On the Process of Gastrulation in Chelonia.

(Contributions to the Embryology of Reptilia, IV.)

By

K. Mitsukuri, *Ph. D., Rigakuhakushi,*

Professor of Zoölogy, College of Science, Imperial University,

Tōkyō, Japan.

With Plates VI.—VIII.

The sea-turtle, Chelonia caouana, *Wagl.* deposits its eggs on almost every suitable stretch of sandy beach in the southern half of Japan during the summer months of the year. During the breeding season of this animal in 1891, I was enabled, by the liberality of the University authorities, to visit Sagara in the province of Tōtōmi, with my assistant, Mr. T. Tsuchida, for the purpose of collecting materials for the study of its development. With the assistance of several kind friends, we made arrangements to have reported to us every deposit of eggs that might be made along about fifteen miles of sandy beach in that region, and we thus succeeded in getting hold of several good deposits. As each of these contained over one hundred eggs—121 is the least, and 145, the largest number in one deposit in my experience, but 169 has been reported in one case—we had command of over one thousand eggs in all, and as we opened eggs from each deposit at certain intervals of time, we were able to secure unusually fine series of embryos, gaps in one series being often filled up by members

from others. This success was in a large measure due to Mr. Tsuchida, whose zeal and perseverance never flagged, even under most trying circumstances; and I would here express my deep indebtedness to him. My thanks are also due to Viscount Tanuma, Mr. Y. Murakami, the Mayor of Sagara, the Chief Officer of the Sagara Police Station, and several other gentlemen who assisted us in various ways and showed us much kindness during our stay. Messrs. T. Ogasawara and K. Niwa of Shizuoka were also kind enough to furnish me with much useful information.

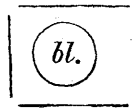
Various observation made by us on the breeding habits of the sea-turtle together with similar facts which I have ascertained in other species, I hope to embody, at some future time, in a separate paper. A short preliminary account of these observations is already published in the Zoölogical Magazine (Japanese) Vol. III., No. 35. I will only remark here for the benefit of those who may attempt a similar study, that Chelonian eggs can be transported with safety for some hours immediately following their deposition, but after that, their removal is apt to bring on death and decomposition. This seems to be due to the circumstance that the white at the upper pole is rapidly absorbed, the blastoderm becomes adherent to the shell membrane, and a large fluid cavity is produced directly beneath the developing embryo. In this condition, slight jarring seems to disturb the delicate arrangements and to cause death. After thirty days or so, when the fœtal membranes have become definitely established, the eggs can again be moved with impunity.

The embryos of Chelonia caouana, thus obtained, together with those of Trionyx japonicus and Clemmys japonica which I already possess or can get in almost any desired stage, afford a good basis for the comparative study of reptilian development, and I intend to use them for this purpose, as I have previously used those

of the two last-named species. Meanwhile I have discovered that when Chelonia caouana deposits its eggs, they are in a far less advanced condition than those of Trionyx or Clemmys and thus enable us to elucidate many points in the much discussed process of gastrulation in the Amniota. The present contribution embodies the results of my own study on this point and, it is hoped, will throw light on some phases of this vexed question.

Preparation and Preservation of the Embryos.

Young embryos were in nearly all cases preserved in Kleinenberg's picro-sulphuric acid. Very advanced embryos were placed, partly in that fluid, and partly in corrosive sublimate. In removing blastoderms from eggs within one or two days of their deposition, at which age there is not yet any large subgerminal cavity in the yolk, the shell was removed and as much of the white as possible. The whole egg was then placed with the blastoderm uppermost in a deep vessel and covered with picro-sulphuric acid. The spot where the blastoderm was to be found was generally marked with a hair since the thin layer of the white necessarily left over it coagulates in the preserving fluid and hides it entirely from view. Proceeding in this way the preserving fluid will be found after three or four hours to have penetrated to the blastoderm and acted on it as also on the upper strata of the yolk. Incisions at right angles were now made with a sharp knife on three sides of the blastoderm, leaving the fourth side and the two corners uncut, as shown in the accompanying diagram. It was then found that a little manipulation with forceps or scalpel easily separates the superficial coagulated white from the blastoderm beneath it. If we then cut the corners, the sheet of the white will roll up of itself towards the uncut side, leaving fully exposed the blastoderm which being already hardened



can then be removed with great ease. The blastoderm thus removed was generally left in a relatively large quantity of the preserving fluid for some hours longer. In more advanced embryos the position of the blastoderm under the shell is easily told in chelonian eggs by the change of colour in the shell. In all the species I have examined, a white patch appears in the shell over the embryo, and increases in extent with the growth of the embryo, or more strictly speaking, *pari passu* with the disappearance of the white over the embryo ; so that, roughly speaking, the size of the patch is a very good indication of the size of the embryo beneath. In these stages the embryo is firmly adherent to the inside of the shell, with a large subgerminal fluid cavity in the yolk beneath it, which can be easily pierced through the shell and the blastoderm with the point of one blade of the fine scissors. By thus piercing the cavity and cutting round just inside the edge of the white patch through both shell and blastoderm, the embryo is removed, firmly adhering to the cut piece ; the latter can then be turned over, exposing the ventral surface of the embryo, and the preserving fluid be poured over it, using the cut-piece as a veritable watch-glass. After half an hour or so, the blastoderm can be easily separated from the shell and placed in a larger quantity of the preserving fluid. This method has the great merit of keeping every part of the blastoderm stretched in its natural condition, and also of making it possible to remove a large number of embryos in an incredibly short space of time.

When the embryo is very much advanced and the allantois has spread itself entirely beneath the shell, it becomes a serious question how to remove the shell without much injury to the fœtal membranes, especially as the shell is leathery, and not brittle as in some other reptiles. In this and similar cases I carefully scrape the shell at one small spot with a knife, until it becomes quite thin, and then apply

to that spot some picro-sulphuric acid, which removes calcareous matter. I scrape again with the knife and again apply the acid. I repeat this process, always using great care, until enough of the shell is worn off to expose a very small patch of the allantoic surface, sometimes not larger than the eye of a needle. However small the opening may be, the acid is able to penetrate through it and harden the tissues for some space around it. The opening may then be enlarged a little, with perfect safety to the parts beneath. The acid is then applied again, a still large area is hardened, and the opening is accordingly made still larger. At length the opening becomes large enough to allow of the removal of the entire shell without injury to the membranes. In removing the shell, it is advisable to use the broad, blunt-pointed forceps and insert them tangentially between the shell and the fœtal membranes. With a little practice, it becomes comparatively easy to obtain in this manner embryos with the fœtal membranes perfect, except for the yellow patch where the picro-sulphuric acid was first applied.

As to staining, imbedding, and cutting sections, there is nothing special to communicate. I generally use borax-carmine for staining. For imbedding, celloidin-paraffin is used.

The methods just described have been used in the case of Chelonia caouana and in those of other species with equal success.

Description of the stages of Gastrulation in Chelonia Caouana.

The first stage to which I wish to call attention is represented in Figs. 1 and 1*a*, Pl. VI. It was taken out of an egg which had been deposited only a few hours before. We notice first the oval-shaped embryonic shield somewhat elongated in the antero-posterior direction. At the posterior end of this, and for the most part, lying outside it,

there is a second much smaller, irregularly circular white patch. This is the structure called the "Primitivplatte" (the primitive plate) by Will* (No. 18); and in later stages, when cells added on from the subjacent yolk form an accumulation, is the "Primitivknoten" (the primitive knob) of Mehnert (No. 8). These names will be adopted and used interchangeably in this paper, for it is after all difficult to distinguish when the state of the plate ends and that of the knob begins. One stage of it is also called the "Sichel" by Will (No. 18). In the dorsal view (Fig. 1) there is already in the middle of this area a large, transversely elongated opening leading into a spacious cavity, For convenience in description, I anticipate my conclusions by stating here that I consider this cavity to be the archenteron and its dorsal opening to be the blastopore. In the ventral view (Fig. 1*a*), the appropriateness of calling the above area a "knob" is clearly seen, for it is a thick accummulation of cells projecting much more than the adjacent parts into the yolk. It is important above all to notice that the archenteron at this stage is not open on the ventral side, although it can be seen from that side through the cell-mass in the specimen figured.

Fig. 9, Pl. VIII. is a median longitudinal section through another embryo of the same stage as that represented in Figs. 1 and 1*a*. In this the yolk was left intact on the ventral face, so that it represents no doubt a more nearly normal state of things than if the yolk had been removed as in Fig. 1*a*. The blastoderm has already extended itself over a wide area and, with the exception of the primitive plate, is divided throughout into two layers : (*a*) the superficial layer (the epiblast of authors, the blastophor of Van Beneden), and

* Also in No. 21. I regret that this paper came to my hands only after the present contribution was nearly in shape to be given to the printer. I could not therefore make as much use of it as I should have liked to do.

(*b*) the lower layer (the cœnogenetic hypoblast of Hubrecht, the paraderm of Kupffer, and of Mehnert, the lecitophor of Van Beneden.) The superficial layer, which I shall call the epiblast, forms a distinct membrane and is composed of columnar cells in the region of the embryonic shield, but changes gradually into low cells in the parts outside the shield. The lower layer is composed of irregular, amœboid-shaped cells and does not probably form a continuous membrane. I hope to show in the sequel that this layer ought to be regarded as only a part of the hypoblast, and might be called the cœnogenetic hypoblast, after Hubrecht (No. 5). In the region of the primitive plate, there is a different state of things. Instead of having two distinct layers, this area shows a thick accumulation of cells. It is composed for the most part of an irregular network of cells with tolerably wide meshes between, so that it is not a compact mass. In the middle of this accumulation, there is seen the invagination-cavity—the archenteron—leading at first downwards but soon forwards and ending blindly. The roof of this cavity shows distinctly a columnar arrangement of cells, and becomes continuous with the epiblast at the anterior lip of the blastopore. On the floor, as well as for some distance behind the blastopore, so long as we are in the region of the primitive plate, we see no columnar arrangement: the general network of the mass extends up to the surface. There is no sharp line of demarcation between the cellular mass of the primitive plate and the subjacent bed of the yolk. The latter is divided into especially fine globules at the boundary line, and we can clearly see many cells arising at this place and adding themselves to the primitive knob. That the nuclei of these cells are the descendants of the segmentation nucleus, there can be no reasonable doubt; in fact I would have this addition of new cells considered simply as the continuation of the process

of segmentation.* As the figure shows, the primitive plate becomes continuous with both the epiblast and the lower layer at its own periphery. I am thus unable to find any independent sheet of cells which lies below the primitive plate, and with which alone the lower layer of the surrounding parts becomes continuous, as Wenckebach (No. 15, Fig. 1) and Mehnert (No. 8, Figs. 20 and 21) and Will (No. 21, Fig. 49) have found. On this point Virchow's observations (No. 14) seem to be similar to mine; Will also sees no such independent sheet of cells in earlier stages (No. 19 Fig. 1, No. 21). I am also unable to find such a sharp line of demarcation between the shield and the primitive plate as is given in Will's Fig. 1 (No. 19†): the primitive plate passes gradually into the epiblast both anteriorly and posteriorly.

The yolk globules in this series of sections are generally spheroidal with a uniform yellow tint. Generally speaking, they are markedly fine immediately below the blastoderm, and become larger farther below (See Fig. 9): of their especially small size underneath the primitive knob, I have already spoken. I agree with Virchow (No. 14, p. 67) in thinking that the dark granules which Mehnert describes in the yolk-globules (No. 8, Figs. 20 and 21) are artificial productions made in the course of preparing sections.

I regret that I was unable to obtain the growing edge of the blastoderm which would no doubt present interesting phases of growth as observed by Virchow (No. 14) and Duval (No. 2). I am therefore unable to throw much light on many questions bearing on cells found in the yolk. I may however mention that in the series from which Fig. 9 is taken, two kinds of cells are found imbedded in the yolk. Their nature is not clear to me. The more numerous kind I

* Will appears to be of the same opinion (No. 21).

† This sharp demarcation is insisted on still more strongly in Will's recent paper (No. 21).

have represented in Figs. 10 and 12. Fig. 10 is part of a section like Fig. 9 from a more lateral part of the blastoderm than that in the latter figure. Beginning from the upper surface, we can easily recognise the epiblast and the lower layer of cells lying immediately below. Under these two layers, there is a rather thick stratum of spherical yolk-globules. We then come to a crowd of cells which are the cells in question. Some of these are large and full of yolk-granules ; while others are smaller and formed of vacuolated protoplasm. The size of their nuclei is tolerably uniform—being about .016 mm. in length. In Fig. 12*b* are shown more distinctly three of these cells from another region. One of them is full of yolk-granules, as is also the unusually large cell shown in Fig. 12*a*. Another is partly full, with an area of granular protoplasm around the nucleus. The third, of which only one-half is seen—having no doubt been cut in two in the process of microtomizing—has no yolk-globules, but is formed of vacuolated protoplasm. Below this stratum of cells there is a layer of closely packed fine granules which represents some liquid coagulated in the course of hardening. Below this, we come to the thick bed of yolk. The globules are here larger than in the upper layer. The conclusion seems to me almost inevitable that the cells above described take up and digest yolk globules and that the stratum of liquid on the edge of which they are found is produced as the result of their digestion. This liquid stratum has probably a genetic relation with the large subgerminal liquid cavity found below the blastoderm a day or two later in the course of development. So much seems tolerably clear ; but whether the cells have for their sole end the digestion and preparation of yolk globules for the nutrition of other cells, or whether they themselves are to form some integral parts of the growing embryo, I am unable to decide.

Cells of the second kind found in the yolk never occur together

in large numbers but are scattered, at least near the surface, indifferently through the yolk-substance. At different points in the yolk we find unusually large nuclei surrounded by a comparatively small amount of protoplasm (Fig. 11*a* and *b*). Sometimes there is only one nucleus (Fig. 11*b*) and then it is very large. The one represented in Fig. 11*b* measures .04×.032 mm. Quite as often, the nuclei occur in a group of two or three, closely adherent to one another (Fig. 11*a*). These cells are no doubt what are called "Merocyten" by Virchow (No. 14). What their nature is, whether they stand in some genetic relation to other kinds of cells or are of a nature *sui generis*, I am unable to say. I have thought it just possible from the frequency with which two or three nuclei are found together, that they are cells dividing by amitosis and possibly undergoing disintegration (Flemming [No. 4] and Ziegler [No. 20]).

