrates, I assume that it is not common. It inhabits the ditches, pools and ponds tributary to the Yakima River in central Washington. My identification is based on three specimens found in the Museum of Natural History at Victoria, British Columbia. The following description may not be out of place here:

Carapace ovate, in the adult considerably elongate; margin flaring, not strongly convex; highest in the middle; length from head to end of tail, ten inches; plastron of twelve plates covering the whole undersurface; lobes not hinged; alveolar surface of jaws medium in width; alveolar groove visible; upper jaw slightly notched in front; carapace

[1] **The Cell in Development and Inheritance.**

depressed, not keeled; toes strong, broadly webbed; carapace dark green; plates of carapace in quincunx, and margined with paler brown below; concentric striation of cestal plates visible but not strongly marked; marginal plates not united in the adult, apparently so in the young; adjacent edges of posterior marginal plates forming a compound curve; marginal plates slightly notched in front; marginal plates twelve, with two narrow supernumerary in front; anterior and posterior marginal plates divided by vertical, yellowish stripe; lateral plates with slightly reticulate, yellowish markings. Head small, hind legs clubshaped, larger than forelegs; four toes of hind feet with long claws, five claws in front. Marginal plates ornamented below with conspicuous, bright red lines; feet and tail black, striped with yellow; head and neck green, covered with smooth skin; side of head and neck marked with yellow stripes converging in front of the eye, and crossing the iris. Plastron red or pink, and marked with a bilaterally symmetrical design of brown, which is very characteristic both in the younger and in the older forms.

Methods.—Preserving fluid, picro-nitric; dehydrated in 15-100 per cent alcohol, passed through chloroform and imbedded in paraffine; sectioned five μ, and stained on the slide. The following stains have been used, and have been found useful about in the order named: saffranin, acid fuchsin, Delafield's hæmatoxylin, picro-carmine, eosin, borax carmine, ammonia carmine, orange G., Bismark brown, Vesuvin brown, violet blue, dahlia violet, iodine green, Congo red, anilin blue, anilin red. Many of these stains were also variously combined. Thus: Hæmatoxylin and picric acid; hæmatoxylin and eosin or acid fuchsin, hæmatoxylin and saffranin, etc. I have also found it profitable to study the egg in the living state or merely hardened and killed without imbedding or sectioning. Iodine applied to eggs in this condition under the coverglass gives valuable and interesting results. It is surprising that iodine, which has been found so valuable in the study of living plant tissue, has not been more extensively used in the cytology of animals.

THE OVARY.

On removing the plastron of the adult animal, at the proper season of the year, after the first of May, the ovary, with its numerous large, yellow eggs, is the most conspicuous internal organ exposed. It lies in the abdominal cavity, and when the eggs are grown, or nearly so, fills the abdominal cavity between the hip girdle and the shoulder girdle. One such ovary contains, besides fifteen or twenty comparatively large spherical eggs, measuring three-fourths of an inch or so in diameter, and having a deep yellow coloration, many stages of the growing eggs down to the very earliest, including the oogonia. The smaller eggs are paler in color, and they are distributed irregularly between the larger eggs, as seen in Plate VII, Fig. 92.

The ovary is covered with a thin membrane, evidently a fold of the peritoneal membrane, and each egg is surrounded by two distinct coats of membranous tissue, which are developed from the stroma of the germinal mass. These latter membranes are richly supplied with blood-vessels.

The Germinal Mass.

I have not traced the origin of the germinal layer in the embryo. From the matured ovary, I infer that it develops in connection with the peritoneal lining of the abdominal cavity, the original germ-cells becoming surrounded by thin membranes apparently continuous with that lining membrane.

The germ-cells form a mass rather than an epithelium; and, in the adult ovary, are divided up into distinct masses having more or less the form of flattened oval ridges, slightly longer than broad, and distributed between the larger eggs. It may be that this separation into ridges is due to the growth of the eggs; and that, in the very young ovary, it forms one continuous mass.

The position of the smallest eggs in Plate VII, Fig. 92, indicates the general arrangement of the germinal ridges, one ridge being usually associated with each of the smaller eggs. There is represented in Plate I, Fig. 1, a longitudinal section of such a germinal ridge; Plate I, Fig. 2, a transverse section; and Plate I, Fig. 3, a horizontal section of a germinal ridge.

The Oogonia.

The germinal ridges consist chiefly of spherical cells, the oogonia each one being surrounded by a layer of cells, forming the stroma of the ovary. Each of these stroma cells has a central flattened nucleus, staining deeply, and all forming a circle around each oogonium, their arrangement is such as to suggest a follicle; but the elongated and flattened shape of these nuclei, as well as their closely packed chromatin and consequently deep staining, renders them easily distinguishable from the true follicle, which forms later within. It is this layer which evidently forms the innermost tunic immediately surrounding the follicle epithelium of the growing egg.

The oogonia are spherical or slightly elongated. The nucleus is large and spherical, and shows, at first, a very distinct network, apparently imbedded in the hyaline karyolymph. The oogonia vary in size. In the larger ones, the hyaline karyolymph becomes turbid by the deposit of chromophilous granules. This renders the nucleus more conspicuous, owing to its increased size, and its greater staining capacity. The cytoplasm of these oogonia is rather hyaline, and does not stain at all deeply in nuclear stains. The cytoreticulum can, however, be seen.

The Centrosome.

In the immediate neighborhood of the nucleus, in the cytoplasm, there is a true centrosome. The amount of cytoplasm is not great, but at one pole of the nucleus there is more of it than elsewhere. The centrosome can here be seen in the form of a tiny body close to the nucleus. It may be more or less conspicuous according to the amount of archoplasm surrounding it. The archoplasm is apparently not always present. The centrosome itself, *i. e.,* the central granule, is an exceedingly minute body which comes into view only on focusing, when it often stands out sharp and clear in the center of what seems to be a clear globule, surrounded by a ring of microsomes. In fact, I have seen this clear globule often when I have been unable to make out the central granule—a fact that may be due to defective focusing. From the circle of microsomes surrounding the clear globule, the cytoreticulum radiates, usually becoming crowded along the sides of the nucleus and forming a thin layer investing the nucleus. My experience with these germ-cells has made me very suspicious about all negative evidence concerning the centrosome. That such a tiny granule is not always visible is not strange, when it is noticed how the cytoplasm varies as regards its density and transparency, not only in variously preserved material, but in the living state as well. As is plain from the later history of the centrosome, to be related presently, the presence or absence of the central granule may or may not be important. What is of greater importance, perhaps, than the central granule is the circle of microsomes surrounding it, and from which the fibrils of the sphere seem to radiate. To say that this central granule is homologous to or identical with the ordinary cytomicrosome does not signify much either one way or another, so long as the radial system of fibrils in its immediate vicinity can be shown not only to exist at this early stage, but to persist throughout subsequent stages of the growing egg. The extreme tenuity, also, of the radial fibers of the sphere, especially in the resting condition of the astral system, often makes it seem more surprising that they can be seen at all than that they should at times become obscured by granules or otherwise become invisible, be it due to reagents used in preparing the tissue or to varying states of the fibers. The fact seems to be that the fibers of the cytoreticulum have the power of contraction by which the microsomes are made to approach one another. The circle of microsomes may thus become closely applied to the central granule, giving rise in that way to the conspicuous centrosome which appears when the oogonium divides.

Division of Oogonia.

The marked difference in size of the oogonia must mean that they grow considerably before dividing to form oocytes. This growth consists in a marked increase in size of the nucleus and the greater amount of chromatin or at least chromophilous granules, as well as in a marked increase in the amount of cytoplasm. During this growth the radial zone of the sphere persists (Plate I, Figs. 1, 2, 3).

The division of the oogonia is mitotic (Plate I, Fig. 3). The chromatin becomes massed into a spireme, a spindle is formed with a centrosome at each pole of the spindle. I have no observations on the division of the centrosome to record. But I presume that the two centrosomes result from division of the original centrosome. This fact may be noted, however, that the centrosomes are now very much more conspicuous than the original centrosome, due, as it seems to me, to the tension of the astral system, the massing of the archoplasm around the centrosome and the concentration of the circle of microsomes so as to make them seem merged into the central granule.

When an oogonium enters the division period, it passes through a series of divisions with a very brief period, if any, between each division (Plate I, Figs. 2, 3). Thus it first divides into two (Plate I, Fig. 6). The two cells thus formed (Plate I, Figs. 8, 9) divide again immediately after the reconstruction of the nuclei, giving rise to four similar cells (Plate I, Fig. 9). After a brief interval, these again divide, giving rise to eight daughter-cells, from the original oogonium (Plate I, Fig. 10). As might be expected, the eight cells thus formed are very small as compared with the original oogonium (Plate I, Fig. 3). As can be seen in Plate I, Fig. 3, not only are the spindles associated into groups, but the progeny of each oogonium lies crowded together in nests of two, four or eight cells, and are surrounded, as was the mother-cell, with the inner layer of the stroma cells.

Formation of Follicle.

One of the eight cells, resulting from the repeated division of the oogonium, becomes the oocyte or egg; the rest become the follicle. The follicle cells are, therefore, the sister-cells of the egg. The oocyte is always the central cell. It differs from the follicle cells, so far as I can see, in two important particulars, namely: First, its central position gives it an environment of similar cells; while the follicle cells have one side adjacent to the egg, the other side adjacent to the surrounding stroma cells. One would naturally expect that, if the differences in surroundings could develop polarity, such should be found in the follicle cells, while the egg should be homaxial. On the contrary, the oocyte differs, secondly, from the follicle cells in having a centrosome at one pole, which is evidently absent in the follicle cells.

Differentiation of the Oocyte.

Seeing that the oocyte resulting from the division of the oogonia is always the central cell, I have endeavored to ascertain the probable cause of this. Is the egg a result of its accidental position amid its sister-cells, and do the follicle cells simply become follicle cells because of their accidental position with reference to the oocyte on the one hand and the surrounding stroma cells on the other? In other words, is it a matter of chance which of these cells shall become an egg, or is there some internal difference in the cells, which results from a qualitative division of the original oogonium? Right here, it seems to me, lies the problem of all problems, that of cell differentiation. The matter presents itself here in its simplest form; for we have here evidently to do with the first of those changes, ecdysis or moults through which the original germ separates off from itself the somatic cells, which nourish and protect it, and of which the development of the fertilized egg is only a more complex process. May not this division of the oogonia be compared to a simple process of cleavage, by which there results the most primitive separation into germ and somatic cells? If intrinsic differences arise in this group of cells from a qualitative division of some sort, it ought to afford a strong presumption in favor of such a process in the development of the fertilized egg; if, on the other hand, the difference between the oocyte and the follicle cells is due to cellular interaction, may this factor not be equally important in the later ontogeny?

I have endeavored to discover the law according to which one of the cells of this group comes to occupy a central position, but I cannot say that I have been successful. There appears to be no regularity in the direction of the spindles in the division of the four cells into eight, which might determine the final position of the central cell. I am not prepared to say, however, that no such law exists. Possibly the following facts are sufficiently important, in this connection, to warrant a statement of them.

