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CONTENTS.

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On the

Morphology of the Enteropneusta.[1]

PART I.

By

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With Plates I—VI.

In the 'Quart. Journ. Micr. Sci.' for April, 1884, I described the early stages in the development of a species of Balanoglossus, common on the shores of the Chesapeake Bay. I am led from various reasons, which I hope to detail when an opportunity occurs for discussing the classification of the Enteropneusta, to regard this species as identical with that described by Alex. Agassiz, and called by him B. Kowalevskii.

I was again enabled, during the summer of 1884, by the great kindness of my friend Dr. W. K. Brooks, to pursue my investigations into the morphology of the Enteropneusta. My warmest thanks are due to Dr. Brooks for once more affording me the hospitalities of the Chesapeake Zoological Laboratory, which was this year situated at Beaufort, North Carolina. While there I collected a number of specimens of a large undescribed species of Balanoglossus resembling B. salmoneus (Giard). This species, a description of which will I hope shortly appear, I propose to call B. Brooksii. My attempts at rearing this species from the egg were unsuccessful, and I was pre-

[1] A note on the subject-matter of this paper appeared in the 'Proc. Roy. Soc.,' No. 235, 1885.

vented by illness from making any additional observations on its development.

On leaving Beaufort, N. C., I returned to Hampton, Virginia, in the beginning of September, in the hope of finding the later stages in the development of B. Kowalevskii, and was fortunate enough to procure a complete series of larvæ from Stage H to individuals possessing ten gill-slits, in which condition the generative organs are first present. It is intended in this paper to give a general account of these stages, together with the histology of the animal, until two pairs of gill-slits are developed (fig. 1). From this point the further histological differentiation of the various organs will be described under separate headings.

With this account of the organogeny of B. Kowalevskii will be given, as far as possible, a comparative description of the same parts in all the species which I have hitherto examined.

External Changes.—The larva of B. Kowalevskii was described (loc. cit.) as having the egg in an elliptical form, divided by two transverse constrictions into three segments. The surface of the body was ciliated, a special tuft of long cilia being developed at the anterior end, while the posterior region was surrounded by a transverse band of long cilia.

From further observations it seems probable that this period (Stage D) assigned as the time of hatching is too early; for embryos kept in aquaria do not break the membranous shell before Stage G is reached. Probably, therefore, the larvæ found swimming in Stage D had escaped owing to an artificial rupture of the shell during the process by which they were found, an account of which is given in an appendix.

The formation of the mouth as a ventral pore in the anterior groove was described (No. 3, fig. 41). It opens directly into the archenteron, which was previously a closed sac, from which five mesoblastic pouches had been given off, forming the anterior, middle, and posterior body cavities respectively.

The external relations of the parts then become changed until finally the larva has the shape shown in No. 3, figs. 16 and 17. In this stage the proboscis is conical and the middle

segment much shortened. In the anterior dorso-lateral region of the third segment are the openings of the first pair of gill-slits, which are simple circular pores leading into the archenteron.

The larva is still opaque, and pale yellowish-brown in colour. In this condition it remains for about ten days, at the end of which time the second pair of gill-slits is formed. The body has become partly transparent, especially in the region of the proboscis, through the walls of which muscle-fibres are visible. Other external changes which occur at this period are the loss of the anterior tuft of cilia and the gradual disappearance of the posterior ciliated ring. At the commencement of this period the larvæ are to be found in Stage G at a depth of about six to eight inches in the sand, but towards its close they work their way into the higher strata of mud, and do not again go down again until the adult condition is reached.

As the cilia disappear a peculiar organ is formed as a small papilla, bearing long cilia and mucous glands, situated at the central part of the posterior surface (fig. 1, *sk.*). This organ serves as a sucker, by which the animal can attach itself to foreign bodies sufficiently firmly to prevent itself being washed off by a stream of water from a pipette. The anterior surface of the proboscis is also slightly suctorial, and by thus fixing itself posteriorly and extending the proboscis it is able to creep slowly about, somewhat in the manner of a leech. The appearance of this organ bears some resemblance to the terminal sucker described by Graaf as occurring in certain Rhabdocœles. It subsequently attains a considerable size, and is traversed by several wrinkles (figs. 3 and 4, *sk.*). This organ afterwards entirely disappears, but as to its mode of disappearance I have no certain observations. It would appear to occur very suddenly at the stage when the animal possesses seven to eight gill-slits. I have found animals with eight gill-slits which possess this sucker, and also animals of apparently the same age without it; hence it may be inferred that it undergoes a rapid atrophy at this point.

Similar suckers[1] occur as larval organs in Tunicata, Ganoids, and Amphibia.

