The Genesis of the Egg in Triton. (With Plates XXII, XXIII and XXIV.) By Mr. T. IWAKAWA.


In the spring of 1880 I began, at the suggestion of my highly-esteemed teacher, Mr. Whitman, to study the early stages in the development of our common Triton (T. pyrrhogaster, Boje) (1).

It was my intention, circumstances favouring, to study the whole series of events, from the original egg-cell to the transformation of the embryo. The spring and summer were mainly devoted to a study of the cleavage and the external changes of the embryo. The appearance of the two important memoirs of Bambeke (2) and Clarke (3), covering the same ground, makes it unnecessary to publish my earlier observations.

The present paper, which treats mainly of the genesis of the egg, was begun in the autumn of 1880, and concluded in June, 1881. Owing to the want of proper aquaria I have obtained very few fresh-laid eggs, and have, therefore, failed to make anything more than fragmentary observations on the changes that follow impregnation.

With reference to method of hardening, I have been most successful with the picro-sulphuric acid of Kleinenburg. For staining fluids I have made use of picro-carmine, haematoxylin, and Beal's carmine. Silver nitrate was employed to obtain surface views of the ovary.

Some General Remarks.

Triton pyrrhogaster is very common in this island, being found in ponds, brooks, ditches, and rice fields. It occurs very abundantly in the pond of Inokashira, located about ten miles west of Tokio, from which place most of my speci-
mens have been obtained. The average length of the adult male is 9.5 cm., female 11.7 cm.

The females are generally larger as well as longer than the males. The only external sexual differences, aside from size, are found in the form and length of the tail, and in the size and form of the cloacal lips. In the male the tail is short and broad with an obtuse apex, while in the female it is elongated and narrow, and somewhat pointed at the extremity. The cloacal orifice of the female is an elongated slit, the lips of which have a swollen appearance. In the male the lips are much larger and the slit much longer, the whole being well adapted for clasping the cloacal slit of the female in the act of copulation.

For keeping specimens I have been obliged to make use of small glass aquaria, the water being kept in a tolerably good condition by such water plants as Chara and Vallisneria.

Their food consists of worms, insects' larvae, small molluscs, &c. They will eat pieces of fish, and even grains of boiled rice. When they are extremely hungry they sometimes bite one another. I observed one instance in which a poor small fellow was swallowed up to the middle part of the body by a larger and stronger one; but the latter had undertaken too much, for he was unable to complete the act of swallowing or to disgorge, and finally died, after vain attempts to free himself from the obstinate morsel. I have also observed that among young specimens reared in an aquarium the larger ones sometimes devour the smaller and weaker ones.

Although I have never seen the act of copulation, I think there can be no doubt that such an act takes place, as I have often found spermatozoa in the oviducts. The manner in which fecundation takes place was made known in 1864 by Professor Nauck, in the "Correspondenzblatt des Naturforscher-Vereins zu Riga." This paper is known to me only through an article by Dr. J. V. Bedriaga in the "Zoologischer Anzeiger," No. 79, 1881, p. 157. As here quoted, Professor Nauck's statements run thus:

"Prof. Dr. Nauck (heisst auf p. 85 des correspondenzblattes zu Riga), berichtete die von ihm einmal beobachtete Begattung zweier Tritonen. Nachdem bei dem sonst Kammlosen Männchen sich ein Kamm über Rücken und

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1 Professor Gascó's recent observations ('Zool. Anz.,' Nos. 85 and 86) on Triton and Axolotl show that no real copulation takes place in these Urodela, since the spermatic fluid is not conveyed to the female by cloacal contact.
Schwanz gebildet hatte und auch das Weibchen eine stärkere Kammauswölbung zeigte, schwammen beide neben einander her, jedoch so, dass ihre Köpfe entgegen- gesetzte Richtung hatten. Die Schwänze beider waren im Halbkreise gebogen und berührten sich mit den Spitzen, so dass das Paar die gestalt eines S. darbot.

"Während die so verbundenen Schwänze lebhaft hin und her vibrierten, sah man die Kloake des Weibchens deutliche Schluckbewegungen machen. Durch die Vibration gelangte der männliche Samen an die Kloake des Weibchens und wurde von dieser aufgenommen.

"Die Tritonen legen also nicht, wie viele Amphibien, unbefruchtete Eier."

The manner of Depositing the Egg.