Let us consider for a moment how such a stage as that described has been reached. What I am inclined to think as probable is as follows :—When the process of segmentation has gone on for some time, the blastoderm separates itself into two layers, the superficial epiblast and the lower layer. This takes place throughout the blastoderm with the exception of the primitive plate.* Here cells not only remain undifferentiated but with the addition of cells from the subjacent bed of yolk form a mass which protrudes into the yolk—the primitive knob. In the middle of this region, an invagination soon appears, which is at first shallow and is directed straight downwards. I have two specimens of this stage but have not figured them because the blastoderms having been peeled off from the yolk to which it is adherent at this stage, the lower part of it is

* And probably also of the growing edge of the blastoderm, but of this part I am not now, speaking———I am gratified to find that what is given above as probable is now verified by Will by direct observation (See No. 21, Figs. 35 & 36).

probably not complete. But as to the above point there is not room for much doubt. The specimens are very much like Fig. 1 of Will (No. 19) with one exception, stated above, viz: that the epiblast of the shield is continuous with the primitive plate and not separate as in Will's figure. One peculiarity of this stage is that both the anterior and the posterior wall of the invagination shows faintly the columnar arrangement as seen in Will's figure. Later on, this feature is confined to the anterior or dorsal wall (Figs. 9, 13, 14). After going straight downwards some distance the invagination cavity takes a forward horizontal direction and reaches the condition shown in Fig. 9. At the anterior lip of the blastopore, the columnar cells are recognizable very early, and the epiblast is here reflected downwards to become continuous with the anterior or dorsal wall of the invagination. In that part of the primitive plate placed behind the invagination the cell-mass remains undifferentiated for a long time, there being later established in this place the rudimentary yolk-plug, as was minutely described in the joint paper of Ishikawa and myself* on the germinal layers of Trionyx. Robinson and Assheton (No. 10) object to our idea of considering the structure in question as the yolk-plug. In the course of this paper, I hope to show that the presence of the yolk-plug at this place is an important feature in homologising the gastrulation of the Sauropsida with that of Amphibia. I may add that several authors, as Van Beneden (No. 13), Wenckebach (No. 15), and Will (Nos. 18 and 21) recognise the yolk-plug in this place.

I shall next describe how the invagination-cavity, as described above, comes to open below and becomes united with the large subgerminal cavity in the yolk. This process has, so far as I am aware,

* Contribution I. I shall refer to the papers in the present series of Contributions by their numbers in order of publication. See the list at the end of the present article.

never been treated with the fullness which its importance deserves. A careful study of this process has given me results which, I venture to think, are of the greatest importance in discussing the problem of gastrulation in the secondary meroblastic egg.

The surface views, Figs. 2–5, and the sections, Figs. 13–17 are introduced to illustrate this process. Figs. 2 and 2*a* are of the stage nearest to that represented in Fig. 1. In the dorsal view (Fig. 2), the dorsal opening of the invagination-cavity has now become a narrow crescent-shaped slit with the concavity directed forwards.* In the ventral view, the primitive knob has become larger. Viewed with a low power, the surface of the knob is tolerably smooth, although the figure represents it perhaps as a little too much so. The longitudinal section (Fig. 13) of this embryo shows distinctly that the depth of the primitive knob has grown greater in this stage than in that of Fig. 9. The invagination-cavity has extended itself much deeper and shows distinctly two limbs, one vertical and one horizontal. The roof of the cavity which is as before continuous with the epiblast, shows a distinctly columnar arrangement which is, however, gradually lost both anteriorly and superiorly. In these directions it merges gradually into an irregular network of cells which is in turn continuous with the lower layer of the embryonic shield. As was the case in the former stage, there is again below the primitive knob, no independent sheet of cells continuous with the lower layer of the shield, as described by Wenckebach or Mehnert. On the contrary, this and the succeeding figures (Figs. 14–17) give the impression that the lower layer of the embryonic shield extends below the epiblast

* Will (No. 21. p. 147) says: "Dieselbe (*i.e.* die Urmundspalte) tritt zuerst im vorderen Abschnitt der Primitivplatte auf, und hat zunächst die Form einer Sichel-rinne, nach Schwund der Sichelhörner aber einer rundlichen Delle" That is, his figures 8 and 9 are less advanced than his figures 4 and 10 so far as the shape of the blastopore is concerned. If the first two figures named are comparable to my figures 2, 3, 4, and the latter figures (his figures 4 and 10) to my figure 1, I can not but think that Will is mistaken in his views.

right up to the angle where the epiblast is reflected downwards at the dorsal lip of the blastopore, and that the primitive knob has been capped on to it from below, although now irrevocably fused with it by a protoplasmic network. The floor of the cavity shows two distinct divisions. In the posterior part (the vertical part in the section) there is a compact mass of cells which have evidently been proliferated from the floor of the cavity. This is the posterior median part of the commencing peristomal mesoblast. In the anterior half of floor, the vacuolated network comes very near the cavity, being separated from it only by a thin sheet of cells.

In the next stage (Figs. 3 and 3*a*), we notice one striking change in the ventral surface view of the embryo. While the top of the primitive knob (spoken of with its ventral surface as uppermost, see Fig. 3 *a*) is comparatively smooth as in the former stage, its base has assumed a honey-combed structure and this structure is spreading itself over the ventral surface of the embryonic shield.

Fig. 14 is a longitudinal section near the median line of this embryo. Compared with Fig. 13, the primitive knob has a longer antero-posterior extension and it will be seen that this increase is due almost entirely to the growth of the anterior half. The forward edge of this half is gradually encroaching on the ventral surface of the embryonic shield (*cf.* Fig. 3 *a*) and is thus giving the primitive knob ever greater extension. Wenckebach (No. 15), Will (Nos. 18 & 19), and Mehnert (No. 8) agree in thinking that the forward growth of the primitive knob takes place by its front growing edge insinuating itself between the epiblast and the lower layer of the shield, and quite independently of these two sheets of cells.* My sections do not allow me to

* In his latest paper (No. 21), Will admits that where gastrulation is completed by the formation of the *Kopffortsatz*, the "primary" and "secondary" endoderm cells cannot be clearly distinguished and that the former may grow by addition of the cells of the latter formed *in situ*. (p. 48).

come to the same conclusion, as a glance at Figs. 13 and 14 will show. Both the surface-views (Figs. 3*a et seq.*) and the sections give us even an impression that the primitive knob is spreading itself under the lower layer of the embryonic shield. In the parts where the primitive knob has once established itself, we can, however, no longer distinguish cells that have come from the primitive knob from those of the lower layer of the shield: they are indistinguishably fused. The invagination cavity at this stage (Fig. 14) has much greater longitudinal extension than in that of Fig. 13. I can discover neither at this nor at any subsequent stage a posteriorly-directed limb of the invagination-cavity, such as is described by Wenckebach (No. 15) in his Fig. 3.

There is nothing special to say of the roof of the invagination cavity, except that the points described in the previous stage are all more pronounced in this one. In the floor, there are some important changes. In the posterior half, where the mass of the peristomal mesoblast, grown much more compact, is easily recognisable, there is not much that is new. But in the anterior half of the floor, the wall of the invagination cavity is no longer so sharply defined as before, and some meshes of the cellular network in the primitive knob even open into the invagination-cavity, so that we can here, already in this stage, pass by a labyrinth of intercellular passages from the invagination-cavity to the subgerminal yolk-cavity. It should be specially noted that the anterior and of the invagination cavity is distinct and does not share in the dissolution of the anterior part of the floor.

With the growth of the embryo, the changes in progress between the stage of Fig. 13 and that of Fig. 14 become more and more pronounced. The primitive knob grows forwards more and more on the ventral surface of the shield, so that its antero-posterior diameter

is ever getting longer (Figs. 14, 15, 16 & 17). In the anterior part of the floor of the invagination-cavity which was already losing its sharp definition in Fig. 14, the disruption has proceeded one step farther in Fig. 15. In this figure, not only this part of the floor is giving away, but the network of cells lying underneath it, and between it and the subgerminal yolk cavity, has been largely absorbed. In Fig. 16, the process of breaking through is seen to be complete, and the invagination-cavity has now a clear opening below. I think it almost certain that such a clear and comparatively large opening has been produced by the running together of several small openings, such as we see in Figs. 14 and 15, which put the meshes of the cell-network in communication with the invagination-cavity. In fact, in Fig. 16 we can still see several such openings in the floor of the cavity in that part of the network situated behind the large anterior opening and in front of the compact peristomal mesoblast mass. Comparison with Fig. 17 makes it probable that this part of the cell-network is to be eventually absorbed, for the single large opening extends in the latter back almost to the peristomal mesoblast. It should also be noticed in Fig. 16 that the extreme anterior end of the invagination-cavity is clearly recognisable and does not participate in the breaking through, which seems to be confined to the floor. We should therefore remember that although the anterior end may not be recognisable in later stages (*e.g.* Fig. 17), it is the floor which is open below. The surface views of the stage at which the invagination cavity has just opened below are given in Figs. 3 *bis*, 4, and 4*a*. There is considerable difference in the appearance of the two embryos which I am not able to explain. I drew them just as they appeared under the microscope. I am rather inclined to think that Fig. 4*a* represents a more normal appearance, if we are to judge from the succeeding stages, although I am unable to detect anything unusual

in the sections of the other embryo (Fig. 3 *bis*), Fig. 16 in fact being one of them.

From the facts given above, the conclusion is reached that the invagination-cavity comes into communication with the subgerminal yolk-cavity by the absorption of the most anterior part of its floor as well as of the cell-network lying underneath this part. In this view, I find myself in agreement with Mehnert (No. 8, p. 411) who says :—" Wenn der Einstülpungssack etwa die halbe Länge des Embryonalschildes erreicht hat, schwindet in dem vordersten Abschnitte seine untere Wand und das mit derselben innig verwachsene Paraderm, so dass durch diesen Vorgang eine freie Communication zwischen der Einstülpungshöhle und der Subgerminalhöhle gebildet wird " I would only remark that this disappearance does not take place suddenly, as Mehnert's words might possibly lead one to suppose. As my sections show, it is already begun as early as in the stage given in Fig. 14. Wenckebach's Fig. 4 (No. 15 p. 60) is very much like my Fig. 16, except for the differences already specified. Although his views are not given in detail, I think, they are probably similar to mine. Will's views are essentially like mine ; only he insists on the greater forward and lateral extension of the invagination-cavity before it opens below. This is especially the case with the tortoise. He (No. 19, p. 191-2) says : " Aus diesen Stadien geht nun die wichtige Thatsache hervor, dass auch der Urdarm der Schildkröte noch in seiner ganzen Ausdehnung hohl ist und dass seine Ausdehnung absolut und relativ diejenige des Gecko noch übertrifft. Während derselbe beim Gecko die vorderen und seitlichen Ränder des Schildes nie vollständig erreicht, nimmt derselbe bei der Schildkröte stets die ganze Fläche des Schildes ein. Der Durchbruch des Urdarms erfolgt auch hier ganz ebenso wie beim Gecko, so dass die Fig.

7 meiner oben zitierten Mittheilung (No. 18 of my list. Same as Fig. 17*b* of No. 21) auch geeignet ist, die Verhältnisse bei der Schildkröte zu illustriren. Es treten zunächst einige wenige isolirte Durchbrechungen der untern Urdarmwand (nebst dem unter derselben wegziehenden Dotterblatt) ein; indem sodann beständig neue Lücken auftreten, die alten sich aber vergrössern gelangt man zu Stadien, bei den von der gesammten unterer Urdarmwand nur noch ein unregelmassiges, bei den verschiedenen Embryonen verschieden gestaltes System von Netzbalken erhalten geblieben ist. Schliesslich kommen auch diese letzten Reste zum Schwunde, wodurch dann das bisherige Urdarmlumen mit dem subembryonalen Raum zusammenfliesst." After seeing his Figs. 55*a* and *b* (No. 21) it seems no longer possible to doubt the great anterior extension of the archenteron in Gecko, before it breaks open. As to this point in Chelonia I shall reserve my judgment until his promised full paper on the tortoise appears.

Van Beneden (No. 13) describes in Mammalia two kinds of openings by which the chorda-canal comes to open below into the blastoderm cavity, viz:—(1) an anterior transverse slit, and (2) several openings which soon run together into a single posterior longitudinal slit. From what has been stated above, I need hardly say that I do not find any such differentiation of openings in Chelonia.

The changes that follow on the breaking through of the invagination-cavity can best be seen in the surface views. Figs. 5 and 5*a*—8 and 8*a* are introduced to illustrate this point. We have seen how the primitive knob, at first confined to a small accumulation of cells at the posterior edge of the embryonic shield (Fig. 9), gradually spreads itself anteriorly until it comes to occupy quite a considerable area on the ventral surface of the embryonic shield (Figs. 13-16), when the invagination cavity breaks through be-

low (Figs. 4*a* and 16). The part that has been covered by the cells of the primitive knob can be very plainly distinguished on a ventral view of the blastoderm, showing mostly a trabecular network (Fig. 4*a*). This gradual spreading of the cells from the primitive knob over the ventral surface of the embryonic shield is continued long after the breaking through of the invagination-cavity. Figs. 5 and 5*a* are only a little advanced on Figs. 4 and 4*a*. The area of the network is not yet very large, but in the stage next introduced (Figs. 6 and 6*a*) it has expanded itself considerably. A great change is now noticeable: a circular area at its centre shows no longer a network but presents a smooth compact surface. This is produced by a continuation forwards of the process by which cells in the roof of the archenteric-cavity, beginning at the dorsal lip of the blastopore, have gradually assumed the columnar shape and formed themselves into a compact sheet (Figs. 13-18)

In Figs. 7 and 7*a* the spreading of the part derived from the primitive knob has gone one step further. Not only is the area occupied by the network larger but the compact part in the centre is considerably enlarged by its extension anteriorly in the median line. There is also another noteworthy new feature: at the anterior end of the median compact area, there is a slight transverse ridge. This is the commencing head-fold. The last stage in which I was able to detect traces of the network is shown in Figs. 8 and 8*a*. At the front end of the embryonic shield, a patch of the network could be faintly traced. I think it almost certain that the part derived from the primitive knob does not extend itself much beyond the area of the embryonic shield, and that it gradually thins itself out and ends by becoming simply continuous with the primitive lower layer at or near the periphery of this area (Figs. 17 & 18). With the exception of the patch above-mentioned, the ventral surface of the embryonic shield

presents now a smooth compact appearance. The head-fold and the chorda-groove have already become conspicuous.

As the head-fold, formed well within the edge of the embryonic shield, marks the anterior end of the embryo, and therefore of the archenteron or the adult alimentary canal exclusive of the stomodæum ; as the primitive knob marks the posterior end of the embryo ; and as the lateral body-wall is formed from the lateral folds, also arisen within the embryonic shield, we are justified in coming to the very important conclusion that the body of the future embryo and consequently the definitive alimentary canal is formed entirely within the area covered ventrally by cells derived from the primitive knob. This speaks in favor of the assumption that the invagination cavity is the archenteron and gives rise to the future alimentary canal. I shall discuss farther on how we ought to regard the breaking through of the invagination-cavity and the gradual spreading of the cells of the primitive knob over the ventral face of the embryonic shield.