At the close of the last division, the cells are arranged as seen in Plate I, Figs. 10, 11. The chromosomes evidently become vacuolated, by the secretion of a hyaline matrix, which separates the chromatin substance into granules arranged on delicate fibres of linin, forming a network. At first, all the nuclei thus formed are about equal in size, all being spherical, and having the chromatin more abundant around the periphery. The cytoplasm is relatively scarce, forming only a thin layer around the nucleus of each cell. Comparing Figs. 10 and 11, it can be seen that all the cells just divided, and before the nuclear reticulum is fully formed, are very similar, each having a centrosome surrounded by archoplasms, situated at one pole of the nucleus. In Plate I, Fig. 11, on the other hand, only the central cell has an undoubted centrosome at one pole. Even in this cell, the centrosome is not so distinct as previously, the archoplasm having apparently spread out along the sides of the nucleus, forming a crescent. The centrosome is now a tiny granule, occuping a clear globule, which is surrounded by a circle of larger microsomes. Evidently this is the resting condition of the sphere, the fibrils being relaxed, and the microsomes of the peripheral ring separated from the central granule, rendering the whole slightly more difficult to see. In the peripheral cells, on the other hand, the slight quantity of cytoplasm surrounds the nucleus equally on all sides. But the nucleus in these future follicle cells show, even in this early stage, a comparatively large central body which resembles a nucleolus. It is difficult to believe, however, that it is a nucleolus, since the true nucleoli develop much later. I am inclined to believe that this is the centrosome of the preceding stage. These cells, so far as can be seen, have a radial symmetry, possibly due to the position of the centrosome within the nucleus. The central cell, the oocyte, on the other hand, shows the centrosome in the cytoplasm at one pole, and hence has a more oval form. A distinct polarity, in other words, exists here; and this seems to be due to the relative position of the nucleus and centrosome respectively. I can see no reason whatever, for doubting that this centrosome is the centrosome of the dividing oogonia (Plate I, Fig. 11), and that the transition from the condition existing in Fig. 10 to that of Fig. 11 is a complete transformation, and a formation *de novo* of the centrosome in the central cell. This centrosome is not a transient body, as the subsequent history of the growing oocyte shows. This fact, too, can hardly be denied, namely: That, first, the nucleus of the central cell or oocyte, now the germinal vesicle, in its earliest stage, is derived directly from the chromatin of the dividing oogonia, and hence is a direct continuation of the nucleus of the oogonia; and, second, that the cytoplasm, instead of being formed *de novo* from the young germinal vesicle, is also a direct continuation of the cytoplasm of the original oogonia. The cytoplasm being so limited, it is easy to regard the sphere as the most essential part of the cytoplasm, and from the later history of this body in the growing egg, one is almost tempted to infer that a chromosome organically connected with a centrosome and sphere is sufficient to develop a nucleus from the former and cytoplasm from the latter, *i. e.*, a cell, in the present case, the egg. In this early stage, immediately following the reconstruction of the nucleus, (now the germinal vesicle), from the chromosomes of the spindle, it is hardly possible that metabolic processes in the nucleus could be responsible, on the one hand, for the central nucleolus-like body in the nucleus of the peripheral (follicle) cells; or, on the other hand, for the accumulation, at one pole of the young germinal vesicle, of the slightly granular centrosome and sphere.

The staining reaction of the chromatin and archoplasm respectively is so different that the origin of the one from the other could not even be suggested by it. The chromatin stains deeply and easily in nuclear stains, the centrosome and archoplasm, on the contrary, are conspicuous chiefly for their resistance to nuclear stains.

I conclude from the above facts that there are important internal differences between the follicle cells and the oocyte at this earliest stage. The principal difference is the position of the centrosome in the oocyte, which not only gives it a polarity, but also seems to confer on the oocyte the capacity for growth. It is this centrosome and sphere which later grows so extensively by the absorption of food and the formation of yolk in the later stages as can be seen by examining Plate VII. The probable function of the nucleus in this later growth is suggested by the origin and history of the yolk-nucleus to be described later on in this paper.

THE EGG.

STAGES OF GROWTH.—The history of the growing oocyte presents three successive phases, which may be used as landmarks for descriptive purposes.

The first period extends from the beginning of growth, to the time when the cytoplasm assumes its characteristic granular appearance; at which time, also, the true nucleoli make their appearance in the germinal vesicle.

The second period extends from the first period to the beginning of true yolk-formation; and the *third period* covers that period of growth in which the true yolk-bodies are formed.

Stage I.

The Follicle.—From the very first, the appearance of the oocyte, differs from the follicle cells in that the chromatin of the latter, at first very similar to that of the young germinal vesicle, being in the form of distinct network of irregular granules suspended in a clear nuclear matrix, increases considerably and consequently stains more deeply. As the oocyte grows, the nuclei of the follicle cells lose their spherical form, and become more or less flattened, the elongation being in a plane vertical to the egg surface.

The young germinal vesicle preserves its spherical form. It seems to grow rapidly—much more so, at this time, at least, than does the cytoplasm. At first, the ground substance of the germinal vesicle is clear, showing the chromatin network beautifully. The increase in size seems to be due to the increased amount of karyolymph. At first the chromatin has the form of granules suspended in or attached to a network of hyaline threads, but this lasts only for a brief period. The granules increase rapidly and soon obscure the hyaline matrix and the nuclear network. Consequently the young germinal vesicle stains more deeply now.

At first some of the granules imbedded in the linin network are larger than the rest; are more spherical; stain more deeply, and are distributed about equally throughout the germinal vesicle (Plate VII, Fig. 91; Plate I, Fig. 17). These spherical bodies become obscured as the irregular granulation of the matrix becomes more marked. I suspect that it is these larger spherical chromatin bodies that are more or less directly responsible for the granules appearing around them.

The Nucleoli.—The whole germinal vesicle being filled with granules, till only traces of the original network can be seen, there appears at the periphery one or two bodies larger than the former spherical chromatin bodies, and having all the characteristics of true nucleoli. The principal characteristics which serve to identify this as a nucleolus are: First, its position, which is identical with that of all the subsequent nucleoli which make their appearance; and second, the appearance, within it, of vacuoles, which cannot be seen in the spherical bodies of the network. As regards size and shape, it is not especially distinguishable from the larger spheres of the chromatin network. In its staining reaction, also, it resembles those bodies. On account of its peripheral position, however, I entertain considerable doubt as to its being one of those early spheres merely augmented in size.

The cytoplasm, also, at first becomes more and more turbid, and increases in amount. The granules causing this turbidity are at first very minute, and do not stain so intensely in nuclear stains as the smaller granules of the germinal vesicle. The cytoreticulum is, however, made evident by such stains as eosin and picro-carmine, and even by hæmatoxylin.

The centrosome retains more or less completely the characteristics which it possesses just after the telophase of karyokinesis of the oogonium. It is not always possible to see the tiny central granule. The circle of large microsomes is more easily seen. It encloses a clear, glassy, round opening or globule (Plate I, Fig. 14). The archoplasm surrounding this usually extends to the germinal vesicle, partly enclosing it, thus forming a crescent-shaped granular area, in the widest portion of which the centrosome and sphere can be seen. The granular archoplasm sometimes obscures the centrosome structure either partly or completely, in which case only an irregular mass of granules marks the location of the centrosome. Occasionally, too, the archoplasm flows around the germinal vesicle, forming a ring (Plate I, Fig. 15), at one pole of which the centrosome and sphere are to be seen. This consists of a small central granule, from which radiate tiny fibers in all directions to comparatively large microsomes which, owing to their size, form a dark ring around a light area immediately surrounding the central granule, and across which the slender radiating fibrils extend. From this first ring of large microsomes, there extend similar radiating fibers to a second ring of microsomes slightly smaller than the first and situated about half way between the inner ring and the periphery of the egg (Plate I, Fig. 17). The entire contents of this second ring stain more deeply than the rest of the cytoplasm, but not nearly so intensely as the germinal vesicle. It is in close contact with the germinal vesicle and is indented at the point of contact, so that it, together with the spherical germinal vesicle, forms an oval area in the center of the young egg, surrounded by a layer of less granular protoplasm of about equal thickness (Plate VII, Fig. 97, *p. z.*).

Cytoplasmic Areas.—As this outer protoplasmic layer is distinguishable throughout the later history of the egg, and must be referred to frequently, I deem it best to give it a name, and shall call it the *peripheral zone* (Plate VII, Figs. 97 and 88, *p. z.*). The outer portion of this zone is further differentiated into a thin layer immediately under the egg-membrane. I shall call this the subcuticular layer (Plate VII, Fig. 85, *s. c. l.*).

The line separating the peripheral zone from the germinal vesicle and sphere, taken as a whole, I shall call the cytocœl (Plate VII, Fig. 97, *cy. c.*); and the sphere itself, because of its many peculiarities, not usually recognized as belonging to the centrosome and sphere, I shall call the *cytocenter* (Plate VII, Fig. 97, *c. c.*, Fig. 85, *c. c.*). I take this cytocenter, in the larger eggs, to be the typical centrosome and sphere of the earlier stages, modified by growth and by the deposit of yolk-bodies and yolk-granules.

I am very reluctant to introduce these names into an already over-burdened vocabulary, but see no way of expressing myself without them.

I have said that there are two rings of microsomes surrounding the centrosome, forming the structural basis of the true attraction sphere (Plate I, Figs. 12, 13, 14, 15, 16, 17). That must be true in the very early stages of development. In Fig. 17 is represented a young growing egg more highly magnified. In Plate I, Fig. 13, is represented a section through the attraction sphere at right angles to the egg-axis. But the same appears to be true, also, of the oogonia (Plate I, Figs. 1, 2, 3, 4, 5, and Plate VII, Figs. 93, 94, 95). The number of these circles seems to increase as the egg grows (Plate VII, Figs. 96, 97; Plate II, Figs. 38, 50, 51; Plate III, Fig. 55).

Stage II.

The nucleolus, having first made its appearance in the preceding stage, the number of these now increases rapidly. They correspond roughly with the size of the germinal vesicle, increasing in number as it grows. From a single nucleolus at the beginning, there may be a hundred or more in the fully-grown germinal vesicle—a fact which has led me to doubt their direct descent from the chromatin spheres of the first stage. They vary considerably in the fully-grown germinal vessicle of the third stage (Plate VII, Figs. 86, 87; Plate II, Figs. 30, 43, 63). Their staining reaction is similar to that of chromatin. Hæmatoxylin and borax carmine make them conspicuous. The larger ones usually show the central differentiation or vacuole common to most nucleoli. It is rare, however, that they possess more than one of these (Plate III, Fig. 63, 64, 65).

The germinal vesicle in this egg presents a somewhat remarkable uniformity as regards form. It is spherical, at times slightly oval, and seems to retain this form from its beginning (Plate I, Figs. 11, 12, 18, 19; Plate II, Figs. 43, 44; Plate III, Fig. 62; Plate VI, Fig. 86?), and even late into the final period of growth when the egg becomes filled with yolk (Plate VI, Figs. 85, 86).

Evidence of the nuclear reticulum is present throughout the three stages, though the granular karyolymph renders the network indistinct, especially in cytoplasmic stains, because of the increasing affinity of its granules for such stains. Hæmatoxylin and picro-carmine, however, make certain aspects of the reticulum very evident. The finer strands of the network, so beautifully evident in the early stage, are not now visible; but bead-like rows of deeply-staining spheres, somewhat resembling the smaller nucleoli, appear as isolated or continuous strands running in wavy lines through the granular matrix (Plate III, Fig. 63; Plate IV, Fig. 68; Plate V, Fig. 75; Plate VI, Fig. 82). From the bead-like bodies of which these chromosomes are composed, there seem to radiate delicate fibrils, giving a woolly appearance to the chromosome bands. This is not visible at all times in the same kind of material. I presume it is due to the finer fibrils of the obscured network.