Only one egg is laid at one time—precisely how often I have not ascertained. The eggs of those specimens kept under observation were generally deposited during the night or early morning on water plants. I have sometimes found eggs on dead leaves at the bottom of ponds, ditches, &c.

During the act the female generally turns upside down, seizes the plant by the hind feet, gathering the leaves around the cloacal orifice, which is protruded and brought into contact with the plant. In this position the egg is ejected and fixed on the plant by means of the outer gelatinous envelope, which is quite sticky when it first comes in contact with the water.

The entire act lasts about five minutes. The purpose of turning upside down seems to be to place the egg in a concealed position under the leaf or stem.

The Structure of the Ovary.

The ovaries of Triton are a pair of elongated closed sacs, tapering slightly towards either end, the hind ends, in our species, bending forwards. From the inner surface of the thin wall project eggs in various stages of growth.

For obtaining surface views—which have been of more use than sections in determining the origin of the ova—I have employed silver nitrate and mounted in glycerine.

Before mounting, all the larger opaque eggs were carefully removed by the aid of needles, leaving only the thin wall with small and transparent ova.

Silver nitrate, while it browns the protoplasm of the epithelial cells and blackens their boundaries, leaves the nuclei for a time transparent, and inclining to a milky whiteness.
THE GENESIS OF THE EGG IN TRITON.

The wall of the ovary (fig. 1) consists of three layers—an external *germinale epithelium* (*g. ep.*), a *lining epithelium* (*l. ep.*), and a middle layer of connective tissue or *stroma* (*st.*).

The cells of the *germinale epithelium*, seen from the surface with a magnifying power of 400 to 500 diameters, generally show smooth hexagonal boundaries, with nuclei (*n.*) lying near the centre, or, more commonly, towards one side of the cell.

The nuclei have a somewhat elongated oval form, the shorter axis measuring about .016 mm., the longer about .023 mm.

By lowering the focus the nuclei of the stroma (*st. n.*) are brought into view, together with fine blood-vessels, which traverse the stroma. In this layer no cell boundaries can be recognised. The nuclei are easily distinguished from those of the superficial epithelium, being smaller (.010 by .013 mm.), sharper in outline, and elliptical in shape.

Placing the focus still lower, the elongated polygonal cells of the lining epithelium (*l. ep.*) are seen. These cells are bounded by fine wavy lines; the nuclei (*l. n.*) have an elongated oval or elliptical form, and are a little larger than those of the germinale epithelium, measuring .017 by .024 mm.

The size of the cells of the germinale epithelium varies from place to place, being much smaller in those areas where primordial ova are forming than elsewhere. This fact may be taken as an evidence that the epithelial cells multiply by division, and that this multiplication is more or less restricted to scattered patches or areas.

Waldeyer (4) speaks of "cell-islands," or scattered aggregations of pavement epithelial cells. In most cases he found these cell-clusters lying beneath the germinale epithelium; but in a few cases (see his fig. 28) some of these cells were found to have a superficial position, the germinale epithelium (endothelium) being absent in such spots. I have made a great many surface preparations, but I have never been able to find any places where the continuity of the germinale epithelium was broken.

If my view of the origin of the ova be correct, it would be difficult to account for such a break in the outer layer as Waldeyer has described and figured, except by supposing that the preparation itself was faulty. Waldeyer's figure

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1. Waldeyer's opinion that the outer layer of the wall of the ovary is not *epithelium*, but *peritoneal endothelium,* rests upon a distinction that finds no support in the latest investigations on the origin of the peritoneum.
which, as he acknowledges, represents a rare instance, would seem to favour his theory of the origin of the ova, according to which entire epithelial cells, in larger or smaller numbers, sink into the stroma, and are subsequently overgrown by the closing up of the epithelium. I have done my best to find evidence to support this view, but my observations have led me to a somewhat different conclusion, as will be seen from the following pages.

**Origin of the Ovum.**

Before stating my own conclusions, I will give briefly those which have been put forward by other authors.

Van Beneden (5), from an extended and comprehensive study of oogenesis, chiefly in invertebrate animals, came to the conclusion that the ovum invariably arises from a nucleated mass of protoplasm.

Waldeyer (4), starting from the other end of the animal kingdom—the vertebrates, arrived at the conclusion that the ovum of every egg-producing animal is at first an epithelial cell. So far as the vertebrate animals are concerned, this opinion has met with general acceptance.