The reason why the advancing edge of the primitive knob is marked by a zone of network is probably, I think, that such a structure allows free and easy access of the nutritive liquid of the yolk to the deeper parts of the tissue.

The network such as is here described, has been seen many times before. For instance, Ishikawa and I noticed it in a Trionyx blastoderm (Fig. 1*b* of Contrib. I) without knowing its significance. Again, Fig. 10, Contrib. III represents the same thing in cross-section in an embryo of Clemmys. Mehnert (No. 8) gives beautiful illustrations of stages showing the network, in his Figs. 4-13. He, however, gives an explanation of it which is at utter variance with the one given above, for according to him, it is concerned with the process of the mesoblast formation. He states

that in the anterior part of the embryonic shield, the dorsal roof of the archenteric cavity divides itself into two layers : (1) a lower one consisting of a single layer of low cells representing the definitive hypoblast, and (2) an upper one consisting of stellate branched cells representing the "Rumpf-mesoblast" (his Figs. 22 & 23). In the course of this separation, the dorsal roof which is at first composed of compact columnar cells becomes permeated by vacuoles, and he says that "das im Flächenbilde eruirte Netz der Ausdruck für die aus dem Verbande des Urdarm-epithelhofes (scl. oberer Urdarmwand) losgelösten Mesodermstränge war, welche sich im Furchungsspalte centrifugal zwischen Ektoderm und Paraderm weiter vorschieben" (p. 434). He thus calls the area of the network with the central compact part the "Rumpfmesodermhof." Moreover he makes this process of the mesoblast formation begin at the cranial end and proceed backwards. He also says that "die periphere Ausbreitung des Mesodermhofes nicht im proportionalen Verhältnisse zur Grösse der Area embryonalis (scl. Embryonalschild) steht" (p. 434). Mehnert's views can not be reconciled with mine: one of us is wrong. Except as to the single point that the network grows centrifugally, I am obliged to differ from him in almost every particular. This network has in my opinion nothing to do with the process of the mesoblast formation. My views on the latter process have already been given in great detail in two former papers (Contrib. I. & III.) and I do not intend to go into them again in this paper. The network is simply the surface expression of cells from the primitive knob spreading themselves over the ventral face of the embryonic shield. In what light we ought to regard this process I shall discuss farther on. But whatever it is, it does not begin at the cranial end and proceed backwards. In obtaining materials for the present investigation, I opened on consecutive days a certain number of eggs from the same deposits, and observed

the progress made during the interval of time between the two successive acts of taking out, making of course due allowance for fast or slowly developing eggs. Fig. 1 and Figs. 4–8 with some intermediate stages, which I have not introduced here, belong to one of the series obtained in this manner, and these show, conclusively so my mind at least, that the area of the network spreads itself gradually underneath the embryonic shield from the spot where the invagination-cavity first breaks through, towards the periphery of the thield *i.e.* from the posterior primitive knob anteriorly over the embryonic shield. It is not the network that is gradually encroaching on the central compact area, as Mehnert assumes, but just the reverse; for the central compact area is formed out of the area of the network. The series, Figs. 5*a*—8*a*, shows also that the area of the network increases with the age of the embryonic shield. I can not therefore accept Mehnert's explanation of the appearance of the network on the ventral face of the blastoderm.

As I said just now, I do not propose to go into the mesoblast-formation again in this paper. I would merely remark that in the stage corresponding to Fig. 17, I already see the establishment of the chorda-hypoblast and the stretch of the epithelium on each side of it which becomes transformed into the gastral mesoblast. (Compare Fig. 11, Contrib. III.).

There are two other points on which I wish to make some remarks.

The first of these is in regard to the position of the primitive knob relatively to the embryonic shield. In Fig. 1, the primitive knob lies for the most part outside of the embryonic shield, only about one-third of its antero-posterior extension being within the shield. In Figs. 2 and 3 it is about one-half, and in Fig. 4, entirely within the shield. This is no doubt brought about by the gradual

extension of the epiblastic area composed of columnar cells. In later stages, (Figs. 6, 7, 8), the mass of the peristomal mesoblast no doubt helps in causing opacity in the posterior region. From the stage of Fig. 6 on, the embryonic shield, which has hitherto passed gradually into the surrounding parts, becomes sharply marked off from the circumjacent transparent area, in which it is eccentrically placed, and becomes apparently diminished in size.

The second point on which I wish to touch is as to the dorsal opening of the invagination cavity. In the earliest stage I possess (referred to on p. 236–7), it is a squarish pit rather elongated in the antero-posterior diameter. In Fig. 1, it is a wide open cavity elongated transversely. In Fig. 2, it is a crescent-shaped transverse slit, no longer gaping, and with its concavity turned forwards. The same can be said of Figs. 3 and 4. In Fig. 5, the blastopore is nearly straight across. It has a slight notch in the median line open backwards. In Fig. 6, the ends of the slit-like opening have turned backwards so that now the concavity faces backwards. In further growth, the backward curvature becomes greater and greater, until it becomes a horse-shoe shaped slit, as can be seen in figures contained in the former Contributions. Of the significance of this change of shape I shall speak later on.* The yolk-plug, which can be traced more or less clearly from the first, becomes very distinct as the backward curvature becomes greater, and sticks out between the two limbs of the horse-shoe.

The yolk-plug in Fig. 8 is very peculiar in that it has a groove in the median line. The cross sections of this region also show it to be

* In his latest paper, Will (No. 21) seems to consider the enclosure of the primitive knob within the embryonic shield as intimately connected with the change of the shape of the blastopore-opening. Both are, according to him, due to the forward growth of the yolk-plug and the consequent shoving forwards of the blastopore-opening (see p. 127 *et seq.*). I find myself unable to accept his views.

a deep fissure cutting the yolk-plug into halves. I am unable to give any explanation of this groove, which is very unusual, this being the sole instance among hundreds of chelonian embryos that have passed through my hands. I think it may probably be teratological. Kupffer (No. 6, Taf. IV. Fig. 40 *f.* & *g.*) gives two figures of Coluber that are strikingly like this.

To sum up the facts of Gastrulation as above described :—

1. When segmentation has gone on for some time, there is established in the blastoderm two layers: (*a*) the superficial epiblast composed of columnar cells, and (*b*) the lower layer composed of irregular stellate cells and probably not forming a complete sheet.

2. This separation into two layers takes place in all parts of the blastoderm with the exception of a small area at the posterior end of the future embryo. Here not only is there no differentiation of layers but a thick knob consisting of a network of cells is produced by the accession of cells from the subjacent bed of yolk. The mass can not be said to belong to either of the two layers above named. This is the *Primitive Plate or Primitive Knob.*

3. In the middle* of the *Primitive Knob*, an invagination cavity is produced, which at first goes straight downwards but soon takes a forward horizontal course. This is the *Invagination-Cavity* or the *Archenteron.* Its dorsal opening is the *Blastopore.* The invagination-cavity

* Will (No. 21) is no doubt quite correct in printing out that the invagination-cavity begins much nearer the anterior than the posterior end of the primitive plate. In front of it there is only the future anterior or dorsal lip of the cavity.

extends itself gradually forwards, *pari passu* with the anterior enlargement of the *Primitive Knob*.

4. The roof of this invagination-cavity which becomes continuous with the epiblast of the embryonic shield at the anterior lip of the blastopore, assumes a colunmar arrangement, the process beginning at the posterior end and proceeding gradually forwards. Out of the median part of it is established the *Chorda dorsalis*, and from a certain stretch of columnar epithelium on each side of it is developed the gastral mesoblast.

5. The floor of the invagination-cavity is divided into two parts:—(*a*) the posterior which proliferates the peristomal mesoblast, and (*b*) the anterior which losing definiteness is finally absorbed, together with the whole thickness of the cell network placed beneath it, *thus putting the invagination-cavity in communication with the large subgerminal cavity in the yolk*.

6. The primitive knob which was gradually spreading itself over the ventral surface of the embryonic shield before the breaking through of the invagination-cavity continues to do so after that event. It spreads from the spot where the invagination-cavity first broke through away towards the periphery of the shield. Its advance in later stages is marked by a zone of cell-network with a compact central area. When the whole of the ventral surface of the embryonic shield has been covered, the process stops. The cell-network afterwards changes into compact cellular sheets.

7. The head-fold is formed some distance behind the anterior edge of the embryonic shield.

8. The future embryo and consequently the definitive alimentary canal is formed entirely within the area covered ventrally by the part derived from the primitive knob.

Putting the results in another way, they may be summed up as follows:—

From the epiblast of the embryonic shield, THE EPIBLAST and ITS DERIVATIVES of the future animal is derived. In the region of the primitive plate and its anterior enlargement are produced the INVAGINATION-CAVITY (the Archenteron), the YOLK-PLUG, the CHORDA, the MESOBLAST (both peristomal and gastral), and the DEFINITIVE HYPOBLAST and ITS DERIVATIVES. The primitive lower layer forms the wall of the yolk-sac, and contributes to the future animal only in so far as some of its cells are unrecognisably incorporated with the cells of the primitive knob, when the latter spreads itself over the ventral surface of the embryonic shield.

On the last point, I find myself at variance with Wenckebach who makes the cœnogenetic hypoblast take part in the formation of the anterior part of the embryo-body, "namentlich an dem cranialen Wachsthum von Chorda und gastralem Mesoderm (No. 15, p. 76).

Theoretical Considerations.

If we represent the chelonian egg diagrammatically in the light of the facts described in the preceding pages, we shall obtain something like that given in Woodcut I.

Woodcut I.

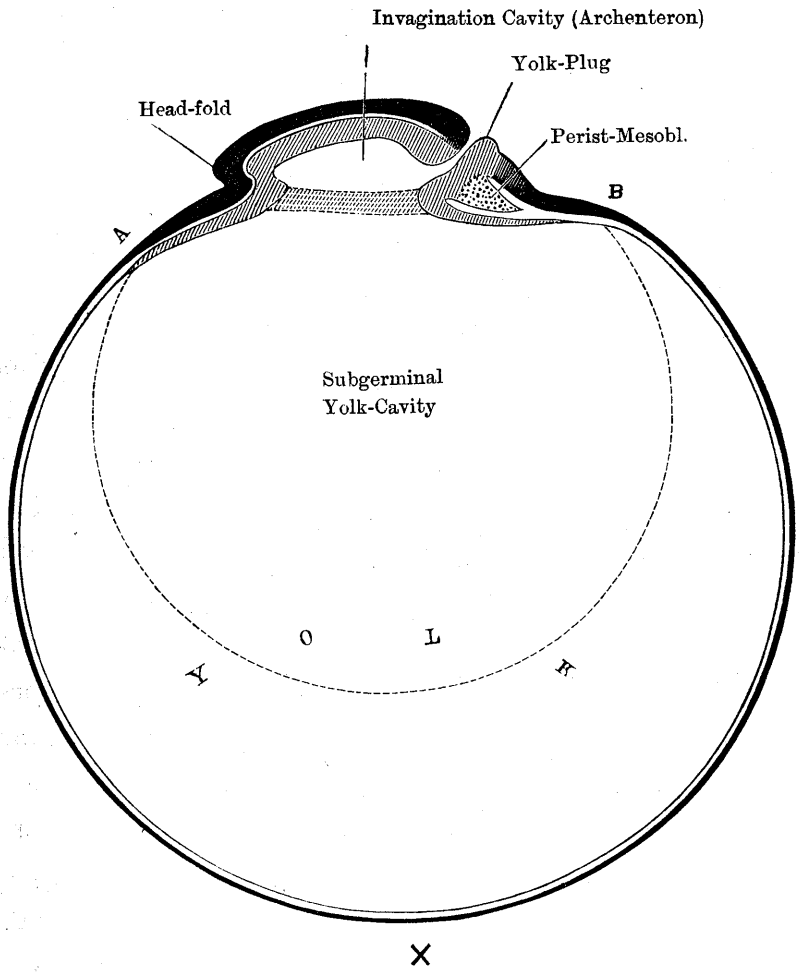
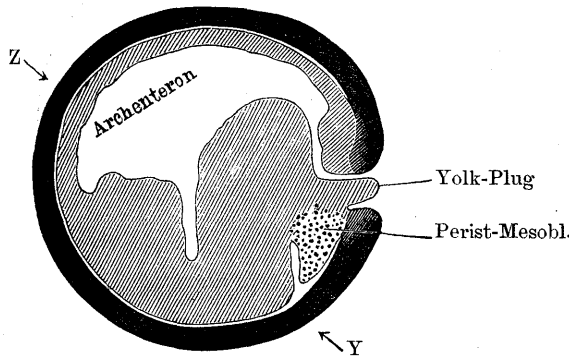


Diagram of a Chelonian Egg.

$A-B$ represents the embryonic shield with the enclosed primitive knob. Within the shield is established the whole of the future embryo. $A-X-B$ is the yolk-bag with the large subgerminal cavity filled with nutritive liquid. The invagination-cavity which has extended forwards *pari possu* with the anterior extension of the primitive

knob has by the absorption of the anterior part of its floor (indicated by dotted lines) been put in communication with the subgerminal cavity in the yolk. The anterior end of the invagination-cavity is clearly recognisable at the time of the breaking through ; it becomes invisible for a time after that event, but is soon marked out again by the commencing head-fold. The thick part of the hypoblast (marked with slant lines) is intended to show the extent to which cells from the primitive knob spread themselves. The structures behind the invagination cavity—the yolk-plug, the peristomal mesoblast—have been fully described in Contribs. I. & III.