With regard to the meaning of the sucker, considering the time of life at which it appears, it is probably not ancestral in origin; as the animal is already, from Stage G onwards, a distinct Balanoglossus in all its characters. It is more reasonable to conjecture that it is of purely developmental importance, and indeed its use to a larva of this kind is sufficiently obvious; for the creatures inhabit shallow pools on the sand-flats, being just buried in the mud, from which position they would be in danger of being washed away by the incoming tide and so be dried up by the great heat of the sun at low tide. On attaining a larger size the body can be, and always is, coiled round a spindle of sand and thus is kept in position, hence the sucker is no more required. In connection with this sucker I observed that nearly all the animals found in these pools were such as are provided with similar means of fixing themselves, which power is probably essential to life in such a habitat. An account of the histology of this sucker will be given subsequently.

During the period which elapses between the appearance of the first and second pair of gill-slits the body gradually acquires, as was mentioned above, a considerable degree of transparency. Owing to this fact several points of internal structure may be observed. This is especially marked in the case of the alimentary canal, which can now be clearly perceived to consist of three regions—an anterior branchial tract, a middle digestive portion, and an intestinal section posteriorly. The digestive section may be at once recognised by the bright yellow-brown colour of the secretion which it contains. This fluid is evacuated after a time when the animal is irritated, but no experiments were made to determine its physiological properties.

The partial transparency of the body wall permits also an indistinct view of the curious supporting rod of hypoblast

[1] Balfour was of opinion that in these forms they might be an ancestral feature (Balfour, 'Comp. Emb.,' vol. ii, "Tunicata").

which projects into the proboscis cavity. For reasons given later in this paper I propose to speak of this structure as the notochord (*Nch.*). The general appearance of the animal at the time when the second gill-slit appears, is shown in fig. 1. The animal is drawn as seen from the actual left side and displays the increased flexion of the body on its ventral surface.

The second gill-slit is shown as a small circular pore.

It will also be observed that that part of the body which lay between the two grooves constituting the middle segment of the body has now assumed an altered shape. At Stage H is found little more than a circular ridge on the body separating the proboscis from the trunk, while in fig. 1 (two gill-slits) it forms a kind of phlange enveloping the base of the proboscis. This change in shape is due to the operation of several causes, which are of great importance in interpreting the processes by which the final form of the adult is reached.

In the first place, after the formation of the mouth as a pore on the ventral surface, the constriction by which the proboscis is segmented off becomes deeper and deeper, until at last it is only attached by the exceedingly slender stalk shown in figs. 2 and 3. As a consequence of this process coupled with a forward growth of the ventral lip of the collar, the mouth comes to be directed anteriorly instead of ventrally (cp. figs. 7, 46 and 57). By this process the anterior phlange of the collar acquires the relation shown in fig. 1, et seq. In addition to these changes a most important structure is first formed at this time, namely, the cavity, which from the relations which it afterwards possesses I shall speak of as the atrial cavity.

In the later conditions of Stage H the body is perceptibly wider in the region of the body immediately anterior to the gill-slits than it is behind them. This increase in width, which is still very slightly marked, is due to a circular thickening which passes all round the animal, being most developed at the sides. By the time of the appearance of the second pair of gill-slits this thickening has considerably increased, and in the

contracted condition of the body is a very marked structure (fig. 2, *op.*). As development proceeds, this thickening increases, and at the same time grows backwards in the lateral regions until (five gill-slits) it has covered half the first gill-slit. The degree of contraction of the body of course alters its relations, but in the extended condition in adult specimens its posterior margin is about on a level with the fourth gill-slit. As then this structure is a process of the body wall which forms an opercular fold over the foremost gills, it appears reasonable to institute a comparison between it and the atrial walls of Amphioxus, &c. It will therefore be alluded to as the operculum, and the cavity between it and the body wall as the atrial cavity.

As previously described, the first gill-slit on its appearance is a simple circular pore. In this condition it remains for some days, but gradually, on about the tenth day, its form changes owing to the growth of a process from the dorsal margin of the pore, which renders the aperture somewhat kidney-shaped (fig. 1). Whilst this process is continuing the second gill-slit appears as another circular pore, similar to the original aperture of the first gill-slit. I was unable to discover any priority in the appearance of the gill-slit of either side in particular, but incline to believing that they appear synchronously on the two sides of the body, as previously mentioned.

After the appearance of the first gill-slit a definite series of changes is undergone, and these form a definite period in the history of the development of the animal, which may be considered as closing with the formation of the second pair of gill-slits.

At this point it may be well to recapitulate these changes. The embryonic life terminates with hatching, and the free larva with one pair of gill-slits escapes, moving about in the mud by means of cilia.

Before the second pair of gill-slits appear, it has undergone the following changes:

(1) Disappearance of ciliated band and apical tuft.

(2) Body walls have become semi-transparent.

(3) Animal has changed its habitat, creeping into the upper layer of mud.

(4) A fixing organ has formed as a ventral posterior sucker.

(5) Owing to growth of collar-fold, and constriction of proboscis-stalk, the mouth comes to be directed forwards.