For Ludwig (6) the chief question is, Does the ovum represent a single cell? His view may be stated in his own words (6, p. 194):

"Our investigations have taught us that in all animals, without a single well-established exception, the egg is a single simple cell from the outset, and that it does not lose this character before maturity."

Götte (7), on the other hand, has maintained that the primordial ovum of Bombinator igneus does not represent a single epithelial cell, but that several such cells coalesce, and thus give rise to a mass of protoplasm, in which are suspended the nuclei of the original cells. Later these nuclei also coalesce, forming a single body. This uninuclear mass does not, according to Götte's interpretation, represent a cell, but an unorganised body, which at this stage contains no "proper egg material," i.e. contains no yolk-substance, which alone is the foundation ("Erzeuger der späteren Entwickelung zum selbstständigen Leben!") of all vital development.

The recent investigations of Dr. Nussbaum (8) have placed this matter in a new light, and have opened up questions of great interest, which can only be decided by embryological studies.

Dr. Nussbaum finds the functional part of the genital organs of Rana fusca, in a larva which has attained the
length of 1·4 cm., to consist of a string-like aggregation of
cells ("Geschlechtszellen") lying on the median side of the
Wolffian duct, in the middle third of the body-cavity. These
sexual cells are at this time easily distinguished from neigh-
bouning cells by the presence of yolk elements, which have
elsewhere dissolved. They are invested with peritoneal
epithelium, which is entirely distinct and independent of
them.

They begin now to multiply by division, and each cell
resulting from this division becomes separately invested in a
capsule of peritoneal cells. Meanwhile the yolk elements
have disappeared. About the time the hind legs bud forth,
the cells thus enclosed split up each into several cells, and
the nucleus in each of these several cells divides, producing
a small heap of nuclei. We thus arrive at a stage in which
a peritoneal capsule encloses several multinuclear cells. A
single nucleus in each of the sister cells enclosed in one
capsule now grows larger, while the others in the same cell
remain small, and take a peripheral position. The larger
nucleus with the protoplasm collected around it forms the
primordial ovum, while the smaller peripherally placed
nuclei, each invested with a share of the protoplasm of the
original cell, become the follicular epithelium.

We have thus a peritoneal capsule enclosing several ova,
each of which is surrounded by its own follicular epithelium.
The concluding step in the formation of the young follicle is
brought about by ingrowths from the peritoneal envelope,
which form a second envelope around each ovum. Each
ovum has now a membrana granulosa, having the same
origin as itself, and a tunica propria folliculi, which consists
of connective tissue derived from the peritoneal envelope of
the ovary.

Nussbaum asserts that the same course of development is
found in the adult as in the embryo. He thus denies any
genetic relation between the peritoneal epithelium and the
primordial ova, claiming that both ova and follicular epithe-
ilium are derived from "sexual cells," which are from begin-
ning to end distinct from the peritoneal layer.

Dr. Valaoritis (9) has published a preliminary paper, in
which he has put forward a new theory of the origin of the egg. He maintains that the ovum is at first a white blood-
corpuscle ("Leucocyte"), which becomes lodged in the
ovarian epithelium, and here matures.

In this paper he appears to base his conclusion concerning
the origin of the egg chiefly on the amoeba-like power of
movement known to belong to white blood-corpuscles, and
assumed to belong also to all young egg-cells. Whatever evidences may be produced in the future in favour of this view, it must for the present be regarded, I think, as a theory without any proper basis.

Automatic action is a character belonging to the protoplasm of young cells in general; and I fail to see any evidence for the assumption that young epithelial cells "execute movements under no conditions."

**Epithelial Islands.**

The epithelial islands discovered by Waldeyer have been mentioned by other authors, and interpreted in various ways.

Spengel (10), describing the ovary of the Urodela, says, "Its outer surface is covered with peritoneal epithelium, which in the adult animal preserves, in places, the character of germinal epithelium, and serves to replace the eggs that have matured."

Brandt (11) succeeded in finding in the frog only a single case comparable with the cell islands of Waldeyer.

Kolessnikow (12) has described "Keim-epithelinseln" found in Triton, Salamander, and Frog. Speaking of the frog, he says:—"Groups of peculiar cells are to be seen from place to place between the endothelial cells; these cells show a granular protoplasm, and larger or smaller nuclei; they have sometimes a round, sometimes a polygonal form, and among them lie single cells, distinguished from them in size, size of nucleus, and richness in protoplasm. The contours of these peculiar cells and the bounding lines separating them from the neighbouring endothelial cells are always plain to be seen. . . . . I regard the groups of cells just mentioned as islands of germinal epithelium, in which the enlarged cells appear as primordial eggs. The size of the islands is .093 to .186 mm., that of the cells .0139 to .0232 mm.