The position of the germinal vesicle, as in the preceding stage, is very constant. It is never exactly at the center of the egg. Its eccentricity seems to be constant, though I cannot say that it is absolutely so. In sections at right angles to the egg-axis, it is central (Plate III, Figs. 57, 58, 59). But in sections parallel with that axis, it is always removed from the center; and, in most if not in all eggs in this and the preceding stage, occupies a position about midway between the egg-center (cytocenter) and the periphery. An inspection of the plates will hardly tend to convince one of the truth of this statement; but in many, if not all cases, the exceptions in this respect are due to the fact that the section does not coincide with the egg-axis, or else does not pass through the center of the germinal vesicle.

The cause of this constant eccentricity of the germinal vesicle is the presence, at the egg-center, of the centrosome and sphere, which in this and in later stages I have called the cytocenter, partly because, although it is a direct continuation of the centrosome of the dividing oogonia, and of the sphere of the earliest stage of the oocyte, it often departs so far from what has generally been understood by the term centrosome and sphere.

The eccentricity of the germinal vesicle is such, that the cytocœl (outer limit of cytocenter) intersects it considerably below the middle (Plate I, Figs. 25, 26; Plate II, Figs. 29, 34, 35, 36, 38, 39, 40, 49, 50, 51). Comparing these figures with Plate I, Figs. 12, 14, 16, 17; Plate VII, Figs. 96, 97, 84, it becomes evident how little this relation has changed even in eggs of the considerable size represented in Plate VII, Figs. 84, 85, 86. I have said that the germinal vesicle is always eccentric. This it must necessarily be so long as the centrosome, and later the cytocenter, occupy the position they do. The cytocenter is always present in this egg, and its persistence throughout this and later stages of the growing egg should be important evidence of the persistence of the centrosome of which it is a direct continuation.

The cytocenter, notwithstanding its many peculiarities, often presents, even in this second stage of the egg, when the cytoplasm has become very granular, the principal features of a typical sphere, with a central granule or granules, such as we find at the beginning of growth (Plate I, Fig. 22; Plate II, Fig. 38; Plate III, Figs. 56, 65; Plate VII, Figs. 96, 97). Furthermore, it often shows very distinctly the surrounding radiations of the true aster (Plate I, Fig. 23; Plate II, Figs. 30, 33; Plate III, Figs. 56, 60; Plate IV, Fig. 68).

The central granule is not always visible. Its place may be occupied by what seems to be a round hole or an unstained transparent body (Plate II, Fig. 49, 51; Plate III, Fig. 65), or by an irregular network (Plate III, Fig. 62; Plate V, Fig. 75; Plate VI, Figs. 78, 82). This network-condition of the center is most frequent in the third stage of the egg, when the yolk-bodies are being formed at the periphery. The network often has a denser central portion (Plate VI, Figs. 81, 83; Plate IV, Figs. 69, 71), in the center of which a deeply-staining body often appears (Plate IV, Fig. 69; Plate VII, Fig. 84). Radiating from this dense central body, are numerous straight fibers passing through the network out into the cytoplasm of the peripheral zone, suggesting most certainly the original sphere with its radial fibers, etc.

A form of the cytocenter, which is more common in the early stages of the second period of growth, is that of a comparatively homogeneous, slightly granular or fibrous mass, as seen in Plate I, Fig. 25; Plate II, Figs. 27, 29, 45, etc. Slight or even pronounced differentiation of this can in most cases be made out as in Plate IV, Fig. 67; Plate III, Figs. 55, 64. The more homogeneous ones of this kind are possibly caused in part by the reagents, for they are occasionally contracted so as to leave an open space extending partly around them (Plate I, Fig. 25; Plate II, Fig. 29). But this does not appear in those represented in Plate II, Figs. 27, 45.

It is difficult to suggest any reason why the reagent should have such effect in one case and not in others. The cytocenter assumes these different forms in the same ovary, treated with the same reagents. Many of the different forms can be seen on a single slide or on a series of slides made from the same serial sections of a single ovary.

While different stains differ in their power of rendering the fibers and granules prominent, the variety of forms can by no means be attributed to the effect of stains.

Staining Effects.—The cytocenter is eminently cytoplasmic in its staining reaction. A center, like that represented in Plate IV, Fig. 69, can be differentiated by acid fuchsin following hæmatoxylin so that alone stands out like a bright red astral body, all other parts of the cell retaining the hæmatoxylin stain.

All parts of the germinal vesicle take the hæmatoxylin stain, and retain it after application of acid fuchsin or eosin. The granular matrix of the germinal vesicle has a paler coloration while the nucleoli are most deeply colored by this stain. When hæmatoxylin is followed by picric acid, the granular matrix is strongly affected, while the nucleoli resist its action, as does also the chromatin network, especially the spherical chromosomes (Plate IV, Figs. 67, 68). Hæmatoxylin has very little effect on the cytocenter. Appearances like those represented in Plate II, Figs. 27, 41, are apparently frequent after this stain. When hæmatoxylin is followed by acid fuchsin, the cytocenter is the most conspicuous part of the section.

Forms like those represented in Plate II, Figs. 29, 45; Plate IV, Fig. 66, are made conspicuous by eosin. A cytocenter of an egg about the size of that represented in Plate II, Fig. 49, from a section stained with eosin, is represented in Plate VII, Fig. 90, as it appears under a high power. That it has the essential structure of the original centrosome and sphere of the very youngest eggs, as that represented in Plate I, Fig. 17, for instance, is quite evident. Owing to the great increase of the amorphous granules of the cytolymph, the fundamental structure is obscured. But it can, nevertheless, be seen that it consists, as in the young egg, of a darker center surrounded by a less dark ring; and this, again, surrounded by definitely limited zones, which again are surrounded by a wider zone of open meshes of fibers apparently in the form of a network. Through this outer network of fibers there can also be seen radial fibers proceeding from the inner zones. I have taken special pains not to exaggerate these features in the section. It is hardly necessary to say that an exact reproduction, in pencil drawings, is difficult if not impossible. Yet Plate VII, Fig. 90, is as near a true picture as I can hope to make it. I feel confident that everything represented in the plates can be seen by any unprejudiced eye, from the slides from which the drawings are made. Indeed, realizing the danger of subjective elements in seeing, I have taken pains to have disinterested parties criticise my drawings from an inspection of the preparations.

Stage III.

The germinal vesicle retains its spherical form, and increases in size with the growth of the egg. Its size, however, does not seem to be constant in eggs of the same size. It also retains its affinity for nuclear stains. The number of nucleoli remains about the same, and they retain their position at the periphery of the germinal vesicle. They still vary in size, and do not seem to grow perceptibly after their formation, being scarcely larger in the large egg, represented in Plate VII, Fig. 87, than in eggs like those represented in Plates IV, V, VI.

The nuclear reticulum remains visible as far as I have been able to trace the germinal vesicle in later stages. After the stage represented in Plate VII, Fig. 87, the egg becomes so filled with yolk that it is difficult to section it successfully.

The distance of the germinal vesicle from the cytocenter increases with the growth of the egg, while its distance from the periphery remains about the same, as is evident from an inspection of the plates. Compare, for instance, Figs. 86 and 87 with Figs. 70, 71, 75. From the very beginning, the germinal vesicle lies in the peripheral zone, between the subcuticular layer and the cytocœl, and continues to occupy that position even as late as those eggs represented in Plate VII, Figs. 84, 85, 86, 87. In Plate II, Figs. 34, 35, 36, 38, 39 and 49, 50, 51, the outer limit of the cytocenter, the cytocœl, is distinctly seen. Note that its relation to the germinal vesicle is about the same in all these cases. It intersects the germinal vesicle at its lower one-fourth. Comparing these figures with the very young eggs of the first stage, as, for instance, Plate I, Figs. 12, 14, 16, 17, it will be seen how closely these relations are maintained throughout the first and second stages. Comparing again these with the eggs of considerable size of the third stage, represented in Plate VII, Figs. 84, 85, 86, it will be seen that the germinal vesicle occupies the same relative position with reference to the cytocœl. The one striking difference between them is the increased distance between the cytocenter and the germinal vesicle. This is especially evident in Plate VII, Fig. 87.

The cytocenter is still visible in eggs as large as that represented in Plate VII, Fig. 87, and in much larger eggs (Fig. 88) where the cytoplasm is crowded with the regular yolk-bodies. The form of the cytocenter in these large eggs is variable. It is still very distinctly differentiated by orange G. (Plate VII, Fig. 86); by acid fuchsin (Fig. 87); and by hæmatoxylin (Plate VII, Fig. 88). In eggs like those of Plate VII, Figs. 84, 85, the cytocenter still retains much of the typical characters of the attraction sphere of younger eggs, it being as yet not invaded by the yolk-bodies. But in eggs like those of Plate VII, Fig. 86, the great increase of the yolk, both around and within it, nearly obscures it. The circular form is still maintained, and distinctly differentiated from all else it is doubtless a remnant of the denser central portion seen in

Plate VII, Fig. 85. So far as my observations extend on these larger eggs, the cytocenter exists wherever the germinal vesicle exists.

The yolk-nucleus is prominent in these eggs. It is especially conspicuous in eggs at the transition between the second and the third stage of growth (Plate IV, Figs. 68, 71; Plate V, Figs. 72, 73, 74; Plate VI, Fig. 78, 79, 80, 81, 82). It is, however, not confined to this transition period, but it is found in eggs of all stages of the second period of growth (Plate I, Figs. 23, 26; Plate II, Figs. 30, 33, 36, 37, 38, 39, 42, 44, 51; Plate III, Figs. 53, 54, 57, 58, 59, 60, 61, 63, 64). The principal characteristics of the second period of growth, besides the appearance of the nucleoli in the germinal vesicle, has previously been stated to be the granular condition of the cytoplasm; that of the third stage, the origin of the true yolk-bodies.

The yolk-nucleus has no such constant morphological feature as the germinal vesicle and centrosome or cytocenter. There is no apparent limit to the number that may exist in an egg (Plate III, Fig. 59; Plate IV, Fig. 69). The size varies greatly even in different sections of the same egg (Plate VI, Fig. 80). They are often circular in section and regular in outline (Plate III, Fig. 54; Plate II, Fig. 37), or they may be oval (Plate IV, Fig. 66); or they may be greatly elongated (Plate IV, Fig. 68); or they may be twisted (Plate II, Fig. 31; Plate IV, Fig. 70); or they may be very irregular (Plate IV, Fig. 71; Plate VI, Figs. 80, 83). In the smaller eggs, they are often located near the periphery (Plate II, Figs. 33, 36, 42; Plate III, Fig. 63; Plate IV, Fig. 68). I assume that these are the bodies that were seen by Clark, **20.** They are also found in the neighborhood of the cytocenter (Plate I, Fig. 23; Plate II, Figs. 37, 38, 51; Plate III, Fig. 54). But their greatest development seems to occur in the neighborhood of the germinal vesicle (Plate III, Figs. 57, 58, 59; Plate IV, Figs. 69, 70), and may partly surround the germinal vesicle (Plate VI, Fig. 79). It is often so close to the germinal vesicle as to make the hypothesis of continuity with the granular nucleoplasm extremely suggestive (Plate II, Figs. 37, 47, and Plate IV, Fig. 69, 70, and Plate VI, Fig. 79, 83). I can discover no law regarding its distribution throughout the egg, except that it usually occurs in that region of the cytoplasm which I have designated the cytocœl (Plate II, Fig. 31, 38, 51; Plate III, Fig. 57, 58, 59, 60, 61; Plate IV, Figs. 67, 69, 71; Plate V, Figs. 72, 75, 77; Plate VI, Figs. 78, 80, 81, 82, 83).