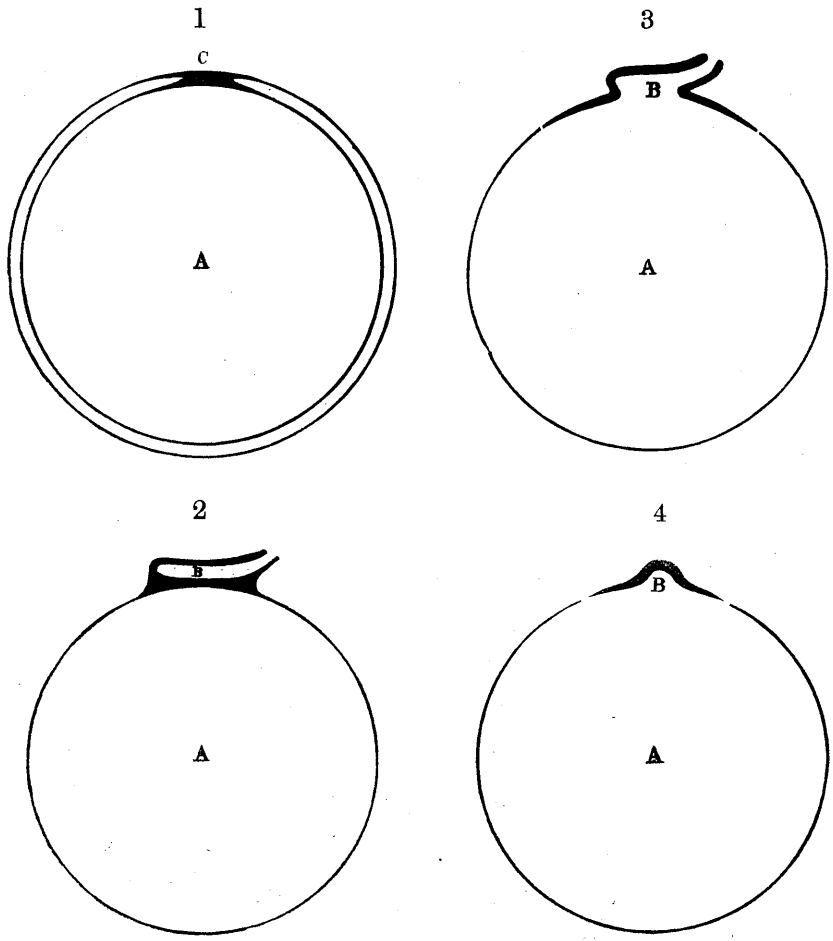
Woodcut II.



When we compare this diagram with the well-known one of an amphibian egg (Woodcut II.) given by Hertwig, their similarity becomes very striking. The structures dorsal to the line $Z-Y$ in the amphibian egg can be identified, part for part, on the embryonic shield of the chelonian egg. In homologizing these two eggs a great deal depends upon the view we take as to the nature of the invagination-cavity, the breaking through of the same, and the large subgerminal cavity into which the the invagination cavity opens. I have already made mention of the assumption that the invagination cavity gives rise to the definitive alimentary canal (p. 245). The following considerations will make my views clear and I trust, justify them at the same time :—There is in the chelonian egg a large yolk reservoir *A* (Woodcut III. 1),

This, let us suppose, is surrounded by a layer of cells (although in point of fact the lower pole is not enclosed until a much later period), except at the point *C* where there is a mass of cells in which both the epiblast and the layer surrounding the yolk are merged. This is the Primitive Plate or Knob. In this knob, there arises an invagination, *B* (Woodcut III. 2) which grows forwards together with the anterior elongation of the primitive knob. Assume

Woodcut III.



for the present the invagination-cavity (*B*) to be the Archenteron. Then the yolk reservoir *A* must from the nature of the thing be an appendage of the invagination-cavity *B*. But owing to its enormous size compared with *B*, the bodily invagination of the yolk is out of the question. It forms the most conspicuous part of the egg from the first, and begins to surround itself with a cell-layer long before the invagination-cavity *B* makes its appearance even. Hence, *A* can only secondarily come into connection with *B*. This happens by the anterior part of the floor of *B* flaring out, so to speak, into a funnel-shaped opening. This is the meaning of the breaking through of the invagination-cavity. The spreading of the cells derived from the primitive knob over the ventral surface of the embryonic shield after the breaking through of the invagination-cavity may be regarded as the gradually thinning wall of the funnel-shaped opening making itself continuous with the cell-layer surrounding the yolk (Woodcut III, 3–longitudinal section, 4–cross section). When the alimentary canal is formed definitely, later on in the course of development, the wall of the funnel-shaped opening of the invagination cavity is again tucked in as the splanchnopleura.

The above course of reasoning explains all the events accompanying the invagination and thus justifies the assumption that the invagination-cavity (*B*) is the Archenteron corresponding to the part marked as such in the amphibian egg (Woodcut II.) and the whole yolk-bag must be regarded simply as a part of its ventral wall that has become bulged out on account of the enormous accumulation of nutritive matter within it. The presence of a large subgerminal cavity in the yolk filled with a nutritive liquid is a physiological accident, so to speak. I agree with Van Beneden, Keibel, and Wenckebach in regarding it as intercellular space in the yolk. It is a cavity arisen solely from physiological necessity

and having a comparatively insignificant morphological value. Although the whole yolk-sac should be regarded as a diverticulum of the archenteron and although it has a definite morphological value, it is a matter of comparative indifference, so far as morphology is concerned, whether its inside is filled with cells charged with yolk-granules, or with free yolk-spheres, or with a nutritive liquid or with a mixture of all three. Looked at in this light, the chelonian egg is nothing but the amphibian egg, with an enormous ventral saccular appendage surcharged with nutritive matter.* The whole yolk-sac (Woodcut I. $A-X-B$) must not, however, be looked on as strictly homologous with the part of the amphibian egg ventral to the line $Z-Y$. For, in the latter, the epiblast of that part becomes the ventral abdominal wall of the future animal, while in Chelonia the epiblast of the yolk-bag becomes later a part of the serous envelope,—the ventral abdominal wall of the embryo being formed within the embryonic shield above the yolk-sac, and the yolk-sac with the enclosing sheet of hypoblast and mesoblast cells migrating within the body of the embryo. When it has done so, nobody has any difficulty in accepting it as an appendage of the alimentary canal which has for its function the storage of nutritive matter. My contention is that as such it should be looked on from the first. The archenteron is at first so utterly insignificant in size compared with the yolk-sac that the true nature of the latter is obscured: none the less the yolk sac is a mere appendage of the archenteron. This view makes it necessary to regard the primitive lower layer enclosing the yolk-sac as a part of the

* It will be seen that further consideration has made me modify in some details my views as set forth in the preliminary notice sent to the *Anatomischer Anzeiger*, and published in that journal, Nos. 12 & 13, 1893.

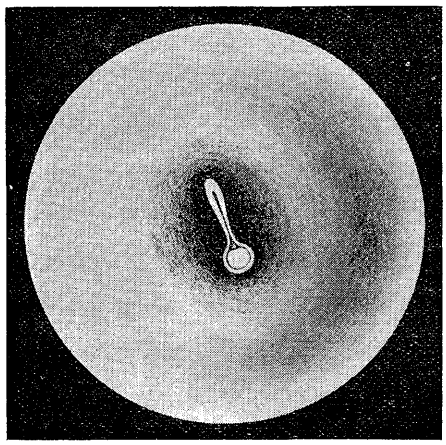
hypoblast. That it arises before the invagination of the archenteron can be explained by the principle of precocious segregation, as has been pointed out by Hubrecht (No. 5). The name "cœnogenetic hypoblast" which he applies to this layer, seems therefore very appropriate as the part derived by invagination may be called the "palingenetic hypoblast."

I think the objection on the part of Robinson and Assheton (No. 10) that the yolk-plug can not be present at the spot designated by Ishikawa and myself is fully answered by comparing the two diagrams (Woodcuts I. & II.). The yolk-plug can not only properly be found at this spot but its presence here is one of the significant, although not the essential, features in homologizing it with the amphibian egg. This is an example of those cases where a secondary characteristic is of great service in identification.

According to the views set forth above, the enormous accumulation of yolk has profoundly affected the course of development in the chelonian egg, especially in the precocious development of a part of the hypoblast, and in the rapid spreading of the blastoderm over the surface of the egg. There is left, however, in the centre of the blastoderm a certain amount of raw undifferentiated materials in the shape of the primitive plate or knob in order to go with it through certain developmental processes of palingenetic character:—the invagination of the archenteron with the consequent establishment of the chorda-hypoblast, the peristomal and gastral mesoblast, the yolk-plug, and the definitive hypoblast. The changes in the shape of the blastopore from a crescent with its concavity turned anteriorly to that of a horse-shoe with its two limbs directed backwards and enclosing the yolk-plug between them must be looked on as the remnant of that process by which the epiblast gradually encloses the endoderm cells in the amphibian ovum or the

yolk in the Elasmobranch egg. If we make a companion diagram to the well-known series given by Balfour (Comp. Embryol. vol. II. Fig. 175) it will be like Woodcut IV. The upper pole of the egg in Reptilia is capped by a small patch where nearly all the changes which in Amphibia are gone through by the whole egg, are performed. Accordingly, the enclosure of the yolk by the blastoderm in the chelonian egg is of a very different nature from the enclosure of the yolk in the Elasmobranch, for while the former is a simple growth of the edge of the blastoderm, and of cœnogenetic character, the latter is a part of the process of invagination and of palingenetic character. That the yolk in Chelonia is not completely enclosed till the embryo has made much progress is due to its large size, and may be regarded as of quite secondary significance. I thus find myself obliged to put aside the yolk-blastopore of Balfour as no longer tenable in Sauropsida.

Woodcut IV.



After what has been given above, I need hardly say that I accept the views of Rabl (No. 9) as to the loss and acquisition of the yolk in vertebrate eggs several times in the course of the phyletic development. All the facts given above tend to prove that Chelonia possesses a secondary meroblastic ovum in contrast to the primary meroblastic ovum of the Selachians.

My views overlap more or less those of previous writers, such as Wenckebach (No. 15), Will (Nos. 18, 19 & 21), Mehnert (No. 8) and Rabl (No. 9). It would, however, be a tedious and useless task

to go over the writings of these authors and point out wherein we agree or differ. The reader acquainted with the literature will be able to do this for himself. The points which I want specially to emphasize are however as follows :—

1. The PRIMITIVE PLATE or KNOB is raw-material left at the centre of the blastoderm, by means of which certain palingenetic processes are gone through.

2. The INVAGINATION-CAVITY is the ARCHENTERON, and gives rise to the alimentary canal and the organs derived from it exclusive of the proctodæum and the stomodæum.

3. The YOLK-SAC must be regarded as a ventral appendage or diverticulum of the ARCHENTERON in which nutritive matter is stored in solid or liquid form.

4. Owing to the enormous size of the YOLK-SAC, it and the ARCHENTERON are formed separately from each other, and come only secondarily into connection.

Having considered the chelonian egg in its relations with that of Ichthyopsida let us now see how it compares with the avian or mammalian egg.

If the process by which the blastopore in Chelonia has assumed a horse-shoe shape (Woodcut V. A) continues on after the state *A* is reached, as actually happens in Amphibia (See Figs. 18 & 19, No. 10), the lateral lips will coalesce* and there will result the primitive streak of the avian egg (B). In cases where the lips have not quite coalesced, we should expect to find the yolk-plug sticking out

Woodcut V.

A B

* I am gratified to find this verified within the group of Reptilia. Will (No. 21) has found in Gecko that the lips of the blastopore approach each other very closely and form a primitive streak.

between them, and such is actually the case as seen in Figs. 15 and 32 of Duval (No. 3). The annexed woodcut will also explain why the posterior limit of the primitive streak is not as sharply defined as the anterior. This view makes it plain that the homologue of the primitive streak in Chelonia is the lips of the blastopore which are, however, still so wide apart from each other that the name "streak" is hardly applicable to it. It should be noted that the primitive plate or knob is not the homologue of the primitive streak. The latter has potentially in it not only that but a great deal more. It is in fact a mass of raw undifferentiated material from which various structures are produced. This view also makes it evident that as the primitive streak is almost the first feature visible in the development of the avian blastoderm, a great many changes of palingenetic character observed in the chelonian egg before the establishment of what corresponds to the primitive streak, are necessarily skipped over in Aves, which are therefore not very good subjects in which to study the process of gastrulation. The removal of the primitive streak to the centre of the blastoderm must also be explained in the way I have indicated above in the case of Chelonia.

Comparison of the reptilian ovum with the mammalian seems easier. The facts given in this paper agree, with the exception of some minor details, very closely with those communicated in Van Beneden's preliminary notice (No. 13). It seems to me that the primitive streak and Hensen's knob together correspond to the primitive plate of Chelonia, and the "Kopffortsatz" to the forward growth of the primitive plate. I can not, however, accept Van Beneden's theory of "Lecitophor" and "Blastophor." Exactly what I cannot accept lies in the emphasized words of the following quotation:—"Wenn diese Auseinandersetzungen richtig sind, wie ich es glaube, so ist es klar dass das sogenannte zweiblätterige Stadium

der Säugethiere der Gastrulation d. h. der Einstülpung, die man von der Epibolie auseinanderhalten muss, vorangeht, und dass die zwei Schichten respektiv dem Ektoderm und dem Entoderm des Amphioxus nicht entsprechen. Dieser Schluss geht schön daraus hervor, dass nicht allein die Organe des Epiblastes, sondern auch die Chorda und der ganze Mesoblast aus der äussern Schicht sich bilden." According to my views, the epiblast of the Amniota is homologous with the epiblast of Amphioxus. The difficulty which keeps Van Beneden from accepting this idea lies in this, that not having for comparison the comparatively simple story of the reptilian development he has reckoned as epiblast what corresponds to the primitive plate of Reptilia. If he had recognised the structure which, as I have shown above, can not be said to belong to either layer and then considered the lower layer as precociously developed hypoblast, the conclusion would have been inevitable that the outer layer corresponds to the epiblast of Amphioxus. Keibel (No. 7) has also shown to what contradiction Van Beneden's theory of the "Blastophor" and "Lecitophor" leads. I think, however, I have now removed the second objection of Keibel:—"Dazu kommt dann ferner, dass uns Van Beneden den Beweis dafür durchaus schuldig geblieben, dass nun wirklich die untere Schicht des zweischichtigen Säugethierkeimes und die Keimhöhle desselben mit der Bildung des definitiven Darms der Säuger nichts zu thun hat." I think, the fact that the whole yolk-sac with the subgerminal cavity within it does not form in Chelonia any permanent part of the alimentary canal, makes it highly probable that the same is also true of the homologous structure in Mammalia. As I have more than once stated above, I accept Hubrecht's view of precocious segregation. In many respects my views are very much like his, but I do not think, he makes a clear distinction between the Archenteron and the yolk-sac. Nor do I know from personal

observation whether such a distinction is possible in Mammalia. I am only inclined to think that, since the reptilian and mammalian eggs are alike in so many points, what is true in the former as regards the development of the alimentary canal will in the main be found true also in the latter. I can also find in Chelonia nothing corresponding to his "proto-chordal plate." As to whether there is such an annular zone of hypoblast as he describes which gives rise to the mesoblast I wish to express no opinion. That the "Rumpfmesoblast" arises entirely within the embryonic shield from the materials derived from the primitive knob I hope to have made at least probable in the preceding pages, but whether some temporary mesoblastic structures of the embryo may not arise in Reptilia from such an annular zone as he describes, I am not in a position either to affirm or to deny.