"In places these epithelial cells are seen to lie under the endothelium, i.e. covered by it. In such places they form sometimes deep and broad, sometimes small groups, which often stand in connection with the superficial germinal epithelium."

In regard to the origin of these islands, Kolessnikow expresses himself thus:

"With the transformation of the sexual gland into an ovary begins a more rapid growth of the stroma on the one hand, and a relatively slower growth of germinal epithelium on the other, and the result (of this unequal growth) is that
the germinal epithelium is distributed in small islands, and
the stroma cells come to the surface of the ovary, where
they take the form of endothelium. The primordial eggs
then arise by a (further) growth of single cells of the
germinal epithelium, while the enlargement of the ovary
results from the growth of the stroma."

Valaoritis (l. c., p. 598) states that these islands are
wanting in immature specimens of *S. maculata*, and that
the period of sexual activity is distinguished from that of
inactivity only by the presence of such islands.

Hoffman (according to Valaoritis) says that “the peri-
toneal epithelium, which covers the ovary of the land
Salamander, is interrupted in places, in which places there
is an ovarian epithelium, which serves to replace the eggs
that have been used.”

From these citations it is apparent that great differences
of opinion exist with reference, not only to the precise
origin of the primordial ova, but also to the character of
the external cell-layer of the ovary.

In regard to the origin of the ovum, we have at least three
quite different theories. While differing in regard to the
mode of origin, the majority of authorities maintain that
the ova are derived from the outer cell-layer of the ovary.
On the other hand, Nussbaum holds that they arise entirely
independently of this outer layer—from so-called “Gesch-
lechtszellen.”

Valaoritis agrees with Nussbaum in denying the epithelial
origin of the ovum, but claims that it is nothing but a white
blood-corpuscle.

The difference of opinion in regard to the character of the
outer cell-layer of the ovary has led to confusion in the
names applied to it. Some call it “peritoneal epithelium;”
some “endothelium;” while others hold that it is made up
of two distinct elements, which they distinguish as “epi-
thelium” and “endothelium.” In this paper it will be
spoken of as “germinal epithelium.” I began the investi-
gation of this point in company with a fellow-student, Mr.
Sasaki, to whom I am indebted for a few figures, which he
has generously allowed me to use. We have made together
a very large number of silver nitrate preparations for surface
views, extending through the winter and spring months;
and also many sections. Our experience has taught us, what
others have learned before us, how difficult a matter it is to
determine the origin of the ovum. Our studies are not as
complete as they might have been, had we not been com-
pelled to drop them at an early date in order to prepare for
examinations and graduating exercises. But we have both arrived at the same conclusion, viz. that the ovum does have an epithelial origin. In regard to the mode of origin, we have arrived at conclusions differing somewhat from any that have yet been published. As before stated, the germinal epithelium forms a complete investment of the ovary, in the form of flat polyhedral cells in a single layer. In surface views (fig. 17) these cells are seen to differ considerably in size in different areas, being generally smaller in those places where newly formed ova are found, or where such are in process of formation. The difference in size, however, is not so great as surface views might lead one to think. In sections passing through such areas (figs. 13 and 14) the cells appear more crowded than elsewhere, and have greater depth. In the least crowded areas the nuclei have a flattened elliptical form, when seen in section, and are placed at considerable distances apart.

In the more crowded areas the nuclei are brought so close together that the cell protoplasm is not always to be recognised between them, and they are tilted up on one end, so to speak, and often overlap one another (fig. 14).

It is in these crowded areas that we meet with the germ-cells,1 which lie at first in the germinal epithelium (fig. 15), and are plainly a part of it. Such a section as that given in fig. 15 would perhaps, at first sight, be supposed to favour the view that the germinal epithelium is composed of two distinct kinds of cells. There are some points in this section, however, that will be more easily explained after a description of surface views.

I have found very often during the winter the nuclei of the germinal epithelium in process of division, as seen in fig. 2. Other cells of the same layer are found with two distinct nuclei (figs. 11 and 12). The two nuclei may be alike in general appearance (fig. 11), or one may present an aspect quite unlike that of the original nucleus. The latter case has been reproduced in fig. 12, in which four epithelial cells are represented. In one cell there is seen, besides the proper nucleus, which is precisely like the nuclei of the neighbouring cells, a second somewhat larger body, which is quite distinctly outlined, and which has a nucleus-like body within. That this body lies wholly within the epithelial cell there can be no doubt. This body represents a germ-cell which has formed within the parent epithelial cell.