There are good reasons for believing that this yolk-nucleus is more or less fluid, and that it spreads throughout the cytoplasm sometimes by ordinary diffusion; but, at other times, by actual currents. These currents or whatever else it may be, sometimes leave a track or channel behind, in which the granules of the matrix are scarce or almost absent. Consequently the cytoreticulum is especially distinct. I do not know how to designate this effect except by the rather awkward term plasma channel.

These channels are rarely straight; they turn and twist in every direction. Consequently a longitudinal section of such a channel is rare. In Plate II, Fig. 31, is represented a plasma channel in the form of a long, bent and twisted body with an enlargement at each end. Another is represented in Plate IV, Fig. 68. If the granular substance of which this is composed should all flow toward one end, it would leave a temporary track in which the cytoreticulum would be evident. I take it that such a transfer of granular matrix actually takes place. Cases can be found where both longitudinal but more frequently transverse sections of such channels occur. Such an one is very evident in Plate VI, Fig. 78. The material having thus flown together would form a more or less spherical body, as appears in Plate IV, Fig. 66; Plate VI, Fig. 80, and Plate III, Fig. 54; Plate I, Fig. 23; Plate II, Fig. 37. It is evident from these figures, also, that several such spherical masses often exist in the neighborhood of the cytocenter (Plate I, Fig. 23; Plate II, Figs. 30, 37; Plate III, Fig. 54, etc.)

Plasma Channel.—Most interesting facts to me have been such appearances as those represented in Plate V, Figs. 72, 73, 74, serial sections of the same egg, where the plasma channel is actually continuous with the germinal vesicle. These figures are not at all exaggerated, incredible as it may seem. The channel is round in section. The very distinct cytoreticulum within this channel is certainly, so far as can be seen, directly continuous with the contents of the germinal vesicle. At the bottom of this channel the granular mass has accumulated, apparently while flowing out from the germinal vesicle and afterward divided into several currents. In Plate VI, Fig. 81, is another, somewhat elongated form, drawn from reconstruction of serial sections. In the different sections the granular mass forms a ring around the oval open space as is indicated in the drawing. This has been seen in other sections also. Most of the material here, it will be noticed, has become scattered in small, irregular bodies throughout the cytocœl, several such bodies also appearing close to the germinal vesicle.

A comparison of Plate V, Figs. 72, 73, 74, and Plate VI, Fig. 81, with Plate VI, Fig. 83, suggests that the latter is similar to the former, in that it is more or less spherical, and is, to all appearances, connected with the germinal vesicle. In this case, however, the granular substance has not yet flowed out of it. The light areas might suggest, perhaps, that it is not a mere reservoir or a single channel into which the liquid substance is poured, which is also suggested by Plate IV, Figs. 69, 70. Its connection, real or apparent, with the germinal vesicle would be strong evidence in favor of the theory of nuclear origin were it not for the marked difference in staining between it and the contents of the germinal vesicle.

I have reasons for believing that it is not fatty in nature. The usual method of imbedding and mounting emulsifies the oil globules which arise in the egg during the formation of the true yolk-bodies, and causes them to disappear entirely in the prepared material, whilst, as I shall show presently, they are very large and numerous in material not so treated.

The stains which bring this yolk-nucleus most prominently into view are acid fuchsin, saffranin and eosin. With these stains it is more conspicuous than any other part of the egg. It often resembles archoplasm very closely. Its granular characteristics are most marked when stained with acid fuchsin and saffranin.

I have no reason to believe that this yolk-nucleus is at all permanent or that it simply accumulates in the cytoplasm as the egg grows. It may, apparently, be present or absent in eggs of equal size. Thus, compare, for instance, the serial sections a, b, c, d, e, Plate III, Figs. 57-61, with Plate III, Fig. 62, an egg of about the same size. The cytocenter is present in both, but not the yolk-nucleus.

The yolk first makes its appearance as definite spherical yolk-bodies when the egg has attained the size represented in Plates IV, V, VI. It is certainly very suggestive that the yolk-nucleus is so very prominent just before the yolk-bodies begin to form (Plate V, Figs. 76, 77; Plate VI, Figs. 78, 79, 80). Yet the yolk-nucleus is by no means peculiar to this stage of growth, as it occurs just as frequently in the very smallest eggs of the second period of growth (Plate I, Fig. 23; Plate II, Figs. 30, 31, 33, etc.).

The yolk-bodies appear as small, bead-like bodies in little vacuoles, one in each, arising between the subcuticular layer and the peripheral zone of the cytoplasm (Plate V, Figs. 72-74, and Plate VI, Figs. 78, 79, 80). At first they are few, with long intervals between them (Plate V, Figs. 72-74). Later they increase, both in size and in number (Plate V, Figs. 76 and 77).

They next arise in the cytocœl, forming a ring around the cytocenter which has now increased greatly in size (Plate VII, Fig. 84). This zone of yolk-bodies gradually broadens, encroaching, on the one hand, on the peripheral cytoplasmic zone and, on the other hand, on the cytocenter. The yolk-bodies then form rapidly inward toward the central portion of the cytocenter, developing even in the central portion of it (Plate VII, Fig. 86). The yolk-bodies first formed in the cytocœl are the largest; and those latest formed in the cytocenter are the smallest at this stage. The yolk-bodies first formed at the subcuticular layer, although the oldest, do not grow so rapidly. They seem to stain differently from the larger spheres nearer the center of the egg (Plate VII, Fig. 86). In a very much larger egg, the cytocenter can still be seen, having now the appearance of a mass of granules (Plate VII, Fig. 87). At some distance from this, there is a zone forming a ring around the center in which the yolk-spheres are still very small and showing the original reticular cytoplasm filled with small yolk-bodies. Notice the light ring surrounding the cytocenter in Plate VII, Fig. 85. The same feature is visible in a very much larger egg, when the yolk-bodies have become very large and nearly uniform (Plate VII, Fig. 88, *i. cy. c.*). It is now a narrow ring, encircling the cytocenter about half way between the latter and the periphery of the egg, and consisting of closely-packed yolk-bodies of minute size and having considerably less affinity for the stain. Both in this stage and in the preceding the yolk-bodies first formed at the periphery have become quite large; smaller spheres have developed outward, so as to encroach on the subcuticular zone, and likewise inward. Yet the yolk-ring first formed has not been merged into that of the second, but is separated from it by a zone of minute yolk-spheres similar to those of the inner ring.

Comparing the yolk of these eggs with the yolk of eggs merely killed with the same preserving fluid, and preserved in 70 per cent alcohol, I found that there are certain bodies in the latter yolk which are not to be seen in the mounted section (Plate VII, Fig. 89). These bodies vary in size, but some of them are large enough to be seen with the naked eye. They are yellow to the naked eye. Under the microscope they appear white or transparent almost like water. They become especially prominent when iodine is applied to the preparation. This solution stains all the true yolk-bodies a deep yellow, but has no effect on the spheres under consideration. The yellow yolk-bodies, especially the smaller ones, seem to cling to the much larger white spheres so as to form clusters with the white spheres in the center. Smaller spheres or vacuoles can sometimes be seen inside the larger ones. Instead of a larger white sphere, there may be a bunch of very little ones having the same optical properties.

The application of chloroform has a peculiar effect on such a preparation. As soon as the chloroform is applied, the white globules, wherever the chloroform comes in contact with them, break up into innumerable tiny droplets, that go spinning in all directions, setting up strong currents in the whole mass. In this way these bodies all disappear. I take this to be somewhat similar to an emulsion, and the white globules to be of a fatty or oily nature. It is doubtless the chloroform in the ordinary process of imbedding, possibly also the heated paraffine which is responsible for the absence of these globules in the mounted specimens.

The true yolk-spheres differ from these globules in being homogeneous throughout (Plate VII, Fig. 100). Others, slightly smaller, are finely granular (Plate VII, Fig. 99), while still smaller ones are coarsely granular (Plate VII, Fig. 101). Comparing the yolk-bodies in the order of their size, as Figs. 98, 99, 100, 101, it appears that the inner granules grow smaller as the yolk-spheres grow larger, till the homogeneous state is attained in the larger spheres. This might be taken to mean that the same sphere changes in this respect as it grows, were it not for the fact that many of the smaller spheres are as homogeneous as the largest, a fact which may mean that there are specific differences between the various spheres throughout their entire history.

The egg-membrane consists of an outer homogeneous layer which when torn has a fibrous appearance. Within this outer layer there is another one having radial striations (Plate VII, Figs. 84, 85, 86, 87). Surrounding the egg-membrane are the follicle cells forming a compact single layer of approximately equal cells (Plate I, Figs. 1, 2).

As the egg grows, it pushes out more and more from the germinal ridge, and becomes surrounded by a second and a third epithelial layer (Plate I, Figs. 1, 2). The second of these seems to be the original stroma cells surrounding the oogonia within the ridge. It remains quite closely applied to the follicle cells, but seems not to be organically connected with the follicle. This epithelial tunic, as well as the third or outer tunic, is richly supplied with blood-vessels. The third or outer tunic is more loosely applied, forming a loose bag, as it were, around the egg. It arises evidently from the peritoneal part of the stroma of the germinal ridge. When the egg is discharged from the ovary, it enters this outer bag, which serves to convey it to the oviduct.

On the Organization of the Egg.

Throughout the entire history of this egg, both the nucleus and cytoplasm exist. At first the cytoplasm is very much reduced, being apparently little more than an attraction sphere with archoplasm extending part way around the nucleus. There is, outside of this, a thin layer of cytoplasm.

The fibrous nature of this cytoplasm is evident, especially in the neighborhood of the centrosome, which is the focus of the astral system. The astral system is a continuation of the cytoreticulum whose fibers consist of microsomes apparently imbedded in a less stainable substance. The network is imbedded in a hyaline matrix, the cytolymph.

This cytoplasmic structure is evidently continuous with a somewhat similar structure in the nucleus. The bead-like stainable bodies embedded in the linin network and which becomes aggregated into chromosomes, are, so far as their relation to the nuclear reticulum is concerned, similar to the cytomicrosomes. They differ, however, in their staining capacity, as is well known in the case of other eggs also.

Like the cytoreticulum, the nuclear reticulum is evidently suspended or imbedded at first in a clear matrix or karyolymph. In both the cytoplasm and in the nucleus the matrix becomes turbid through the formation of tiny granules. The deposit of these granules takes place in the nucleus slightly earlier than in the cytoplasm, and seems to be accompanied by the formation of nucleoli, just as in the cytoplasm it is accompanied by the formation of yolk-nuclei, and considerably later by the formation of true yolk-spheres.

Evidence tending to show that these granules belong to the matrix both of the germinal vesicle and of the cytoplasm, is afforded, in the first place, by the fact that the nuclear reticulum can be seen even when the egg is filled with yolk, and even so late as when the germinal vesicle lies close under the egg-membrane (Plate VII, Fig. 87), and in the second place, by such appearances as are represented in Plate V, Figs. 72, 73, 74, and Plate VI, Fig. 81, where the granules have temporarily accumulated in one spot, and have left the meshes clear behind them. Here the fibrous cytoreticulum comes again prominently into view. If this is due to a flowing movement in the interfilar substance, it should afford evidence in favor of the reticular theory of protoplasm, as contrasted with the alveolar. The fibrous structure of the cytoplasm becomes again prominent, also, in connection with the cytocenter.

I conclude that this reticulum, both of the nucleus and of the cytoplasm, is the real organized substance of the egg, and that, on the other hand, the matrix with its contained granules possesses no organization, no permanent form, but is like any other chemical mixture of organic substances, the culture medium, so to speak, of the organized substance.