Postscript.

The foregoing article was nearly finished in January of this year. I made an extract of it in the early part of that month and sent it to the *Anatomischer Anzeiger* as a preliminary notice.* As I was giving final touches to the article I received from Dr. Ludwig Will an article of his own entitled "*Die Anlage der Keimblätter beim Gecko*" (*Zool. Jahrbücher; Abth. f. Anat. u. Ont., VI Band, 1 Heft*). As I mention in a previous page, I was not under the circumstances able to make full use of Dr. Will's paper, but inserted remarks on it mostly in footnotes. The foregoing article has since then been lying ready for the press, but its publication was greatly delayed, owing to various extraneous circumstances. When it was at last to be put in the printer's hands, I received a second article by Dr. Will: "*Die Anlage der Keimblätter bei der menoquinischen Sumpfschildkröte*" (*Zool. Jahrbücher: Abth. f. Anat. u. Ont., VI Band, 3 u. 4 Heft*). As it is too late to go over my article again in the light of the facts brought out by Dr. Will, I have decided to add here as a postscript a few remarks on Dr. Will's two papers, as well as on some other articles which have appeared recently.

Will's observations on the two species, Platydactylus facetanus, *Schreib.* and Cistudo lutaria, *Gesn.* coincide throughout. They, I am glad to see, agree also in many essential points with the results I have brought out in this and previous contributions. There are however, several points on which we differ and some of these, it must be confessed, are by no means insignificant.

1. According to Will, a stage in which a sickle is present precedes the establishment of the primitive plate in both the species. Since receiv-

* Published in *Anat. Anz., VIII Jahrg., No. 12/13.*

ing his second article, I have again gone through the chelonian embryos in my possession in order to examine this point. In Chelonia caouana, the two youngest embryos which I possess (referred to on p. 236) are not probably much older than that corresponding to Will's figs. 1 and 13 (II Art.) but neither in the sketches I had made of surface views, nor in the sections, was I able to detect any structure resembling the sickle. In Trionyx, I was not more successful. But in Clemmys japonica, some embryos which I had taken out of the oviduct showed a structure which on surface views looked very much like a sickle.

The annexed figure (Fig. A.) represents one of these in which the sickle is seen to extend to the sides more than in the others. This stage is more advanced than that in which Will figures a sickle, (Fig. 1, II Art.) inasmuch as the invagination cavity has already broken through below. On cutting sections of this embryo, the sickle was found to be due to an accumulation of the lower layer cells continuous with the primitive plate. (See Fig. B.). The epiblast is sharply marked off from this mass, so that it can not be regarded as a part of the primitive plate—at least not in this stage. After becoming familiar

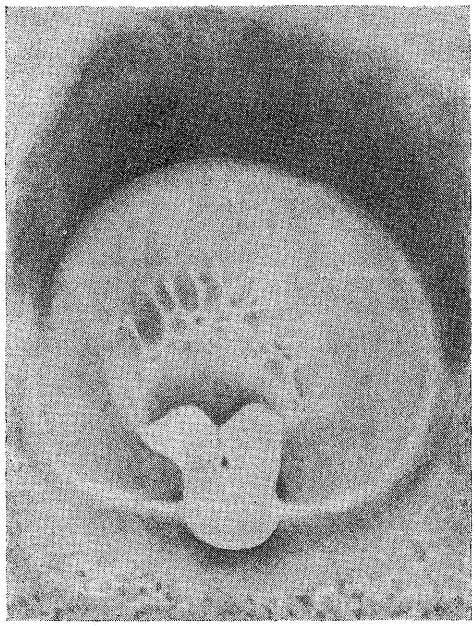


Fig. A.

Ventral View of a Clemmys Embryo taken from the Oviduct.

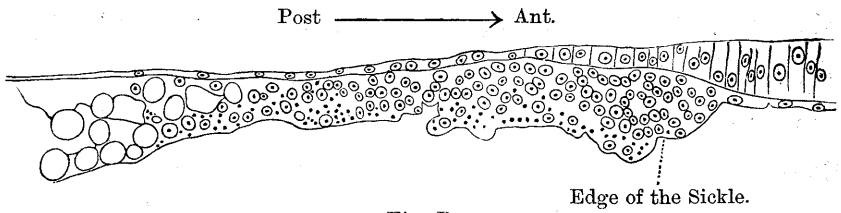


Fig. B.

Fig. B. Posterior part of a longitudinal section of the Embryo given in Fig. A.

with the appearance of the sickle in this series of sections, I was able to detect the same structure persisting in the sections of some older embryos of Clemmys. There is a great deal of variation in the degree of development to which this structure attains in different individuals as well as on the two sides of the same individual. It seems to disappear entirely later. As the mesoblast develops afterwards quite independently of this, it is not what Will calls Kupffer's sickle. For the present, I think, it corresponds probably to the sickle (Koller's sickle) which Will describes in the earliest stage, although there are some features of it which I do not yet quite comprehend and which may finally establish its difference from Koller's sickle.

Apart from the structure which I have described, I can detect nothing comparable to Koller's sickle in my materials.

2. Will makes out a sharp line of demarkation between the ectoblast and entoblast at the edge of the primitive plate (I Art. Figs. 43, 44, and others ; II Art. Figs. 13 *a.* and *b.*). Since reading Will's second article, I have again carefully gone over the sections of my earliest stages, but I am unable to make out such a line at all. As this line is figured in Will's papers as persisting to quite late stages, I am surprised that I do not see it at least in some of my sections, if it really exists.

3. Perhaps the most serious point of difference in the observations of Will and of myself is in regard to the extension of the invagination cavity, before it breaks through below. In Cistudo, Will states that

the invagination cavity becomes exactly co-extensive with the epiblastic embryonic shield, before that act takes place (II Art. figs. 6*b*, 7*b*, 8*b*). In Platydactylus it is said to be only slightly less. In Chelonia caouana, which I have studied, the invagination cavity breaks through, as I have stated in the foregoing article, when it is quite small compared with the epiblastic shield. Will accounts for this discrepancy by assuming that Cistudo and Platydactylus on the one hand and Chelonia on the other are really different in this respect. (*II Art., Nachschrift.* Also in a note "*Ü. d. Gastrulation v. Cistudo u. Chelonia,*" *Anat. Anz., VIII Jahrg., No. 18/19*). In Trionyx, I possess several embryos which are like Fig. 1*b*. of Contrib. I. or Fig. 6 of the foregoing article, so that I think I am justified in concluding that Trionyx is like Chelonia in this respect. In Clemmys there seems to be individual variations as to this point. For instance, if we compare Fig. A. in this postscript with that given in Fig. 1 of my Contrib. III, we find that in the latter, the invagination cavity must have advanced farther forwards, nearer the anterior end of the embryonic shield than the former. So that it is an actual fact that there are variations in different species or within the same species in the proportion of the invagination cavity to the shield. For the present, I am therefore willing to accept Will's assumption as the correct explanation of the disagreement between his statements and mine. And yet I can not help having some doubts lurking in my mind that his Figs. 6*b*, 7*b*, and 8*b* (II Art.) are expressions of something other than the breaking through of the invagination cavity. That in Cistudo the invagination cavity becomes both in length and breadth exactly coextensive with the embryonic shield—not one whit more or less—seems to me very extraordinary. The figures Will copies from Clarke do not certainly show the lateral extension of the in-

vagination cavity to be equal to the width of the shield. In this connection we must remember another fact which Will brings out in another place and which I believe myself able to corroborate, that "die gesammte dorsal Urdarmwand zur Bildung der Chorda und des gastralen Mesoderms aufgebraucht wird" (II Art. p. 612).* If the invagination cavity becomes, as Will maintains, really coextensive with the embryonic shield, it follows from the above-mentioned fact that the lower layer covering the entire ventral surface of the embryonic shield is used up for the above-mentioned purpose and the gut-hypoblast (Darm-Entoblast) must come from outside the shield. But this can not be reconciled with the fact which I have brought out in the foregoing paper and of which there can not be any doubt, that the gut-hypoblast comes from the cells derived from the primitive plate and arises within the embryonic shield. It can not be urged that there are actual differences in this respect between Cistudo which Will has studied and Chelonia which I have studied, for his Fig. 9 (II Art.) is very much like my Figs. 6*a* or 7*a* in the foregoing article, and shows, beyond a shadow of doubt, that in Cistudo as well as Chelonia the gut-hypoblast arises within the embryonic shield. These considerations force me to suspect that possibly there is no great difference in the actual facts between Cistudo and Chelonia in this matter.

4. As I have stated in the foregoing article, I was unable to detect in Chelonia any differentiation of the primary and secondary endoderm such as Will and several others describe.

5. In one place Will does me injustice. On p. 587 (II Art.) he says: "Die Bedeutung dieses Flächenbildes, von dem ich in Holzschnitt Fig. 7. (my Fig. 2, Contrib. III.) eine einfache Skizze

* I am indebted to Dr. Will for pointing out the inaccuracy of my expression on this point under Heading 4 of my preliminary notice (*Anat. Anz., VIII. Jahrg., No. 12/13*).

gebe, konnte von unserm Autor (i.e. by Mitsukuri) nicht erkannt und auch nicht interpretirt werden, weil demselben damals die ähnlichen Oberflächenbilder vom Gecko noch nicht bekannt waren, die allein dieses vereinzelt stehende Bild deutbar machten. Wir erkennen in die Skizze zwei in ihrem hintern Abschnitt nahezu parallel der Mittellinie verlaufende Linien, welche vorn plötzlich stark divergiren. Ich kann dieselben nur als die Insertionsgrenzen des gastralen Mesoderms ansehen." That I was aware of the significance of my figure referred to above is shown by the following words in my Contrib. III (p. 46). "This inward extension* of the gut-hypoblast is probably the cause of the grooves converging posteriorly into the single median chorda groove seen in the surface view Figs 2 and 3*a*." If these words are read in connection with what precedes and follows, I think, it will be plain that I had in my mind the significance of this figure to which Will refers above. I am, however, willing to admit that Will has made this point very clear and his fig. E. (II Art. p. 586 or fig. 4, I Art. p. 94) is certainly a very suggestive one.

6. I may perhaps be allowed to make remark on a part of Will's observation on Cistudo. In his second article (p. 542) he says: " Während der Embryonalschild bisher noch vollkommen im Niveau der übrigen Keimscheibe lag, tritt dieselbe auf diesem Entwicklungsstadium zuerst als deutliche, wohl umschriebene Erhebung von herzformiger Gestalt aus der Keimscheibenoberfläche hervor. Dementsprechend macht sich diese Wölbung an der Dotterseite (Fig. 3*b*.) durch eine leichte Concavität bemerkbar." In another place (p. 568), he is surprised that Mehnert's embryos are not more vaulted or bulged out. When Ishikawa and I first undertook the study of Trionyx, we used to open the shell and try to cut the blastoderm out as is usually

* Perhaps the words I used were not entirely happy. If I had said the "inward movement," it would have expressed my meaning more clearly.

done in taking out chick-embryos. At that time, we used to find Trionyx embryos vaulted dorsally, just as Will describes in the quotation given above. Since adopting the method given in the foregoing article of preserving embryos stretched in their natural condition, I have never found the shields vaulted in this manner at any stage : they were always on a level with the rest of the blastoderm.

7. In a note entitled "On Mesoblast Formation in Gecko," (*Anat. Anz.*) No. 12 u. 13, 1893), I ventured to criticize Will's views on the mesoblast formation of Reptiles. Will replies to my criticism in the postscript to his second article, and also in a note "Zur Frage nach der Entstehung des gastralen Mesoderms bei Reptilien" in the *Anatomischer Anzeiger* No. 20, 1893, which has just come to my hand. I must refer the reader to Will's original papers as well as to the above note for his views. Suffice it to say here that Will considers the gastral mesoblast to be formed by a fold which arises in the outer wall of the archenteron and grows towards the median line, thus cutting off the dorsal portion of the archenteron from its lower main portion. The wall of the small dorsal portion thus cut off is said to become the mesoblast. This is put forth in opposition to the view which was first propounded by Hertwig and which appears true to me, viz : that the mesoblast is formed from two diverticula of the archenteron arising directly on each side of the chorda.

Will considers that Fig. 23 of my Contrib. III. which shows a distinct diverticulum on each side of the chorda can not be held to prove the "Divertikelbildung" as it comes from an old embryo which has the chorda already cut off in the middle dorsal region. In his own words* : "Hier sieht man thatsächlich rechts und links neben der Chorda ein kurzes Divertikel, von dem die solide Mesoblastmasse ausgeht, jedoch lässt sich an einem solchen Bild aus dem Ende des

* The note above referred to. *Anat. Anz. No. 20.* p. 681.

ganzen Processes natürlich nicht mehr erschliessen, ob es sich um echte Divertikelbildung oder um Unterwachsung von Seiten der Urdarmfulten handelt, ob das Divertikel das Primäre und die solide Mesoblastmasse das Secundäre ist, oder umgekehrt." Now, the fact is familiar to every embryologist that at a given stage of development a structure, one part of which is already finished may show at another portion of its length only the commencing phases of the process of formation, so that one can see in one and the same specimen the whole process from the beginning to the end. Such seems to me to be the case with the mesoblast in the Clemmys embryo from which my figure 23 is taken. The fact that the mesoblast formation is complete and the chorda is cut off in the middle dorsal region, does not necessarily vitiate what is seen in the head region: here the process of the mesoblast formation is in a less advanced phase, and if a diverticulum is seen there, it is highly probable that a diverticulum is a feature of the mesoblast formation. That there is no such distinct diverticulum seen earlier in the middle dorsal region is because the epiblast presses closely down, and there is no space for the diverticula to curve upwards to any large extent as in the head region. I think, I have sufficiently demonstrated in my Contrib. III, that the diverticulum in the head region corresponds to that part of the primitive hypoblast in the dorsal region which Will calls the "Zwischenplatte," and that this must therefore be regarded as a shallow diverticulum.