The question at once arises, How does this endogenously

1 As I am uncertain whether these cells give rise to both the primordial ova and granulosa, I call them germ-cells.
formed cell escape from the parent cell? By comparing the surface views represented in figs. 3, 6, and 7, it will be seen that the nucleus destined either to be, or to give rise to, the nucleus of the ovum, increases in size and becomes coarsely granular. In fig. 3 it occupies about one half of the original cell, and that end of the cell in which it is lodged is bounded by a curved line. The second nucleus of this cell differs in no particular from those of surrounding cells, except that it has a concavo-convex form, which it has probably assumed in adjustment to the larger nucleus. In this case I found no well-defined body of protoplasm enveloping the larger nucleus, as in figs. 5, 6, 7, and 12. In fig. 7, which represents a single epithelial cell with a rounded outline due to the action of acetic acid, the larger nucleus is surrounded by a distinctly marked area of protoplasm, in close apposition with which lies the smaller nucleus, which has here nearly the same form as in figs. 3 and 6.

In fig. 6 the larger nucleus, together with its protoplasmic envelope, has taken the first step towards freeing itself from the mother-cell. It is still partly within the original cell, but dips at one end under two other epithelial cells. It is simply the expansion by growth of the young germ-cell, and not any active amoeba-like movement, which causes it to get under the neighbouring epithelial cells and beyond the limits of the mother-cell. In some cases I find the boundary of the germ-cell passing directly into that of the original cell, always so when the dipping just begins, as seen in fig. 1.

The germ-cell at this stage has no membrane, although its protoplasm is clearly bounded. In the minds of those who hold a different opinion in regard to its origin, fig. 6 may be said to admit of another interpretation. It may be said that the germ-cell possibly lies wholly under the germinial epithelium, having no connection with it whatever. This mode of explanation was the first to occur to me, and I have endeavoured to test it, not only by the study of a very large number of surface preparations, but also by sections. I find it quite impossible to reconcile this view with my observations. Sections confirm in a very positive manner the explanation which I was led to adopt from the study of surface views. The youngest germ-cells are always found in the epithelial layer. Two of these are seen in fig. 15, one of which (on the left) corresponds exactly with what is seen in fig. 7. On the left of the germ-cell is a nucleus, elongated in a vertical direction,
lying in close contact with the germ-cell, and to all appearances answering to the concavo-convex nucleus of fig. 7.

In fig. 6 the germ-cell has already begun to get under the neighbouring epithelial cells. This section shows also that the epithelial layer thickens around the growing germ-cell, the epithelial nuclei being more crowded and sometimes lying beneath the surface. This crowding of the nuclei around the germ-cell is seen in all cases, and is well illustrated in figs. 4, 5, and 17. Fig. 6 shows what I have often met with, the nuclei of the surrounding epithelial cells lying each in that side of their respective cells which is in contact with the proliferating cell.

In regard to the origin of the follicular epithelium, I have not been able to come to any definite conclusion. I am quite certain that the germinal epithelial cells cluster about the germ-cell, and that they form a more or less perfect envelope around it, as is seen in the right germ-cell of fig. 16. In fig. 9, outlined with the camera from a preparation in Kleinenburg's fluid, are seen some small germ-cells, around which are clustered epithelial nuclei, and a young ovum invested with what I regard as the young follicular epithelium. The epithelial nuclei around the germ-cells differ in no respect from those around the ovum. In fig. 18 is seen a section of an ovum of about the same size, around which are seen five nuclei, belonging to as many cells, whose limits are not recognisable. On the inner side of this ovum (lower side in the figure) three distinct layers are recognisable, the lining epithelium of the ovary, the stroma, and the follicular epithelium; while on the outer side only two layers are to be seen, the germinal epithelium and the follicular epithelium. In an ovum having a diameter about two and a half times that of fig. 9 (fig. 19) we find the same three layers on the inside (left side of the figure) and one additional layer (stroma) on the outside. The stroma layer has now become very thin, and the nuclei of both the follicle and the lining epithelium are much smaller than in fig. 9.