This theory of the permanence of the reticulum of the nucleus and cytoplasm, about which there seems to be some difference of opinion, is supported by the facts observed in this egg regarding the permanency of the attraction sphere and cytocenter and the resulting polarity of the egg.

The Reticulum.—There is certainly good reason to be skeptical regarding the permanency of this reticulum, and consequently of its real morphological value. Reagents are often held responsible for its artificial production. To test the possible effects of reagents in this regard, I have made permanent preparations, by the ordinary histological methods, of the striated muscle of an insect larva, in which the longitudinal fibers and the transverse striations in their minutest details are beautifully shown. On examining the living larva with the same magnifying power, I found that I could see every detail about as plainly in the living contracting muscle. These details in the living muscle were not altered in the least in the prepared material except that the fibers and their verrucosities were made more conspicuous.

The Centrosome.—The regular arrangement of the microsomes and radial fibers, immediately surrounding the centrosome in the resting state, points to a primitive and permanent architecture in the midst of this complex system of fibrils. In my work on the egg of Limulus, **61**, I came to the conclusion that the vitalline-body in that egg is a direct continuation of the centrosome of the dividing oogonia, just as I have been forced here to believe that the cytocenter in the later stages of the egg of the tortoise is a continuation of the centrosome of the dividing oogonia. Wilson, **84**, has intimated that these bodies, like ordinary yolk-nuclei, may be the result of metabolic activity of the nucleus, and that the entire cytoplasm may be derived from the germinal vesicle. The evidence of continuity of the cytocenter with the centrosome is more conclusive in the egg of the tortoise, and it is, furthermore, so radically different from the yolk-nucleus as previously described, that it seems rash to insist on any identity between the two. I can readily admit that so much of the facts as could be shown in the plates of my work on Limulus was not sufficient to establish such a vital point, and that even all that I could gather in four years of continuous study of that egg was not equal to the amount of labor expended. In a prolonged study of this kind, one naturally, I suppose, forms certain general conclusions which cannot be gained from a mere inspection of the plates. Yet, what is evidently needed is positive, not negative evidence of normal not abnormal or pathological conditions.

The evidences, so far produced by writers, of the disintegration and disappearance of the centrosome are all of a negative rather than a positive nature. Negative evidence of such a body as the tiny granule of the centrosome, or even of the surrounding microsomes and radial fibers in the midst of a granular cytoplasm, may well create doubt rather than conviction, to say the least. The few cases of multiple centrosomes, with which we have been made familiar, were either admittedly pathological, or else would seem to be temporary aggregations of the cytoreticulum having no connection whatever with a normal centrosome. About all that can be said concerning pathological centrosomes, if they be centrosomes at all, is that they are what they are admitted to be, namely, pathological. Such evidence must be of doubtful value in estimating normal structures. And when a few such abnormal structures are made the foundation of a whole system of beliefs, as sometimes seems to be the case, what assurances have we that the whole system is not as abnormal as the foundations on which it rests? In the granular cytoplasm, like that of the egg, it is a comparatively easy matter to find centrosomes almost anywhere, especially if one has multiple centrosomes in the eye to begin with. Thus, in mounted sections of eggs like that represented in Plate VII, Fig. 87, a very regular and pretty radial system of fibers surrounds those large yolk-bodies that do not lie too closely packed for it to be seen. But who would say that these yolk-bodies are centrosomes, or that such a system is homologous to a true aster or even comparable to the cytocenter as seen in these eggs?

The evidence of the continuity of the centrosome of the dividing oogonia with that of the growing oocyte, is more satisfactory, it seems to me, in this egg than in the egg of Limulus, and has tended to strengthen my belief in the correctness of the views expressed in my paper on that subject.

That there is some constant relation between the cytoplasm and the germinal vesicle, and that the latter is not merely a chemical mixture, is suggested, first, by its constant position in the cytoplasm, its constant relation to the cytocœl, and hence the cytocenter; second, by its constant form, the persistence of the chromatin network, as well as the peripheral arrangement of the nucleoli. I can find no evidence that these nucleoli are influenced by gravity. No matter in what plane the germinal vesicle is sectioned, the nucleoli are about equally distributed at its periphery.

Chemical Processes.—One is almost forced to believe that the nucleoplasm, which makes its appearance after caryokinesis of the oogonia on the reconstruction of the nucleus is due to metabolic activity of the chromosomes. The granules, which accumulate in this hyaline karyolymph in the second stage of the egg, seem also to be the result of chemical action of some kind.

It is claimed by many observers that chromatin passes out from the germinal vesicle in some eggs and becomes changed in the cytoplasm either directly into yolk, or assuming temporarily the form of yolk-nuclei, is either finally absorbed by the cytoplasm or else later converted into yolk. No satisfactory proof of this elimination of chromatin has yet been given. In the present case, it would be natural, perhaps, to infer that the yolk-nucleus in this egg has such an origin. Its relation to the germinal vesicle is such as to suggest such an origin. But its staining reaction is such as to render that interpretation doubtful. All parts of the germinal vesicle stain deeply in nuclear stains. With possibly the exception of borax carmine, the yolk-nucleus resists these stains more than any other part of the egg. Saffranin, acid fuchsin, and eosin differentiate it, but stain also the granules of the germinal vesicle. Saffranin is especially favorable in this regard. With these stains, therefore, one is strongly impressed by the similarity of the granules of the yolk-nucleus with those of the germinal vesicle, and would very easily be convinced that the presence of the yolk-nucleus in the immediate neighborhood of the germinal vesicle means the origin of the former from the latter. The application of carmine or hæmatoxylin, however, changes matters entirely, for, while the nucleus is deeply affected, the yolk-nucleus is not in the least affected by these stains. Wilson seems to recognize this difficulty, but avoids it by assuming that a chemical change takes place in the chromatin on entering the cytoplasm. It must be evident that such a chemical change would involve a contribution of some sort by the cytoplasm through which the chemical change, if such there be, is brought about.

In my work on Limulus, 61, I differentiated the substance in the neighborhood of the germinal vesicle by means of Lyon's blue. I further noticed that, in certain phases of the germinal vesicles of that egg, a clear zone appeared around it, which I took to mean the extrusion of karyolymph. I believe that interpretation is the correct one. and that the granular yolk-nucleus, even in this egg, is due to chemical union of karyolymph with some substance in the cytoplasm. It is, so far as I can see, an amorphous chemical substance in the cytolymph, more or less fluid and capable of a flowing movement between the fibers of the reticulum The frequency with which it occurs in eggs of the second stage, as well as its frequency in the cytocœl, and its scattered condition would certainly suggest that it has something to do with yolk-formation as is frequently asserted. It is often found in rather small patches scattered throughout the cytoplasm, especially in the peripheral zone. And this condition seems to be most frequent at about that period in the egg's history when the true yolk-bodies arise.

There, are, however, other facts which almost preclude the possibility of this substance being converted into yolk-bodies directly. In the first place, the yolk-bodies arise, first between the peripheral zone and the subcuticular layer where the yolk-nucleus is rarely to be seen. In the second place, the yolk-nucleus exists in the egg when the cytoplasm is merely granular. The yolk-nucleus makes its appearance as soon as the cytoplasm assumes its granular appearance and may be found up to the time of true yolk-formation.

I am, therefore, led to the following conclusion regarding the yolk-nucleus: *It is a kind of metaplasm (or archoplasm) arising in the neighborhood of the germinal vesicle through the combined influence of the nucleus and cytoplasm.* From the place of its formation, it diffuses or flows throughout the cytoplasm where it serves as a culture medium of the living substance of the egg; in other words, it serves as food. The true yolk-bodies are a secretion of the living substance of the cytoplasm.

The growth of the egg seems to be due largely to the growth of the cytocenter, originally the centrosome. As this expands, the germinal vesicle approaches more and more the periphery, and is consequently greatly removed from the cytocenter formerly so near to it. It still retains its relation to the cytocœl, and this is possible because the peripheral zone becomes greatly thinned out owing to the expansion of the cytocenter and the accumulation of yolk-bodies within the latter. Reference to the plates will make this clear. By comparing the different regions of the cytoplasm in the earlier stages with the large eggs represented in Plate VII, the region of greatest growth is easily seen to be the central portion corresponding to the original sphere. The region of greatest growth is also the region where the greatest amount of yolk accumulates; hence the vegetative pole.

Polarity of the Egg.—The polarity of this egg is marked from the beginning and is determined by the relative position of the cytocenter and the germinal vesicle. In the young oocyte, immediately after the telophase of caryokinesis of the oogonium, the centrosome remains, as already stated, at one pole of the nucleus, now the germinal vesicle. The uniaxial feature of the spindle in that division remains in the young oocyte, being determined, in this stage, as in later stages, by the position of the nucleus and centrosome respectively. The pole at which the centrosome is located becomes the vegetative pole, due, as I have shown, to the fact that it especially is the center of cytoplasmic growth.

The egg axis has no fixed relation to other parts of the ovary. I found this to be true, also, of the egg of Limulus. Nor does the eccentricity of the germinal vesicle show any fixed relation to the source of food so far as this can be determined in this egg. The egg of Limulus being related to a germinal epithelium rather than to a follicle, was especially favorable for the determination of that question, the source of food being there easily determined. In that egg, also, the eccentricity of the germinal vesicle bore no constant relation to the source of food. I was led in the study of that egg to the conclusion, which I am forced to accept here, that the egg axis is determined from the beginning by the position of the germinal vesicle and centrosome, and that neither gravity nor the topographical relation of the egg to other tissues has any important influence in the matter.

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REFERENCE LETTERS.

p. z.—Peripheral zone.
c. c.—Cytocenter.
cy. c.—Cytocœl.
i. cy. c.—Inner cytocœl.
i. y. l.—Inner yolk-layer.
o. cy. c.—Outer cytocœl.
o. y. l.—Outer yolk-layer.
sc. l.—Subcuticular layer.
st. m.—Striated membrane.
h. m.—Homogeneous membrane.
fl.—Follicle.
g. v.—Germinal vesicle.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1. Longitudinal section of a germinal ridge of the ovary of the tortoise, showing the stroma cells, nuclei of oocytes and caryokinesis of oogonia; also varying stages of growing oocytes with the beginnings of nucleoli in the germinal vesicle; the centrosome and its transformation into the cytocenter of the large eggs; also the variation in direction of the egg axis. The largest egg is a section at right angles to the egg axis showing the cytocenter, and the yolk-nuclei in the cytocœl. In the next largest egg are also seen various larger forms of the yolk-nucleus. Here also is seen the relation of the egg to the two outer epithelial tunics, in the outer of which a blood vessel is seen.

FIG. 2. Transverse section of germinal ridge near the proximal end, showing various stages of the egg with its follicle, and its tunics as in Figs. 1, with the centrosome and sphere variously developed, and yolk-nuclei scattered throughout the cytoplasm of one of the large eggs. This shows also the variation in the egg axis.

FIG. 3. Horizontal section of the germinal ridge showing oogonia of various sizes, their final division by caryokinesis, and the formation of follicle and oocytes.

FIG. 4. Large oogonium showing surrounding stroma cells, a centrosome with two rings of microsomes—the essential structure of an oocyte at the beginning of growth.

FIG. 5. Oogonium with surrounding stroma cells and centrosome previous to division.

FIG. 6. Spindle stage of the oogonium, first phase of the division period.

FIG. 7. Two-cell stage of the oogonium previous to the second division.

FIG. 8. Second division of the oogonium leading to the four-cell stage.

FIG. 9. Four-cell stage of the oogonium after the second division.

FIG. 10. A group of cells probably resulting from the third division of the oogonia.