Will also objects to my views on the following grounds: "Bei der Auffassung der Zwischenplatte als ein gestrecktes Divertikel müsste der solide Teil des gastralen Mesoderms (*mgr.* in Fig. 1 B.) nicht an dem Rande der Zwischenplatte inserirt, sondern aus der Mitte der letzteren hervorgewuchert sein." (*Anat. Anz. No.* 20, 1893. p. 681) Again "Wäre die Zwischenplatte ein abgeflachtes Mesodermdivertikel, so musste aus ihr sowohl der somatische wie die splanchnische Mesoblast

hervorgehen." (*Ibid*) The first of these objections occurred to me, while writing my Contrib. III. If one examines the cross-sections of Amphioxus as given, for instance, in Hatschek's Taf. IX. (*Studien ü. Entw. d. Amphioxus. Arb. a. d. Zool. Inst, Wien, Bd. IV.* See also Hertwig's Lehrbuch fig. 72), we shall find that the mesoblast pouch spreads ventrally and laterally not from what corresponds to the apex of the earlier diverticulum, but from its outer or lateral wall. The same thing takes place in Reptiles. Although I did not express this distinctly, it was present in my mind, as a reference to the middle of p. 41 (Contrib. III.) will show. These two objections on the part of Will are, I think, fully answered by this consideration.

Will again says : " Die Urdarmfalte würde bei der Mitsukuri'schen Auffassung überhaupt belanglos für die Mesodermbildung und deshalb unverständlich sein " (loc. cit. p. 681). I do not quite see the force of this objection. A fold is needed to mark the outer limit of the diverticulum,* and when the diverticulum is finally to be cut off from the main portion of the archenteron, it takes place by this fold advancing towards the median chorda. I described this inward movement of the fold† in my contrib. III. (pp. 42 and 46 ; also Figs. 16-17). It is this last phase of the mesoblast formation which Will emphasizes above all others, and on which he builds what he considers to be a new theory of the mesoblast formation (I Art. p. 102). Even in his own views the part of the mesoblast which is formed by " Septenbildung " is only a small proximal portion near the chorda, for the part *mgr* in his fig. 1 B. C. D. (*Anat. Anz.* No. 20, 1893) is according to himself not formed by " Septenbildung " but proliferated from the archenteric

* In a sentence similar to the above, in my note " On the Mesoblast Formation in Gecko " (*Anat. Anz.* No. 12-13, 1893) the word " mark " is by a most unfortunate oversight in proof-reading misprinted " snack "—a mistake which makes my sentence well nigh incomprehensible.

† I admit that I used then the expression " gut-hypoblast "—instead of the word " fold " which I ought to have adopted. But a reference to figs. 16-17 will show that as a matter of fact I had observed a *fold.*

wall. This course of reasoning reduces Will's views practically to the same thing as mine as given in Contrib. III. with the exception of the single point that I consider the "Zwischenplatte" as a flattened diverticulum, while he does not. I have already urged above the reasons for my views, so that I will not again go into them. I must refer the reader to it as well as to my Contrib. III. Notwithstanding that Will says, I have fallen into a fundamental error in confounding "Septenbildung" and "Divertikelbildung," I still think, I was not without reason, when I said in my note (*Anat. Anz.* No. 12 and 13, 1893. p. 434). that "* * * whether the presence of the fold is emphasized or the diverticulum is pointed out as the essential feature does not alter the facts of the case much. Will's objection to Hertwig's theory may therefore be only an apparent one." The difference between "Septenbildung" and "Divertikelbildung" which Will points out is exactly like that between the process of budding and of division. It is not possible to draw a hard and fast line in one case as in the other.

Finally I would like to add that while Will and myself agree as to the essential features of the reptilian development, the above discussion shows that on many minor points we must for the present "agree to disagree," (as I heard the late Prof. Balfour remark on a similar occasion), until fresh observations bring out new facts and enable us to settle these vexing points.

I have very recently received through the kindness of the author, Keibel's "Studien zur Entwicklungsgeschichte des Schweines." (Morphologische Arbeiten. III). It would perhaps be going out of my way too far to offer any extensive remarks on this article interesting though it is to me. The foregoing paper shows that, like himself, I divide the gastrulation into two phases, but these two

phases are different in his case and mine. My own views are (1) that the cœnogenetic hypoblast is formed by precocious segregation, and (2) that the definitive hypoblast is produced by the formation of the invagination cavity which gives rise to the definitive alimentary tract as well as to the chorda and the gastral mesoblast. According to Keibel, "In der ersten dieser Gastrulationsphasen wird bei den Säugern das Entoderm des Darmes und des Dottersacks gebildet, in der zweiten Mesoderm und Chorda" (*loc. cit*, p. 108). That is, the second phase which corresponds to the formation of the invagination cavity in Chelonia gives rise simply to the mesoblast and chorda and has nothing to do with the formation of the alimentary canal. On the latter structure he says, quoting from an earlier work, "* * * so habe ich doch wohl festgestellet, 'dass,' so sagte ich damals, 'wir das Homologon des Urdarmes unter der zweiten Schicht des zweiblättrigen Säugethierkeimes zu suchen haben. Doch wurde demselben nicht die ganze Höhle des Bläschens entsprechen, sondern nur ein ideeller Spaltraum zwischen der unteren Keimschicht und dem Inhalt des bläschenförmigen Keimes, welchen Inhalt ich dem Dotter homologisiren möchte. Die untere Keimschicht des zweiblättrigen Säugethierkeimes entspricht aber nicht dem gesammten Urdarmepithel des Amphioxus, sondern nur den Theil desselben, welche zum definitiven Darm werden. * * *." (pp. 1—2) Lwoff* has also come to a somewhat similar conclusion. I would not like to be understood as opposing this view in a dogmatic spirit. On the contrary, I think, there are several points which seem to favour such an interpretation. For instance, when the invagination cavity breaks open below in Reptiles, the dorsal wall, which alone remains, gives rise only to the chorda and the gastral mesoblast, as Will points out, and if we looked

* Basilius Lwoff:—Ü. d. Keimblätterbildung bei den Wirbeltieren. *Biologisches Centralblatt, XIII. Band. No. 2 & 3.*

simply at such a figure as Fig. 7*a* of the foregoing article, we might naturally come to the conclusion that the invagination cavity gives rise only to the gastral mesoblast and the chorda, and has nothing to do with the formation of the definitive alimentary tract. But we should always remember a fact which I hope to have proved conclusively in the foregoing article that the bottom of the invagination cavity has been removed. When the bottom is still present, we may

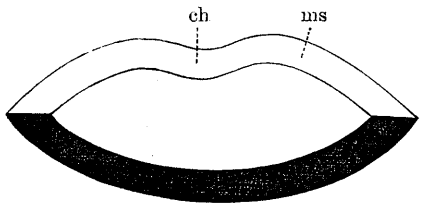


Fig. C.

Diagrammatic Cross-section of the Invagination-Cavity in Chelonia.

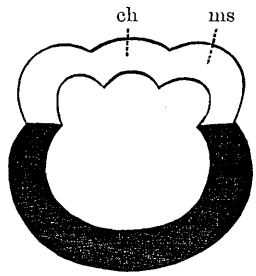


Fig. D.

Diagrammatic Cross-section of the Archenteron of Amphioxus. (*After Götte, from Born. Ergebn. d. Anat. u. Entw. I Bd. p. 494.*)

represent the cross-section of the invagination cavity as in Fig. C. The dark portion is the part that is removed. When we compare it with a homologous section of Amphioxus (Fig. D.) we are struck with their similarity and I think, we are justified in concluding that the invagination cavity of the reptilian ovum is homologous with the archenteron of Amphioxus, and has potentially present in it not only the chorda and the mesoblast but also the definitive alimentary tract. The removal of the bottom, or that part which represents the definitive hypoblast, must in any case be regarded as a secondary process, and can not stand in the way of homologizing the two structures. These considerations, together with the reasons which I have brought out in the foregoing article, incline me more towards those views which I have set forth in the preceding pages than to those of Keibel and of Lwoff.

On another point I would like to say a few words. Referring to the structure in Mammalia which Van Beneden homologizes with the yolk-plug in Amphibia, Keibel says :—" Der Dotterpfropf hat bei den Amphibien keine grosse morphologische Bedeutung, er ist ein Entwicklungshinderniss, er hat kein Funktion. Warum sollte gerade diese Bildung so zäh festgehalten werden, während doch so vieles Andere, das von ungleich grösserer Bedeutung ist, undeutlich wird und verschwindet." As I have insisted on the presence of the yolk-plug in Reptilia, ever since Ishikawa and I first discovered it, I may perhaps give my own view on Keibel's objection. I have in a previous page tried to explain the change of shape in the blastopore in Reptilia as repeating that process by which the lower half of the Amphibian egg becomes enclosed by the epiblast. Taking this in connection with the presence of the yolk-plug, I think that what is inherited is not simply the yolk-plug but the whole process of the epibolic invagination which is gone through in the region of the primitive plate. This is certainly important enough to persist for a long time.

Tōkyō, Oct. 1893.

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PLATE VI.

Plate VI.

FIG. 1.—Dorsal view of an embryo of *Chelonia caouana* a few hours after its deposition. Zeiss aa×2 (B1a)

FIG. 1*a*.—Ventral view of the same. aa×2

FIG. 2.—Dorsal veiw of an embryo of *Chelonia caouana* 1½ days after its deposition. The lateral parts of the embryonic shield are not represented. Zeiss aa×2 (D1a)

FIG. 2*a*.—Ventral view of the same. aa×2

FIG. 3.—Dorsal view of an embryo of *Chelonia caouana* 1½ days after its deposition. Only a small part around the primitive plate is represented. aa×2 (C2)

FIG. 3*a*.—Ventral view of the same. aa×2

FIG. 3, *bis*.—Dorsal view of an embryo of *Chelonia caouana* about 2 days old. Only a small part around the primitive plate is represented. aa×2 (O?)

FIG. 3*a*, *bis*.—Ventral view of the same. aa×2

FIG. 4.—Dorsal view of an embryo of *Chelonia caouana* 2½ days after its deposition. Only a small part around the primitive plate is represented. aa=2. (B4a)

FIG. 4*a*.—Ventral view of the same. aa×2.