It would be easy to interpret all this in favour of the opinion that the granulosa takes its origin from the germinal epithelium. But I am not prepared to deny Nussbaum's theory, according to which both the granulosa and the primordial ovum are derived from the germ-cell. I have found germ-cells with multiple nuclei, as seen in figs. 9 and 10; but I have not succeeded in ascertaining the fate of these nuclei. I have never found a "mulberry stage" of the nucleus in germ-cells as large as the ovum seen in fig. 9. I think
there can be little doubt that some of the nuclei seen on and around the ovum in fig. 17 correspond to the follicular nuclei seen in fig. 18.

The nuclei around the ovum (fig. 19) are precisely like those around the small germ-cells, and these nuclei are to be found around the germ-cells which have multiple nuclei, as well as around those with simple nuclei. While thus my observations incline me to the theory of the epithelial origin of the granulosa, I cannot regard them as conclusive.

The origin of the ovum, according to the above observations, may be briefly stated in the following words:

A nucleus of a germinal epithelial cell divides into two; one remains as the nucleus of the proliferating cell, the other enlarges and becomes the centre of a well-defined portion of the protoplasm belonging to the parent-cell. The germ-cell thus formed lies at first wholly within the parent-cell, but as it increases in size it expands beyond the limits of the latter, and sinks beneath the surface, becoming at the same time free from the mother-cell.

Epithelial cells crowd around the germ-cell, and some; sinking beneath the surface with it, form a follicular (?) wall around it. The germ-cell represents a primordial ovum, or a cell from which the primordial ovum plus the follicular epithelium arise.

**Formation of Yolk-spherules.**

In the young germ-cells the protoplasm is clear and transparent, like that of the epithelial cells. The first plain indication of the presence of yolk-spherules appears about the time the egg has attained a diameter of '25 mm. At this time the ovum appears as represented in fig. 20.

The protoplasm has become somewhat clouded with very minute granules; and scattered patches, consisting of larger or smaller aggregations of small yolk-spherules, are seen on one side of the ovum lying near the periphery. These aggregations are quite opaque, although the single spherules are not so. Viewed singly, after being expelled by pressure of a cover from a ruptured ovum, they appear to be minute shining spherules, measuring about '001 mm. They have a refrangibility much like that of particles of fat, and show the usual Brownian movement. Along with aggregations numbering from 20—25 spherules are found others consisting of only two or three. The spherules of the patches do not vary much in size; but outside these patches are found single spherules, varying from the size of those in the
patches to that of the most minute granule that we are able to recognise.

Seen by reflected light these masses of yolk-spherules appear whitish. These patches are found at a little later date scattered throughout the peripheral portion of the ovum, but a little larger and more closely arranged on that side of the ovum where they presumably first appeared than on the opposite side, as seen in fig. 22.

In this section of an ovum, measuring '57 mm. in diameter, and hardened in picro-sulphuric acid, the yolk patches present an appearance quite different from that which they have when examined in a fresh condition. The fine granular protoplasm here appears darker than the patches themselves—just the reverse of what is seen in the living egg. The size of the spherules is now '002 to '003 mm. In fig. 21, which represents an optical section of a portion of an ovum measuring a little less than 1 mm., examined in a fresh condition, some of the aggregations appear to be more or less elongated in a radial direction. The further history of these deutoplasmic masses can be learned only by the study of sections of hardened ova; the protoplasm stains deeply red in picro-carmine, and the same is true of the narrow boundary lines between the radially elongated masses of yolk-spherules. The deutoplasmic elements and the protoplasmic matrix are thus well defined by the action of the staining fluid. The persistence of the bounding lines even after the greater portion of the ovum has become thickly crowded with yolk-spherules is quite remarkable, and I am not aware that such a feature has been described before. That it is not an artificial product is perfectly certain, for I constantly meet with it in eggs varying in size between '5 mm. and 1 mm., and meet with it in all phases intermediate between figs. 20 and 23, and between fig. 23 and later stages of complete obliteration.

The disappearance of these radial bounding lines occurs soon after the egg has attained a diameter of 1 mm., some time before the yolk masses have reached, in their inward growth, that stratum of protoplasm which lies next to the germinal vesicle. The latter event is usually accomplished by the time the ovum has attained a diameter of 1'5 mm.

That these young yolk-spherules are not cells is of course quite certain.