FIG. 11. The first differentiation of the oocyte from follicle cells, showing archoplasm and centrosome in the cytoplasm of the oocyte, and a central body in the nucleus of the follicle.

FIG. 12. Growing oocyte, showing traces of the centrosome and the crescent-shaped archoplasmic body at one pole of the nucleus.

FIG. 13. Section of growing oocyte at right angles to the egg axis through the centrosome and sphere, showing its central position in the cytoplasm, a very distinct sphere with its distinct central body, centrosome, in the center of a lighter area.

FIG. 14. Oocyte with pronounced polarity, showing its oval shape, and by the position of the circle of microsomes with indistinct central granule, its relation to the germinal vesicle, a relation which is maintained throughout its succeeding history.

FIG. 15. Oocyte showing the archoplasm forming a ring partly enclosing the germinal vesicle, probably also the first beginning of a nucleolus in the germinal vesicle.

FIG. 16. Oocyte showing the relation of the centrosome and sphere to the

germinal vesicle, a clear area around the centrosome, and an accumulation of granules in the nucleus, probably the beginning of a nucleolus.

FIG. 17. Oocyte more highly magnified, showing the nuclear reticulum, the bead-like chromatin bodies of various sizes, and in the cytoplasm a centrosome with its two rings of microsomes and their relation to the germinal vesicle.

FIG. 18. A more advanced oocyte with the first undoubted nucleoli in the gërminal vesicle, and the cytoplasm filled with granules that obscure the microsome rings of the sphere.

FIG. 19. A still more advanced egg with a conspicuous sphere.

FIG. 20. Growing egg with a more or less fibrous archoplasmic sphere.

FIG. 21. Egg with large, almost homogeneous cytocenter, probably due to the kind of stain (hematoxylin) used.

FIG. 22. Egg showing a typical sphere consisting of a central body, centrosome, a clear zone surrounded by a circle of microsomes, which again is surrounded by a zone of radial fibers extending to the cytocœl, or outer circle of microsomes.

FIG. 23. Growing egg with true peripheral nucleoli in the germinal vesicle, and in the cytoplasm a cytocenter with astral radiations and two oval yolk-nuclei in its immediate vicinity.

FIG. 24. Egg with a rather large, homogeneous centrosome, surrounded by a zone archoplasm.

FIG. 25. Egg with a very large, apparently homogeneous protoplasmic cytocenter with a clear ring around it, and bearing a definite relation to the germinal vesicle.

FIG. 26. Egg with a distinct centrosome, cytocenter with astral radiations surrounding it; also in the cytoplasm a yolk-nucleus.

PLATE II.

FIG. 27. Egg with a large homogeneous cytocenter having very much the appearance of archoplasm.

FIG. 28. Egg with an indistinct circle of microsomes and astral radiations packed into a bundle on one side giving the cytocenter an elongated appearance, and extending nearly to the periphery of the egg.

FIG. 29. Egg showing the open cytocœl, the outer limit of the cytocenter, and its relation to the germinal vesicle. The peripheral zone of cytoplasm extending from this cytocœl to the periphery, is here clearly seen.

FIG. 30. Section of an egg showing a cytocenter in form of an aster with a large yolk-nucleus on one side. The multiplication of nucleoli and their variation in size is here evident.

FIG. 31. A somewhat larger egg with an elongated body, probably a combination of the archoplasm of the cytocenter with yolk-nuclei.

FIG. 32. Section of egg showing a simple spherical cytocenter, possibly a yolk-nucleus.

FIG. 33. Section of egg at right angles to the egg axis, showing cytocenter with astral radiations, and peripheral yolk-nuclei.

FIG. 34. Section of an egg showing cytocenter with twó circles besides the inner one, and the relation of the outer circle, cytocœl, to the germinal vesicle; a slight indication of astral rays.

FIG. 35. Egg showing the cytocenter with a clear open space part way around it, the cytocœl, with some indications of radial striations in the cytoplasm of the peripheral zone.

FIG. 36. Egg showing a cytocenter with a centrosome, and a yolk-nucleus in its immediate neighborhood, and several at the periphery.

FIG. 37. Section of an egg showing an aster-like cytocenter, with a larger yolk-nucleus between it and the germinal vesicle and two opposite, not very distinct in the plate.

FIG. 38. Section of an egg, showing the cytoreticulum very distinctly and a cytocenter composed of circles of microsomes, in the midst of which are several yolk-nuclei.

FIG. 39. Section showing cytocenter with central granules; its relation to the germinal vesicle; yolk-nuclei at the periphery, and one near the center.

FIG. 40. Section of an egg showing cytocenter in form of a sphere, with clear central globule, circle of microsomes, and astral radiations; also many concentric zones in the cytoplasm.

FIG. 41. Section of an egg, showing the chromatin network in the germinal vesicle, numerous nucleoli, and a homogeneous cytocenter having the appearance of archoplasm.

FIG. 42. Section of an egg, showing cytoreticulum, a cytocenter with radial striations apparently continuous with the cytoreticulum, and bounded by a circle of large microsomes, the cytocœl; in the peripheral zone a large yolk-nucleus.

FIG. 43. Section of an egg with a cytocenter in which the central granules are most marked; a clearer zone surrounding it in which the granules of the cytoplasm are not so marked.

FIG. 44. Section of an egg showing three large yolk-nuclei and some smaller ones. The cytocenter of this egg is in another section, not here represented.

FIG. 45. Section showing a large cytocenter apparently homogeneous and feebly stained. It resembles archoplasm.

FIG. 46. Section of an egg, showing cytocenter with an unstained central globule, surrounded by archoplasm, and this again surrounded by an outer irregular ring of archoplasm shading imperceptibly into the general cytoplasm; in the germinal vesicle, nucleoli and chromatin network in the midst of a granular caryolymph or ground-substance.

FIG. 47. Section of an egg, showing cytocenter consisting of a central, granular spherical body, surrounded by a ring of similar substance, a lighter ring separating them; a similar ring bounding the outer circle from which radial striations are evident on two sides, a small yolk-nucleus close to the germinal vesicle.

FIG. 48. Section of an egg showing a homogeneous cytocenter with slightly darker central portion; in the germinal vesicle a headed nuclear reticulum.

FIG. 49. A section showing a cytocenter with a somewhat indistinct central portion and an outer zone of reticulated fibrils.

FIG. 50. Section of an egg of the tortoise showing germinal vesicle with peripheral nucleoli, chromatin bodies and a typical cytocenter resembling an attraction sphere with a central centrosome, and surrounded by an indistinct zone bearing a constant relation to the germinal vesicle.

FIG. 51. Section of an egg showing a cytocenter similar to the preceding as regards the number of circles or zones, but in which the reticulum of the outer zone is more distinct; also yolk-nuclei in the cytocœl or outer limit of the cytocenter.

PLATE III.

FIG. 52. Section of an egg at right angle to egg axis, showing the cytocenter consisting of a dark central body surrounded by a light ring which again is surrounded by a system of radial fibers like an aster; a number of yolk-nuclei in the cytoplasm.

FIG. 53. Section of egg showing the zones of the cytoplasm, the subcuticular, peripheral, the cytocœl and finally the zones of the cytocenter; yolk-nuclei in the cytoplasm.

FIG. 54. Section of an egg showing germinal vesicle with peripheral nucleoli, some of which are vacuolated; and besides the bead-like nuclear reticulum; a fibrous cytocenter resembling an aster; one large, round yolk-nucleus, and two smaller ones.

FIG. 55. Section of egg at right angles to the egg axis showing central body, cytocenter and the cytoplasmic zones around it.

FIG. 56. Section showing cytocenter in the form of an aster and a central body, centrosome and surrounding granular zone; a germinal vesicle showing peripheral nucleoli and chromatin bodies arranged in rows.

FIGS. 57, 58, 59, 60, 61. Serial sections of the same egg, at right angles to the egg axis, showing (a) yolk-nuclei near the germinal vesicle; still more of them in (b) where the section passes through the center of the germinal vesicle, yet more in (c) where many of them are grouped around the pole of the germinal vesicle next to the cytocenter; the aster-like cytocenter in (d) surrounded by numerous yolk-nuclei; the central sphere (e) with radial fibres on one side and numerous yolk-nuclei arranged in a circle around it.

FIG. 62. Section of an egg, showing germinal vesicle, peripheral nucleoli, nuclear network, and a cytocenter consisting of a network of deliate fibres.

FIG. 63. Section showing germinal vesicle, with peripheral nucleoli, and bead-like chromosomes imbedded in a somewhat granular karyolymph or nuclear matrix. A large spherical cytocenter with distinct astral rays evidently continuous on one side with the cytoreticulum, at the periphery a large yolk-nucleus.

FIG. 64. Section of an egg showing germinal vesicle; cytocenter with archoplasmic zone and astral rays in the cytoplasm and numerous yolk-nuclei in the peripheral zone of the cytoplasm.

FIG. 65. Section of egg showing germinal vesicle and cytocenter; section not parallel with egg axis; cytocenter and germinal vesicle in different sections hence the closeness of one to the other. The cytocenter appears diagrammatic, but a true representation of very many of these centers, the large microsomes being slightly exaggerated.

PLATE IV.

FIG. 66. Section of an egg showing a single large vitelline-body of homogeneous substance resembling archoplasm occupying a somewhat eccentric position in the cytoplasm and staining very similarly to the granular matrix of the germinal vesicle.

FIG. 67. Section showing germinal vesicle with distinct nuclear reticulum, composed of spherical chromosomes of various sizes, and also numerous peripheral nucleoli; a cytocenter with a clear center and two archoplasmic zones; in the cytoplasm, also, numerous small yolk-nuclei arranged principally in the cytocœl.

FIG. 68. Section showing germinal vesicle with chromatin network; cytocenter with astral rays; an elongated yolk-nucleus connected with the periphery and a smaller one at opposite pole similarly connected.

FIG. 69. Section showing germinal vesicle; a cytocenter with central body surrounded by a denser zone, and an outer reticular zone. In the cytocœl, are numerous small yolk-nuclei and near the germinal vesicle a large irregular body staining like the smaller ones and apparently continuous with the germinal vesicle.

FIG. 70. Section of egg showing a reticulated irregular cytocenter; a distinct germinal vesicle in the neighborhood of which there is a conspicous irregular yolk-nucleus apparently continuous with the granular matrix of the germinal vesicle.

FIG. 71. Section showing germinal vesicle with distinct nuclear reticulum, and peripheral nucleoli; a reticulated cytocenter with central condensation; a large round yolk-nucleus and several very irregular ones forming a more or less continuous mass in the cytocœl; several smaller yolk-nuclei in the neighborhood of the germinal vesicle.

PLATE V.

FIGS. 72-74. Three serial sections of an egg, showing sections of the germinal vesicle, plasma channel, yolk-nuclei and cytocenter; a few true yolk spheres near the periphery close to the subcuticular layer.

FIG. 72. Section showing connection of the plasma channel with the germinal vesicle; the cytoreticulum in the plasma channel very distinct, and an irregular yolk-nucleus apparently connected with it; two other large yolk-nuclei and many small ones in the cytocœl, and true yolk-bodies at periphery.

FIG. 73. Section of same egg as 72, showing further the connection of the plasma channel with the germinal vesicle on the one hand and the yolk-nucleus on the other hand; several yolk-nuclei in the cytoplasm; a distinct cytocenter with evident astral radiations, showing the contrast between a true cytocenter and the yolk-nucleus; the first yolk spheres as in the preceding section.

FIG. 74. A section of the same egg as Fig. 72 and 73, showing yolk-channel; and the various forms of yolk-nuclei and their distribution.