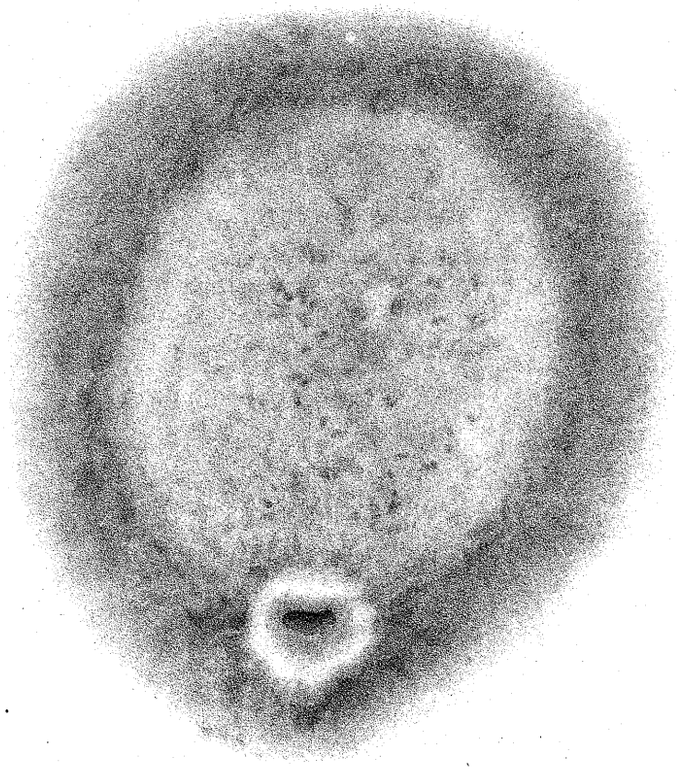

Fig. 1

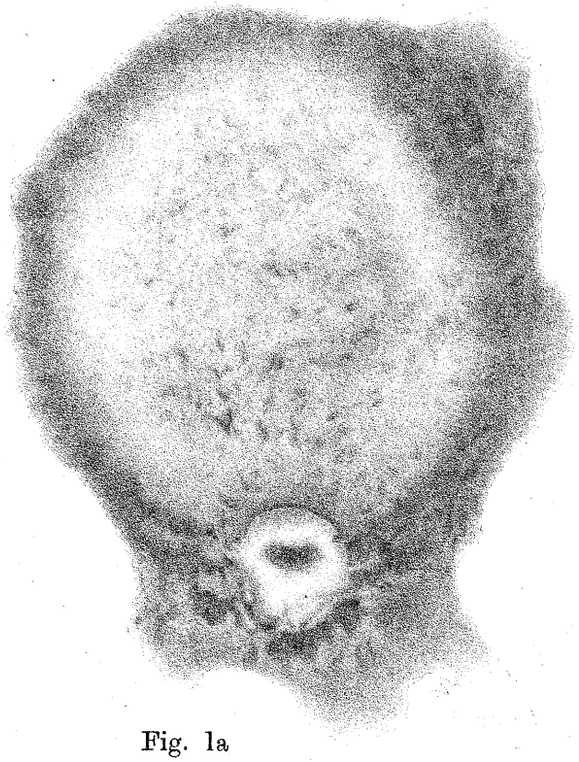

Fig. 1a

Fig. 2

Fig. 2a

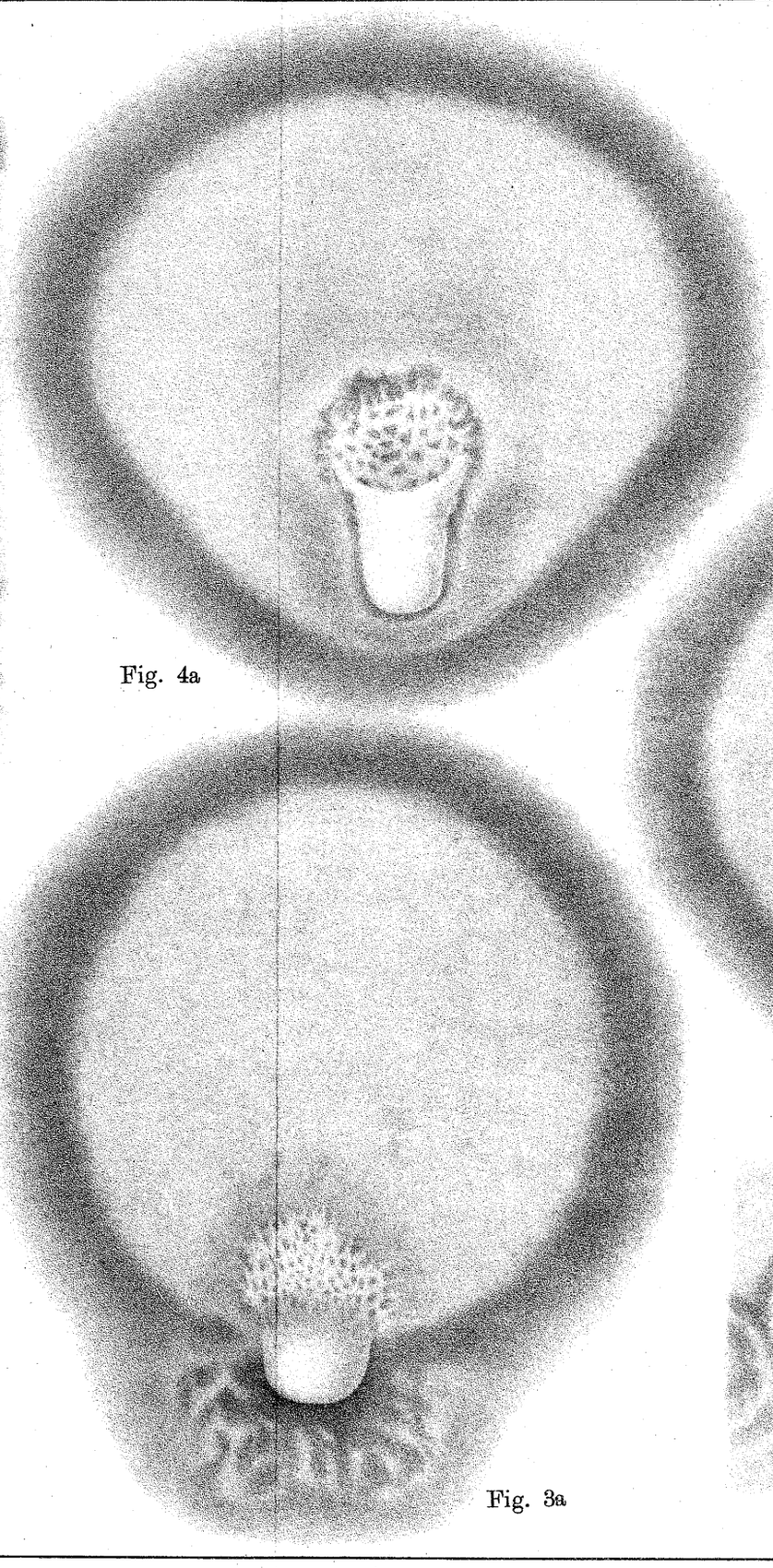

Fig. 3a

Fig. 4a

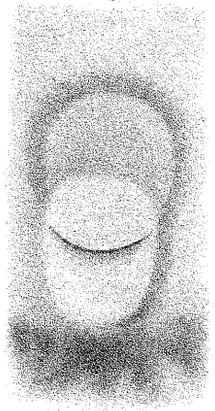

Fig. 4

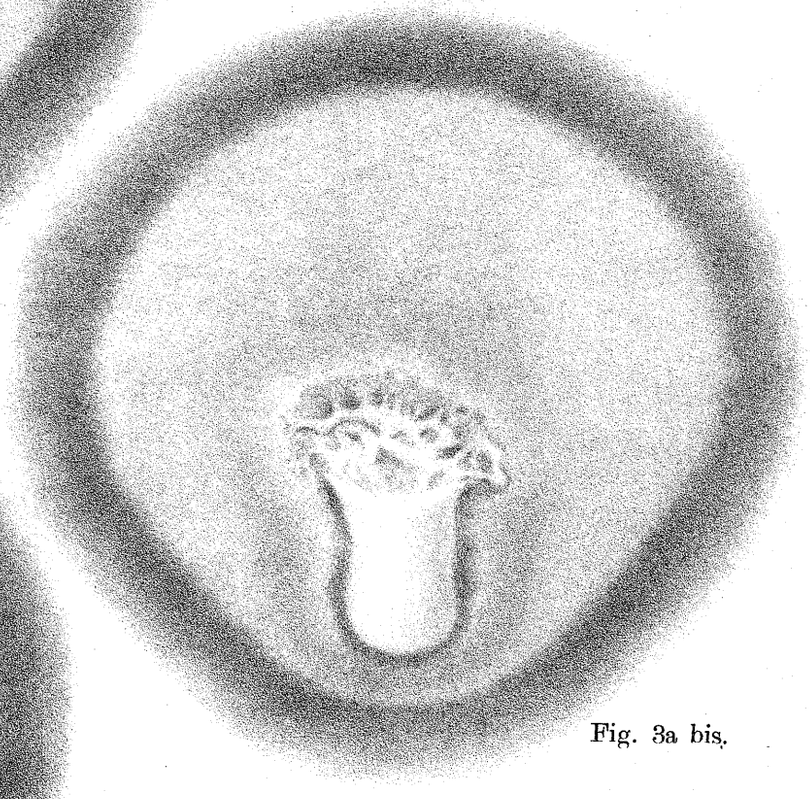

Fig. 3a bis.

Fig. 3

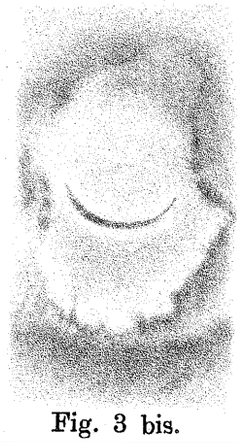

Fig. 3 bis.

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PLATE VII.

Plate VII.

FIG. 5.—Dorsal view of an embryo of *Chelonia caouana* 3½ days after its deposition. aa×2 (B5a)

FIG. 5*a*.—Ventral view of the same. aa×2

FIG. 6.—Dorsal view of an embryo of *Chelonia caouana* 5½ days after its deposition. aa×2 (B7a)

FIG. 6*a*.—Ventral view of the same. aa×2

FIG. 7.—Dorsal view of an embryo of *Chelonia caouana* 5½ days after its deposition. aa×2 (B7b)

FIG. 7*a*.—Ventral view of the same. aa×2

FIG. 8.—Dorsal view of an embryo of *Chelonia caouana* 7⅓ days after its deposition. aa×2 (B9a)

FIG. 8*a*.—Ventral view of the same. aa×2.

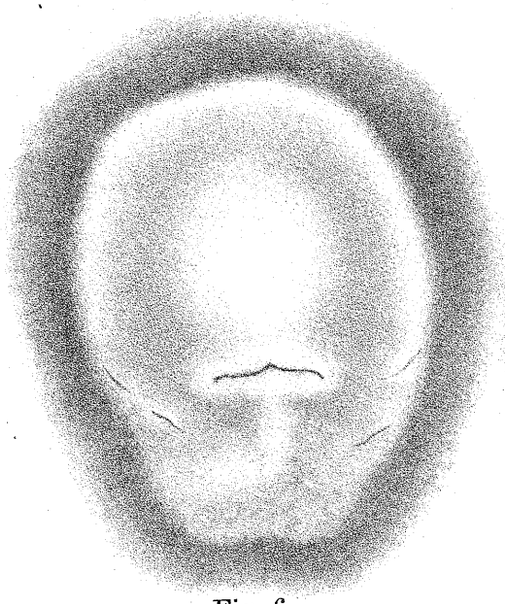


Fig. 6

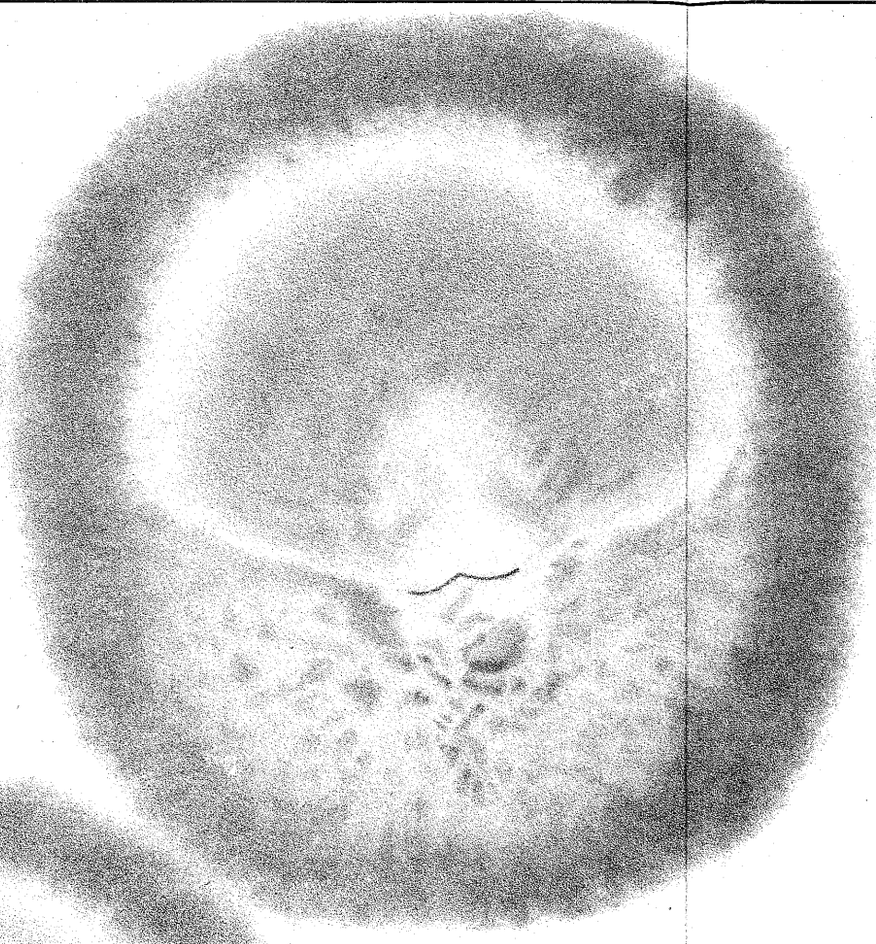


Fig. 5

Fig. 7

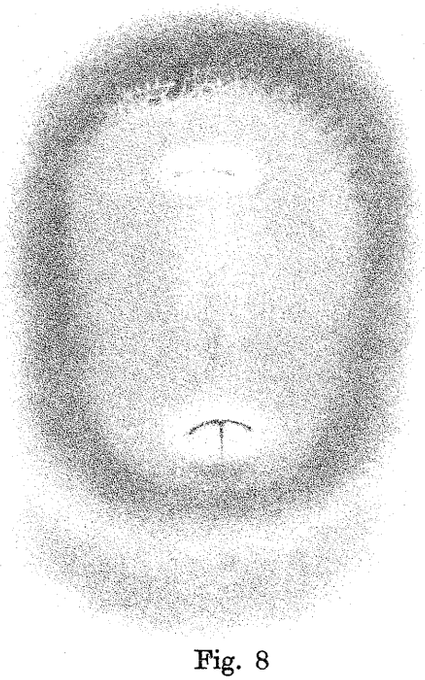


Fig. 8

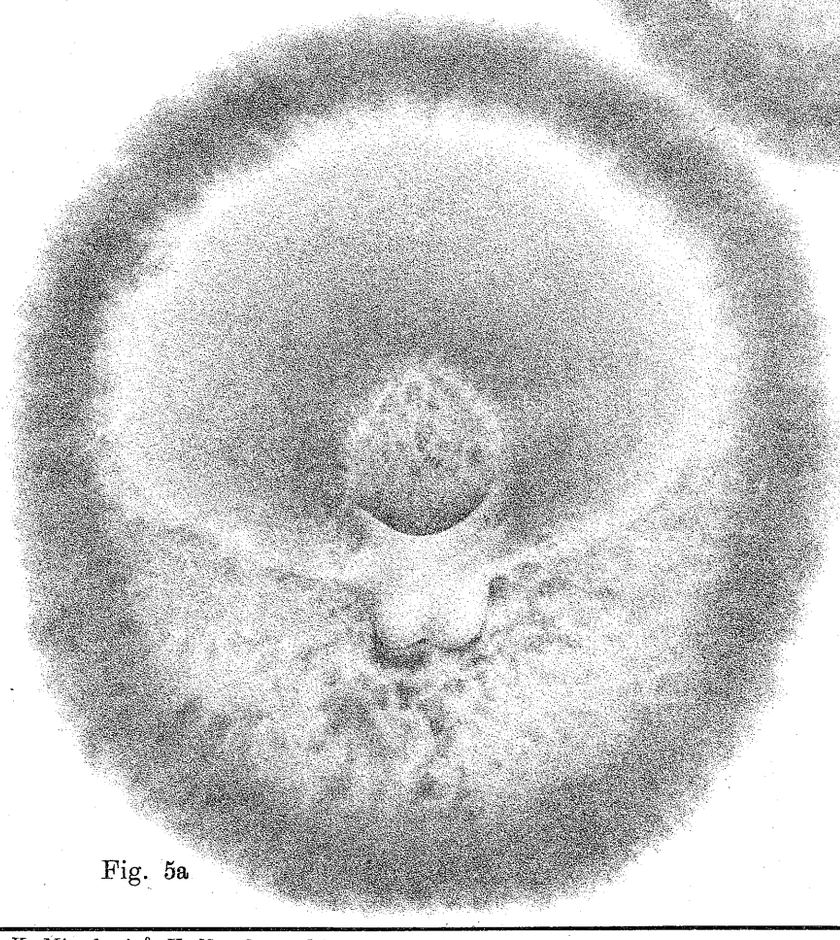


Fig. 5a

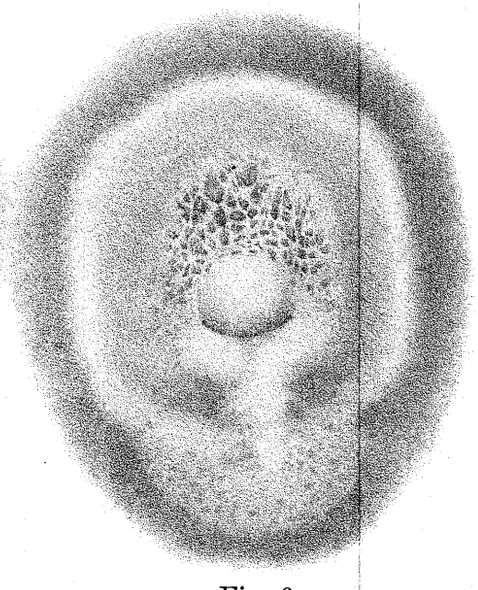


Fig. 6a

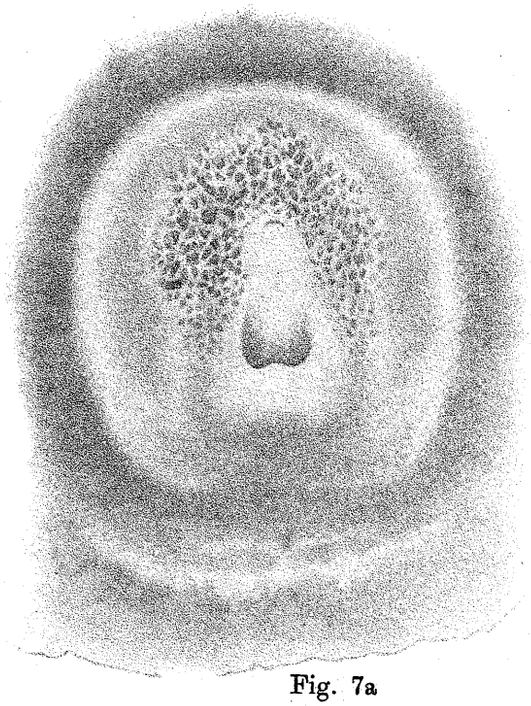


Fig. 7a

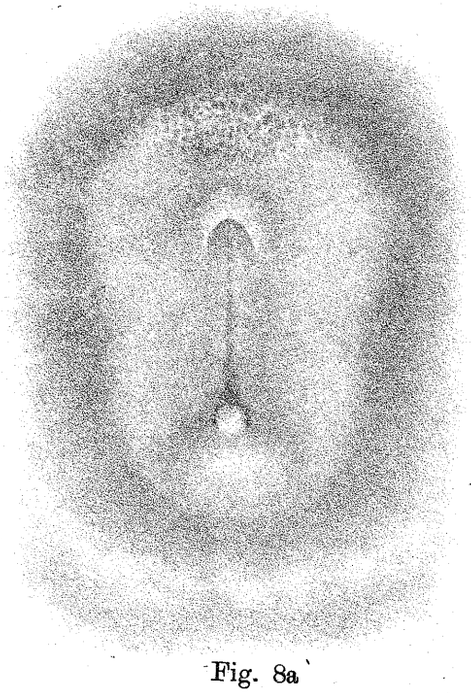


Fig. 8a

PLATE VIII.