That they originate within the protoplasm is proved by the fact that there is always a cortical zone entirely free from them, and that no such corpuscles are to be found at any time outside the protoplasm. That they arise in the
manner described by Gegenbaur (13) seems most probable.¹

The persistence of bounding lines, by means of which the individuality of the yolk masses is maintained for a remarkably long time, makes it clear that there is a centripetal growth of these masses. It is probable—we may say certain—that this growth is due, not only to the expansion of the individual spherules, but also to the formation of new spherules, which are added to the mass on the central side.² Just before the complete obliteration of the boundary lines of the yolk aggregations, the entire body of deutoplasmic spherules may be said to be made up of a considerable number of pyramidal masses, the bases of which lie in the subcortical zone, while the apices lie in the perinuclear zone. The basal portions of these pyramids are formed first, and the apical portions, which are farthest removed from the nutritive supply, are the latest to develop. It should be added, however, that the boundary lines never extend to the innermost, last formed, zone of spherules; hence the outlines of the yolk pyramids are never complete.

The distribution of the earlier formed yolk-spherules in small masses or aggregations in the subcortical zone has been observed by Gotte in Bombinator igneus (7, p. 17); by Balfour (14, p. 410) in Scyllium canicula; and by His (15, p. 31, figs. 32 and 40, pl. iv) in Salmon.

The form of the yolk-spherules is from the outset ellipsoidal. In the mature egg the larger spherules measure \(0.11\) mm. by \(0.17\) mm.; they are clear and homogeneous, showing nothing which could give any support to the idea that they are of a cellular nature. The cortical layer of protoplasm, which has a thickness of \(0.007\) mm. in fig. 23, becomes very thin in the mature egg, but does not wholly disappear. In fig. 26, which represents a ripe follicular egg, the yolk-spherules have encroached much upon the cortical layer, and here, as in earlier stages (figs. 24 and 25), the size of the spherules diminishes in the peripheral portion.

Vitelline Membrane.

As before remarked, the primordial egg-cell is membraneless. Nothing in the form of a membrane appears for some time after the ovum becomes enclosed in a proper follicular

¹ Kolessnikow (12, p. 400) makes a slip in saying that Gegenbaur refers the origin of the vitelline spheres to the follicular epithelium.

² I have seen nothing to favour the idea suggested by Gotte (7, p. 18), that the first formed spherules move toward the centre of the ovum, and that their original places are occupied by newly formed spherules.
epithelium. In all ova under 1 mm. in diameter, the cortical layer of protoplasm follows closely the boundary of the cells of the granulosa, as seen in fig. 21.

In ova measuring about 1 mm. may be seen a thin superficial portion of the cortical layer, quite transparent and free from granules (fig. 24). The outer contour of this thin layer is sharply defined, but there is as yet no line of division between it and the cortical zone. This surface stratum of the cortical layer, which is about .001 mm. thick, is the vitelline membrane in process of formation. In an ovum of 1.5 mm. (fig. 25, v. m.) it has taken the form of a double-contoured membrane, which appears to be perfectly homogeneous. In this section, which was treated with chromic acid (½ per cent.) and Beal's carmine, the membrane was stained more deeply red than the underlying protoplasm. There is no doubt in my mind that the membrane seen in fig. 25 is identical with the clear surface stratum in fig. 24; and as in the latter figure it is continuous with the protoplasm, I regard it as a modified part of the protoplasm, and hold that the vitelline membrane is produced by the egg itself, and not by the granulosa.

The Germinal Vesicle.

My observations on the germinal vesicle, especially on its earlier stages of growth, are quite incomplete. In ova measuring about 0.26 mm., examined in a fresh condition, the germinal vesicle appears perfectly clear, is spherical, and has a somewhat excentric position (fig. 20).

Numerous germinal dots (.003 mm.) lie at the very surface of the nucleoplasm (Van Beneden), apparently in contact with the inner surface of the very thin delicate membrane of the vesicle. In ova hardened in picric-sulphuric acid and stained with haematoxylin the nucleoplasm has a reticulate appearance (figs. 19 and 27). Cases like that seen in fig. 19 occur not infrequently, in which the nucleoplasm has contracted more than the membrane, in which case the germinal dots remain adhering to the membrane. In other cases the membrane follows the contracting nucleoplasm, as seen in fig. 27. In fig. 22 is seen a germinal vesicle, of about the same size as that seen in fig. 27. The ovum from which fig. 27 was taken corresponded also very nearly in size with the one given in fig. 22. In fig. 22 the germinal vesicle appears to lie in a large cavity, or rather to be suspended from one side of the cavity. This cavity, if it existed in the living egg, was undoubtedly filled with a watery fluid, upon which the staining fluid had no visible effect. In fig. 27 the
vitellus lies in close contact with the germinal vesicle, except at one side, where a small space, probably caused by contraction, is seen.