FIG. 75. Section of an egg showing germinal vesicle; yolk-nuclei and their relation to the germinal vesicle and the cytocœl; a large oval yolk-nucleus; a reticulated cytocenter.

FIG. 76. Section of egg showing numerous small yolk-nuclei and their apparent connection with the germinal vesicle; numerous true yolk-spheres near the subcuticular zone.

FIG. 77. Section of egg showing nucleoli distributed apparently throughout the germinal vesicle, but really due to the fact that the section has passed near one pole of the germinal vesicle which on that account is smaller than

usual, numerous yolk-nuclei distributed throughout the cytoplasm; an irregular cytocenter; first yolk-bodies at the subcuticular zone of cytoplasm.

PLATE VI.

FIG. 78. Section of an egg showing germinal vesicle; numerous yolk-nuclei in cytocœl; a plasma channel; a reticular cytocenter; the first true yolk-bodies.

FIG. 79. Section of an egg through the germinal vesicle, nearly at right angles to the egg axis, showing the germinal vesicle and the relation to it of the large yolk-nucleus forming an incomplete ring around the germinal vesicle; numerous smaller yolk-nuclei scattered throughout the cytoplasm; also the first yolk-bodies at the subcuticular layer of cytoplasm.

FIG. 80. Section of an egg, showing germinal vesicle, peripheral nucleoli, and many small yolk-nuclei surrounding it; a conspicuous aster-like cytocenter, and numerous large yolk-nuclei both regular in outline and also very irregular and staining very deeply; yolk-bodies at the periphery.

FIG. 81. Section of an egg, showing a germinal vesicle with peripheral nucleoli and many small yolk-nuclei surrounding it; a plasma channel apparently connected with the germinal vesicle, as seen from reconstructed serial sections; numerous yolk-nuclei occupy the cytocœl and seem to surround the plasma channel as if issuing from it; a somewhat reticulated cytocenter having no similarity to the yolk-nuclei.

FIG. 82. Section showing the germinal vesicle with bead-like chromosomes, peripheral nucleoli; yolk-nuclei, and their relation to the germinal vesicle and to the cytocœl; a spherical, definitely bounded cytocenter with granular center and reticulated outer portion.

FIG. 83. Section showing germinal vesicle with nucleoli; and a large spherical yolk-nucleus connected with the germinal vesicle; numerous smaller ones in the cytocœl; a spherical cytocenter.

PLATE VII.

FIG. 84. Section of an egg, showing germinal vesicle; a fibrous cytocenter, surrounded by a zone of cytoreticulum; the order of yolk formation in zones—the outer yolk zone just under the subcuticular zone, and the second in the cytocœl.

FIG. 85. Section of an egg considerably larger than the previous, showing the relative increase of the two yolk zones and the relation of these to the germinal vesicle and to the cytocenter, *c.c.* The cytocenter is surrounded by a zone of protoplasm, the inner cytocœl, *i. cy. c.;* this again surrounded by the inner yolk layer, *i. y. l.*, this surrounded by the outer cytocœl *o. cy. c.*, followed by the outer yolk layer, *o. y. l;* outside of this the subcuticular layer, *sc. l.* Surrounding the subcuticular layer is the striated membrane, *st. m.*, outside of which is the homogeneous membrane, *h. m.*, the two constituting the egg membrane or chorion. The follicle, *fl.*, forms a single layer of cell surrounding the egg.

FIG. 86. Section of an egg more advanced than the preceding as is evident from the greater development of the yolk. The yolk-bodies have encroached on the cytocenter which is reduced to a crescentic mass of granular substance staining differently from the rest of the cytoplasm. The section shows

the relation of the yolk to the cytocœl and the relation of the latter to the germinal vesicle.

Fig. 87. Section of large egg, showing the germinal vesicle at the periphery, and the cytocenter, now an irregular mass of deeply staining granules. The outer zone has become narrowed by the encroachment of the inner yolk layer; the inner cytocœl still visible as a spongy zone of protoplasm.

Fig. 88. Section of a large egg, showing the cytocenter surrounded by fully developed yolk granules; the inner cytocœl, *i. cy. c.*, now a narrow zone of uniformily small yolk-bodies; the peripheral zone, *p. z.*, now filled with well developed yolk-bodies especially in the inner yolk layer, *i. y. l.;* the outer cytocœl, *o. cy. c.*, evident from the less perfectly developed yolk-bodies resembling those of the inner cytocœl, *i. cy. c.;* the outer yolk layer, *o. y. l.*, with well developed yolk-bodies: and the subcuticular layer, *sc. l.*

Fig. 89. Portion of the yolk of the largest eggs showing yolk formation and oil globule. Killed in the usual way, but not imbedded. Mounted on the slide and treated with iodine, showing oil globules unstained by iodine; arrangement of yolk-bodies around these large unstained globules, and their occasional breaking up into clusters of smaller globules often containing a large one in the center.

Fig. 90. Section of a cytocenter highly magnified, showing a small dark central body, surrounded by indistinct zones of granules, the outer less dense, and this again surrounded by a third outer zone apparently loosely reticulated in the meshes of which there seems to be a system of astral rays proceeding from the central mass.

Fig. 91. Section of a germinal vesicle in the early stage of development before true nucleoli have developed, and before the nuclear matrix has become opaque by the formation of granules.

Fig. 92. The ovary of the tortoise as seen with the naked eye, showing the various stages of the developing eggs. The smallest eggs mark the position of the germinal ridges lying between the larger eggs.

Figs. 93, 94, 95. Oogonia showing increase in size previous to division to form oocytes; stroma cells, with no follicle yet formed; nucleus in various stages of growth; the attraction sphere; the relation of the latter to the nucleus and to the outer protoplasmic zone of cytoplasm, the peripheral zone.

Figs. 96 and 97. Young growing oocytes, showing in the young germinal vesicle, bead-like chromatin network apparently imbedded in band of unstained linin substance; nucleoli forming in the interior, some of them already vacuolated; typical spheres with indistinct centrosomes, but distinct circles of microsomes with evidence of radial striations proceeding outward from the center; the relation of the sphere (cytocenter) to the germinal vesicle; the evident cytocœl, *cy. c.*, Fig. 97, separating the cytocenter, *c. c.*, from the peripheral zone of cytoplasm, *p. z.*

Fig. 98. Yolk-bodies of various sizes stained in iodine and showing in the larger a central body that is not so strongly affected by the stain and appearing like vacuoles but evidently some differentiated solid substance.

Fig. 99. Yolk-body composed of minute spherical granules throughout.

Fig. 100. Yolk-sphere homogeneous throughout.

Fig. 101. Smaller yolk-body filled with smaller yolk spheres but larger than those of Fig. 99.

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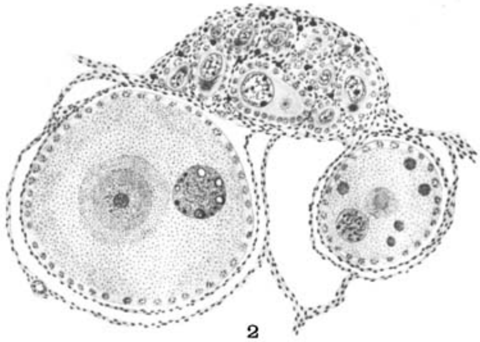

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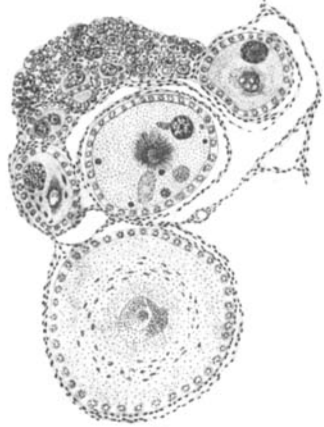

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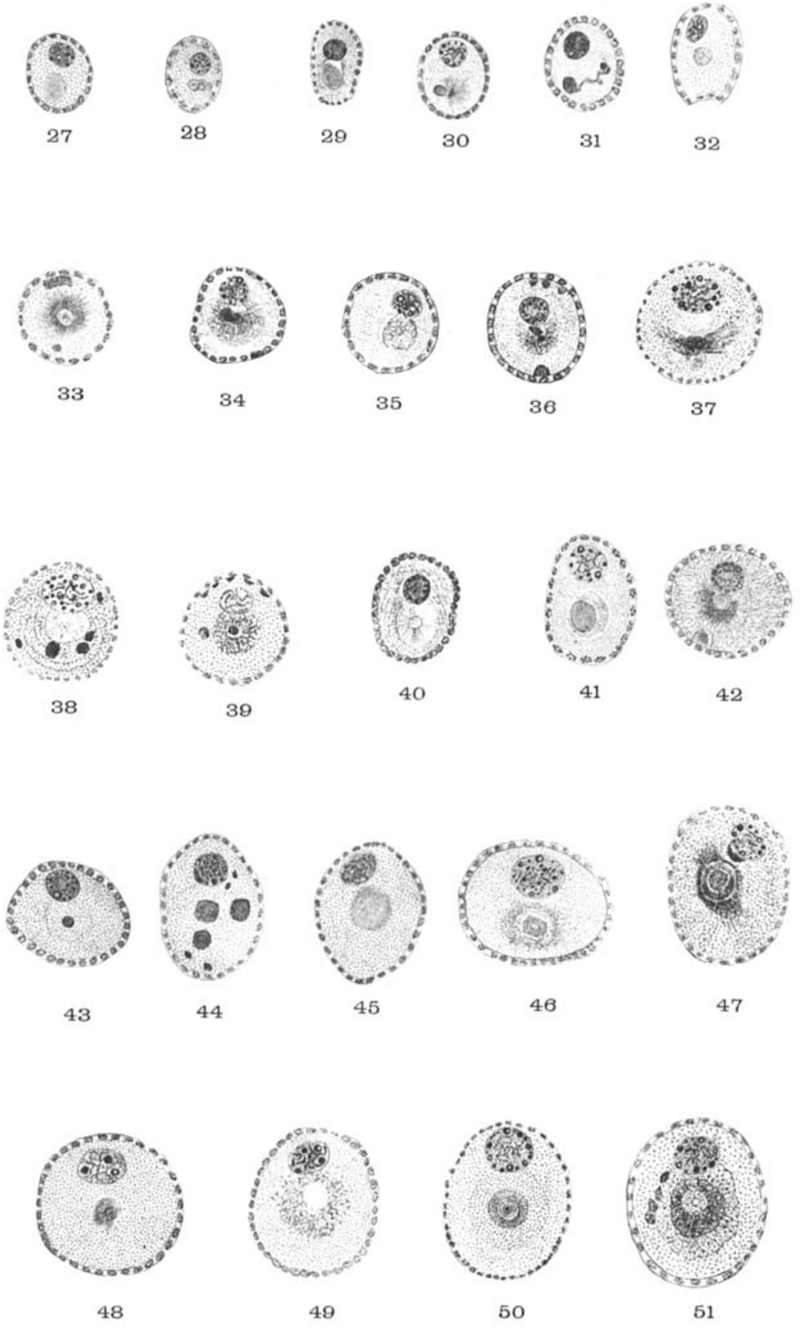

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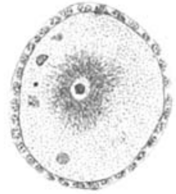

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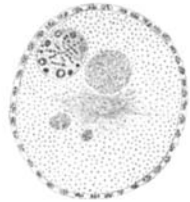

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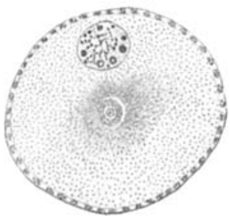