Plate VIII.

FIG. 9.—Longitudinal section near the median line of an embryo of the same lot and stage as that represented in Figs. 1 & 1a. CC×2 (B1b, 4l. 2c. last—2)

FIG. 10.—Part of a longitudinal section of the same series as Fig. 9. From more lateral parts. DD×4 (B1b, long. 2, 5l., last—2)

FIG. 11 *a* & *b*.—Two merocytes from the same series as Fig. 9. DD×5 (B1b. {a. long. 2, 5l. last—2 / b. long. 2, 5l. 7.s)

FIG. 12 *a* & *b*.—Cells of the same kind as those represented in the middle stratum of Fig. 10. DD×4 (B1b. {a. long. 2, 5l. last—2 / b. long. 2, 3l. 6s.)

FIG. 13.—Longitudinal section near the median line of the embryo represented in Figs. 2 & 2a. CC×2 (D1a, 3l. 2c. 5s.)

FIG. 14.—Longitudinal section near the median line of the embryo represented in Figs. 3 & 3a. CC×2 (C2. long. 1, 1l. 2c. 7s.)

FIG. 15.—Longitudinal section near the median line of an embryo 1 day older than Figs. 1 & 1a and 1 day younger than Fig. 4 & 4a. CC×2 (B3. long. 1. 4l. last)

FIG. 16.—Longitudinal section near the median line of the embryo represented in Fig. 3 *bis*. and 3*a*. *bis*. CC×2 (O? long. 1. 2l. 2c. 2s.)

FIG. 17.—Longitudinal section near the median line of an embryo slightly younger than Figs. 6 & 6*a*. CC×2 (B6. 3l. 10s.)

FIG. 18.—Longitudinal section near the median line of the embryo represented in Figs. 7 and 7*b*. BB×2 (B7b. 3l. 2c. 1s.)

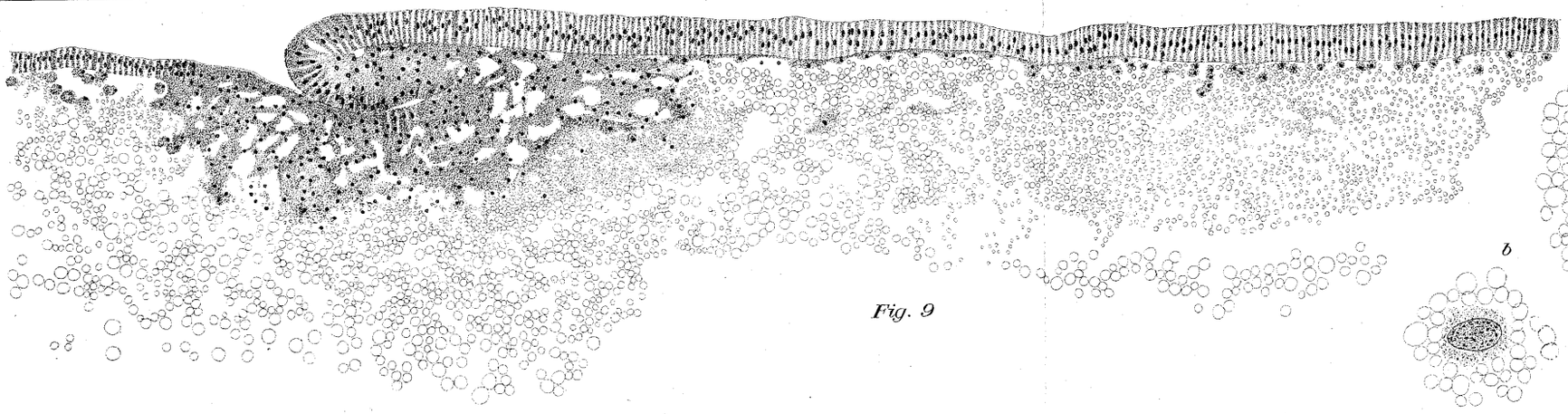
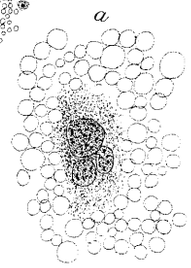


Fig. 9

Fig. 11



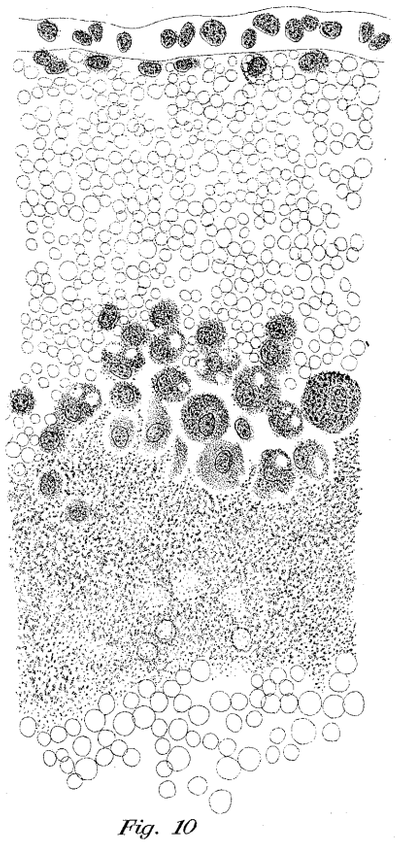


Fig. 10

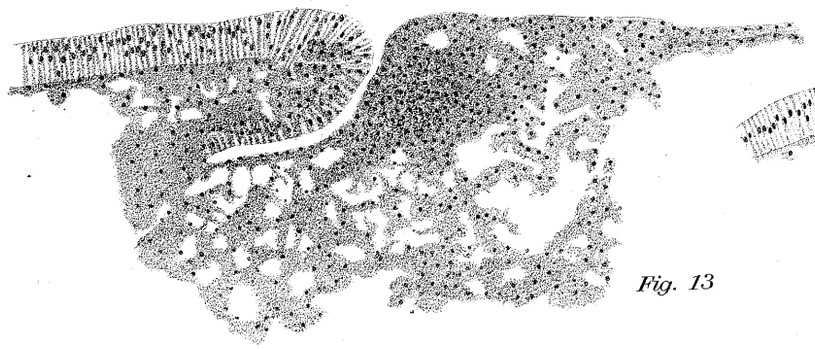


Fig. 13

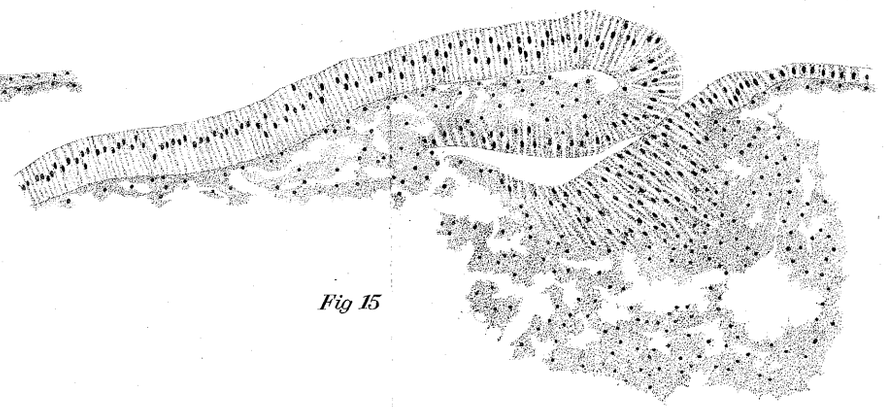


Fig 15

Fig. 12

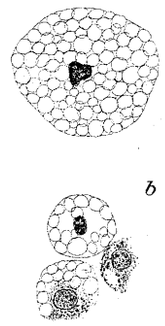
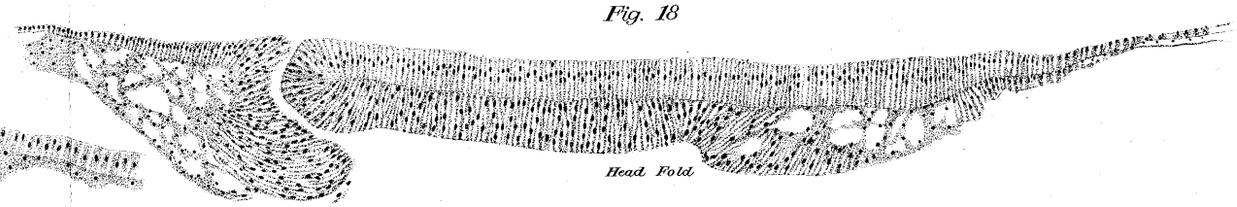


Fig. 18



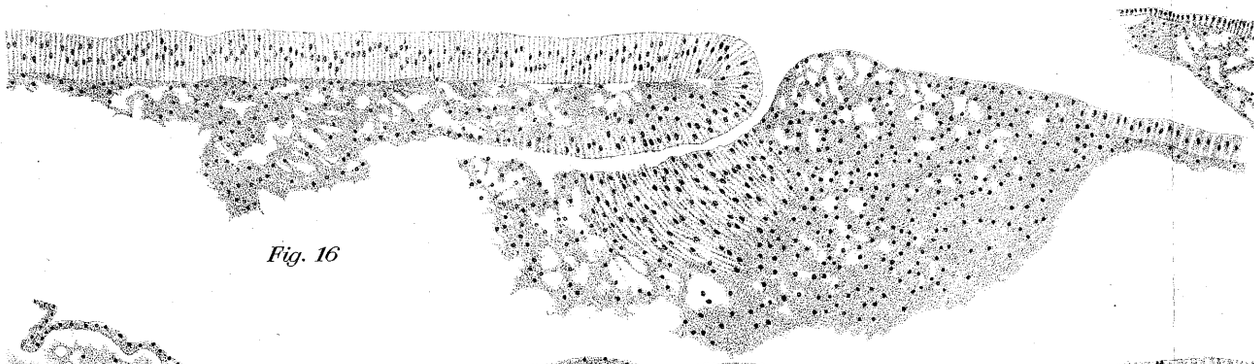


Fig. 16

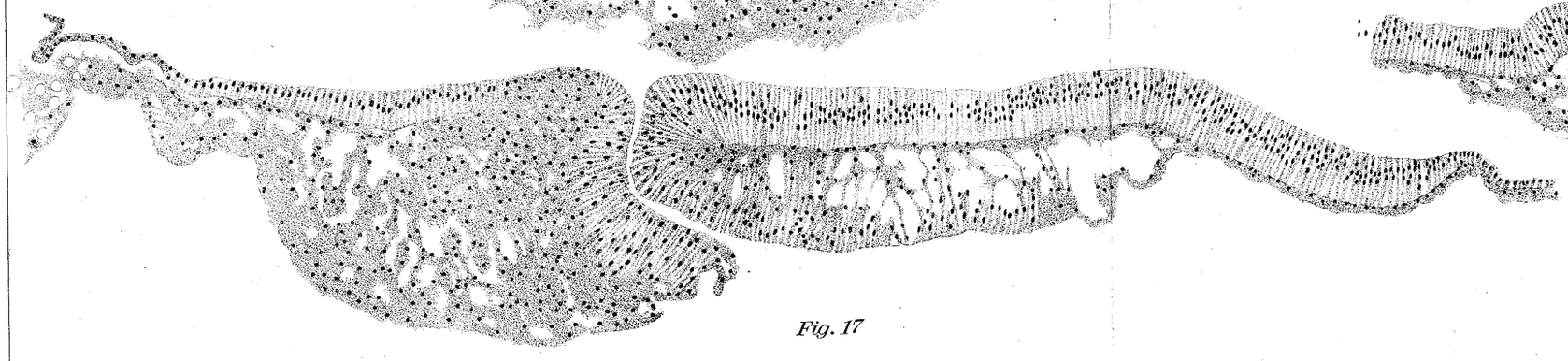


Fig. 17

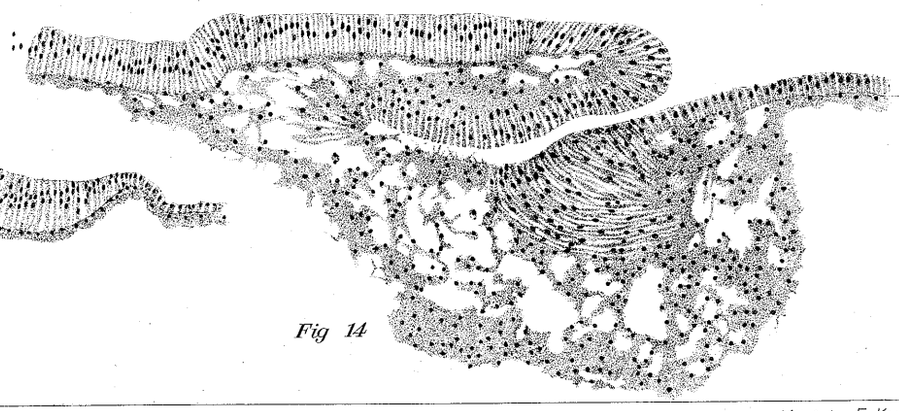


Fig 14