That the germinal vesicle in fig. 22 filled the whole cavity in the living ovum is very improbable, since it is not at all abnormally small—in fact is about equal to the germinal vesicle in fig. 27.

In fig. 22 the nucleoplasm is a little more coarsely granular than the egg-protoplasm, except the peripheral portion, which contains the nucleoli, and which is less coarsely granular than the central portion.

The germinal vesicle has everywhere except on the side of contact a sharp outline, but there does not appear to be a double-contoured membrane.

On the side of contact is seen an elongated area of perfectly homogeneous, non-granular, deeply-stained substance, which extends a little beyond the limits of the germinal vesicle in both directions. In this substance are seen two or three of the germinal dots, and the substance is continuous with the nucleoplasm, from which it differs only by having no granules. On the side of contact there is certainly nothing that could be called a membrane. It seems probable that this non-granular substance is a part of the germinal vesicle, and that the germinal vesicle itself is upon the eve of certain internal changes, some of which are seen in fig. 28. This germinal vesicle (fig. 28), belonging to an ovum measuring 82 mm. in diameter, is still more flattened than in fig. 22, and the outline is less regular, and shows no sign of a membranous envelope. The germinal dots have taken on peculiar forms, and appear to be moving towards the centre of the vesicle. Several elongated forms are seen, some of which are constricted about the middle, evidently in process of division. In some cases the division is just on the point of completion, the two halves still being joined by only a slender thread.

Another peculiarity, not observed in earlier stages, is the vacuole-like space seen in most of the germinal spots. In the elongated forms generally two such vacuoles, located at the two poles, are seen, and sometimes the same number is seen in the non-elongated massive forms. Hertwig (16) has described similar appearances in the egg of Hæmopis and in that of the Frog, and v. la Valette St. George (17, p. 58) has observed the same in the egg of one of the Libellula.

Together with this breaking up and centripetal movement of the dots there is also a concentration of the coarser
nucleoplasmic elements, leaving a wider peripheral portion than is seen in fig. 22, containing the less granular part of the vesicle.

In fig. 29 these changes are carried still further. The germinal dots have been reduced by division to coarse granules of various sizes, and have coagulated in a central mass, leaving a broad peripheral portion almost as homogeneous as the small elongated mass at one side of the vesicle in fig. 22.

Scattered dots are seen in this peripheral portion. The germinal dots appear to have undergone some change in composition, judging from the effect of the staining fluid.

In fig. 28 the nuclear substance is stained, but much less strongly than the dots. In fig. 29 the nuclear substance is coloured as before, but the fragments of the dots have a dull yellowish-brown colour instead of the deep red of fig. 28.

In fig. 29 the vitellus is in immediate contact with the germinal vesicle, the clear space seen in fig. 22, which was also present in fig. 28, having entirely disappeared.

Owing to the limited amount of time which I have had to devote to this part of my study, I have not been able to learn the further course of events in the germinal vesicle.

The "Yolk-nucleus."

In small ova, measuring from 15 mm. to 10 mm., I have several times met with an oval body at one side of the germinal vesicle (fig. 8). I do not find this body in all eggs of this size; indeed, it may be said to be a rather rare occurrence. This is undoubtedly the Dotterkern (not to be confounded with the "Dotterkern" of Goette) of German authors.

Hertwig (16, p. 37) has proposed to designate this body as "Dotterconcrement," in order to avoid the comparison implied in the term "yolk-nucleus." I can say nothing in regard to the origin or fate of this body, but as it is not constant, no special importance can be attributed to it. It has a granular composition, but this fact does not warrant the opinion that it is a mere aggregation of yolk-granules.

Van Bambeke refers the origin of this body to the follicular epithelium, one of the cells of which gets loosened and embedded in the ovum.

Waldayer (4, p. 75) remarks that this body is found only in young ova; later it appears to disappear completely.

Leuckart (18) and others have made similar observations.
THE GENESIS OF THE EGG IN TRITON.

List of Papers.

15. His, ‘Untersuchungen ueber das Ei und die Eientwicklung bei Knochenfischen,’ Leipzig, 1873.