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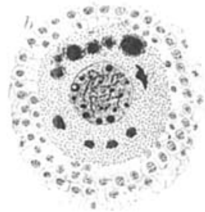

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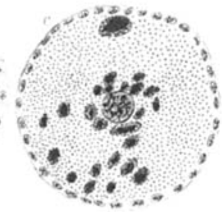

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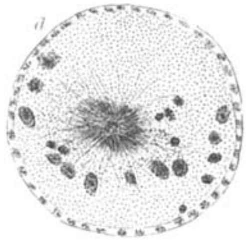

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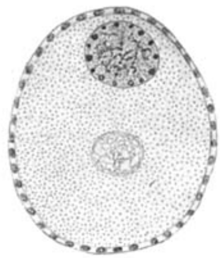

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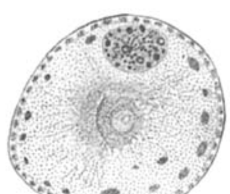

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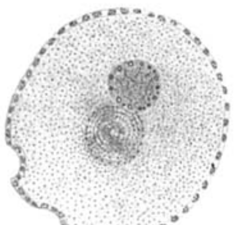

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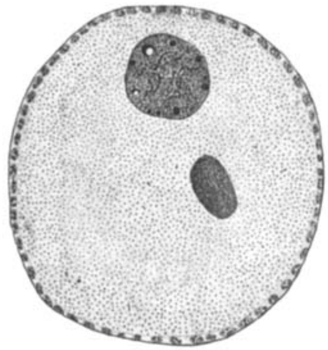

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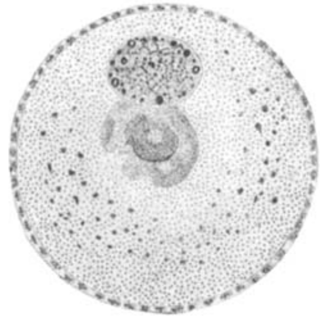

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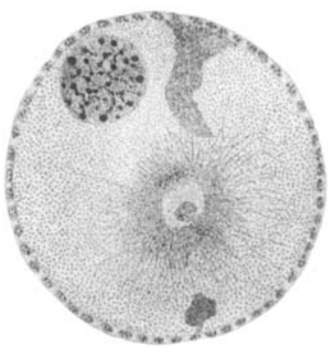

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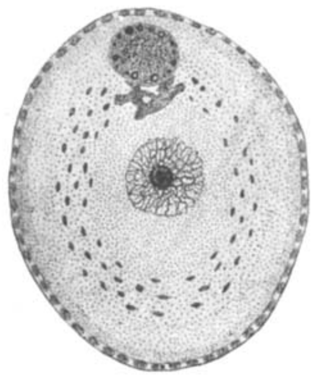

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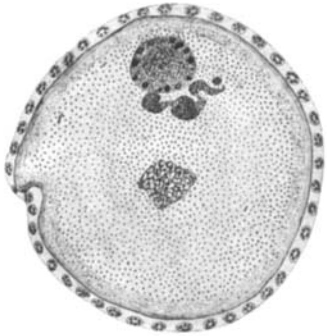

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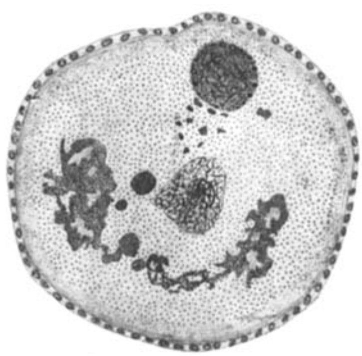

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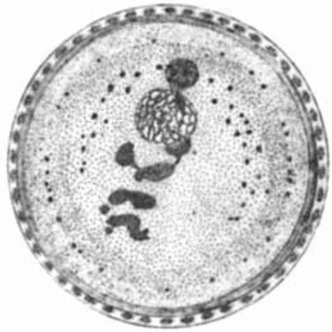

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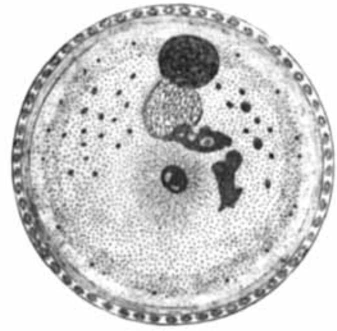

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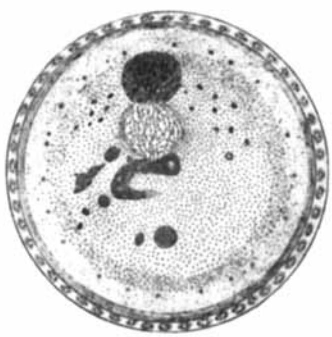

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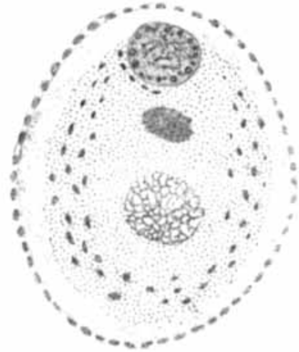

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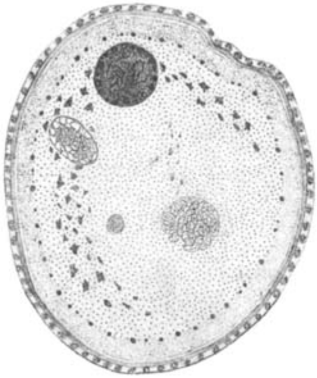

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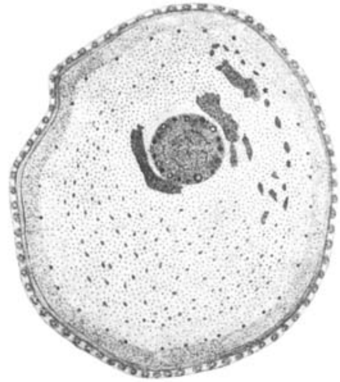

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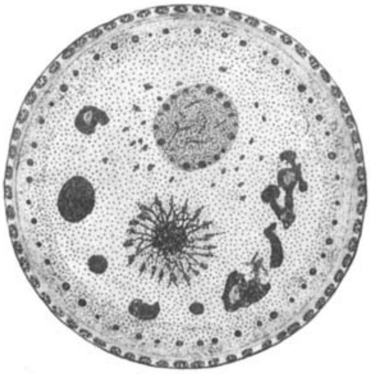

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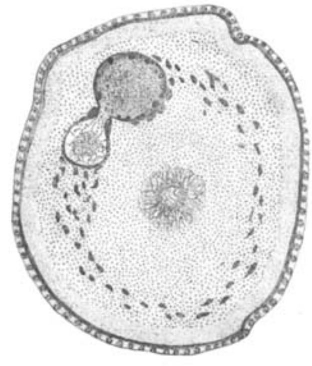

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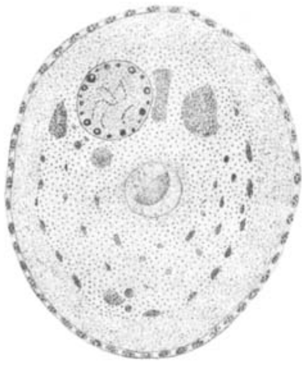

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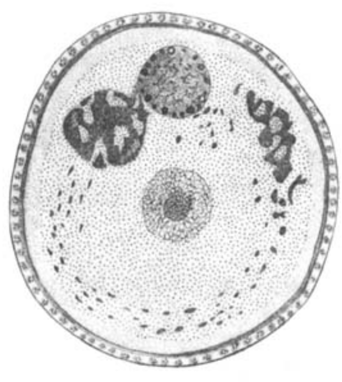

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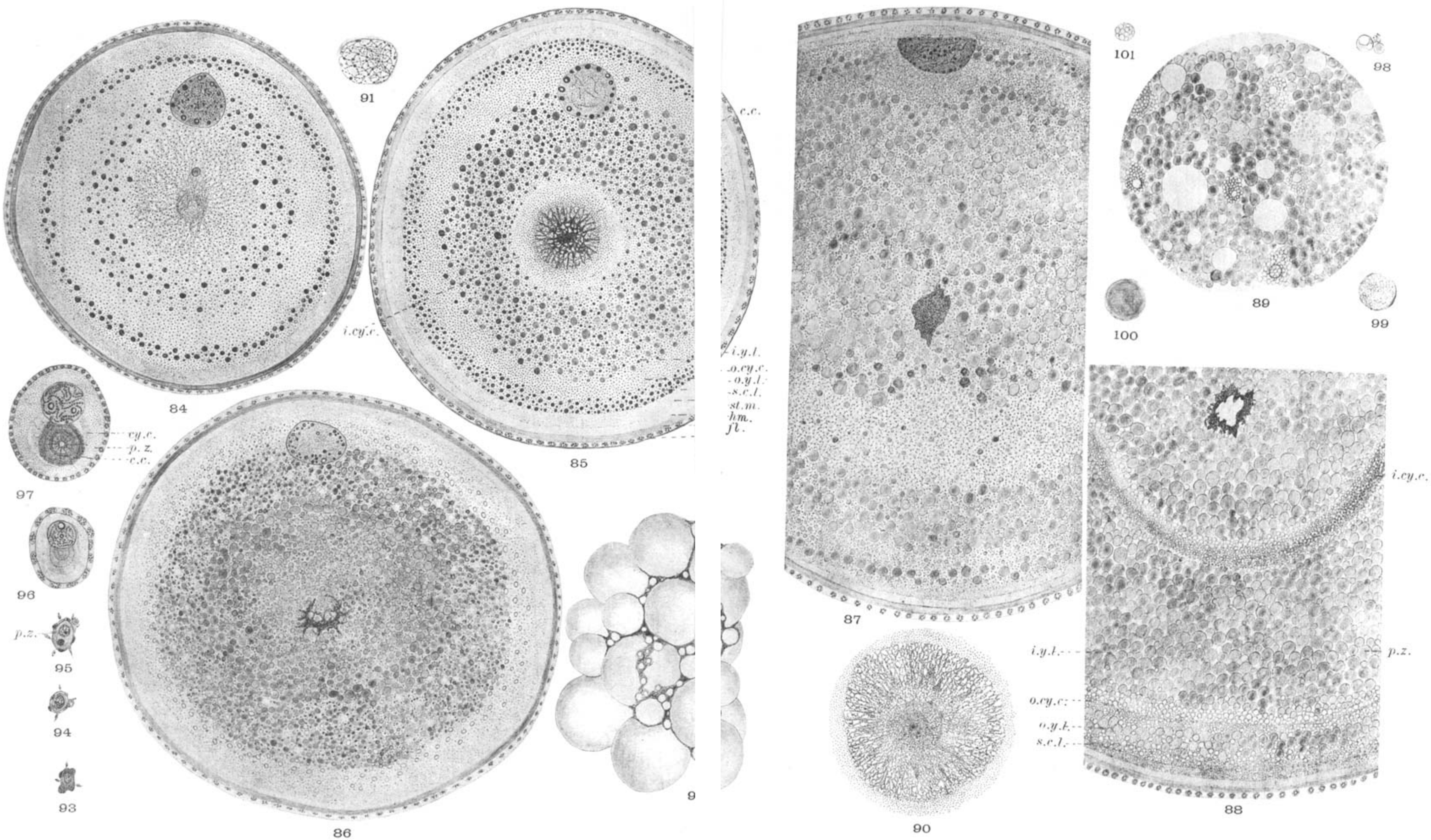

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