(6) Differentiation has occurred in the hypoblast cells, marking out the alimentary canal into three regions, viz. branchial, digestive, and intestinal.

(7) Notochord is distinctly visible.

(8) Anal perforation present.

(9) Second pair of gill-slits arise.

(10) Opercular fold forms, as a circular thickening.

From this point onwards the principal changes in external features consist chiefly in an increase in size, and in continual progress towards transparency. This latter is so marked a feature that when three pairs of gill-slits are formed the walls of the body behind the collar, which were originally opaque, become perfectly clear and glassy, so that the internal structures are quite distinguishable. The walls of the proboscis and of the collar never entirely lose their primitive opacity. An attempt is made in figs. 1, 2, and 3 to show this gradual transition. It is presumably due to the consumption of the food particles which in the earlier condition were distributed almost uniformly among the cells of the body. This great transparency is not usual among forms that do not lead a free-swimming existence, and is possibly, so to speak, accidental, and correlated to the rapid growth which now occurs. Numerous glandular patches and spots are to be seen in the skin. They are developed chiefly on the proboscis, collar, and sucker, those on the latter being refractive and differing somewhat in appearance from the rest.

The increase in size seems to be rapid, but as to the length of time that is taken in passing through subsequent stages I have no record, as the specimens were caught from time to time, and not reared in aquaria.

As the body grows, the number of gill-slits increases. They are always added in pairs behind the last formed. The newest has a circular orifice, while the growth of the "valves" (fig. 4) from the dorsal margins of the anterior ones continues to modify their shape. From being circular they then become, first, kidney-shaped, then horseshoe-shaped, and next, by a diminution in width from before backwards, together with a great elongation dorso-ventrally, their openings are made U-shaped. When this condition is attained, the "valve" continues to grow downwards, its free end lying inside the pharyngeal cavity, as will be described when the histology of the gills is treated of. Frequently, in contracted specimens, these valves are washed outwards through the gill-slit, and hang freely out in the water. This condition often occurs during life. The gill apertures are from the first strongly ciliated. The cilia move in a constant direction, driving a current dorsalwards on the anterior line of the U, then down the anterior margin of the "valve" and up the posterior, and finally ventralwards on the hinder edge of the gill-slit. The currents have the same course before the formation of the "valve," viz. on looking at a circular gill-slit of the left side, if the animal's head is directed to the observer's left, the course will be round the aperture in the direction of the hands of a watch. By this current the water which passes in at the mouth is carried out of the pharynx; probably, therefore, the motion of the cilia is in a sort of spiral converging outwardly, and not circular as it appears to be on looking down upon a gill-slit.

From the fact that the number of gill-slits varies with the length of the animal, together with the constant presence in the posterior branchial region of a regularly arranged series of gills in all stages from a complete U-shaped opening to a terminal one which is always circular, I am led to believe that these structures increase in number throughout the greater part, if not the whole, of the life of the animal. The greatest number of slits which I have observed was fifty-seven pairs. Figures illustrating the development of the branchial

skeleton, &c., will be given when the histology of the gills is described.

Together with the increase in number of the gills the differentiation between the digestive and intestinal region becomes more prominent, the bright yellow-brown of the former showing through the transparent body wall, being a most striking feature in the appearance of the animal. When first perceptible, the digestive tract is a simple tube, separated by a slight constriction from the intestine. As growth proceeds this constriction becomes more marked, and when the second gill-slit is fully formed the separation between the two is sharply defined (fig. 2). Subsequently a fold arises in the digestive region which gives it the appearance of being made up of two saccules (two gill-slits). This condition becomes more and more marked, and then a third saccule appears posteriorly (4—5 *g. s.*). When, however, the animal is seen from the dorsal side the alimentary canal is seen to have a wavy contour, the two saccules being thus parts of a slightly bent tube. Moreover, in longitudinal sections the divisions between the saccules are parts of a spiral fold which traverses the whole digestive region. When five gill-slits are formed there are three saccules, and in animals with ten gill-slits they are five in number. After this the body walls become much more opaque, attaining the condition which they present throughout adult life. It is consequently not possible to follow the internal development after this stage by means of surface views.

The walls of the intestinal region are also thrown into folds, but their arrangement would appear to be irregular. The cells in this tract bear long flagelliform cilia which appear to drive a current through the anus.

The anus is first found at about the time of the formation of the second gill-slit. As previously mentioned it is almost if not quite in the position in which the blastopore closed, being posterior, median, and dorsal. When the tail is formed the anus is immediately dorsal to it, in fact, its ventral margin is formed by the dorsal side of the tail. In ordinary conditions

the anus remains open widely, but when the animal is irritated it contracts its body and draws in the intestine, closing the anus, over which there project two flaps of the body wall. These flaps are very thin and transparent presenting an appearance as of a posterior vesicle (fig. 3*a*).

In some specimens a curious separation between the mesoblast and epiblast occurs in this place, which may be due to re-agents or may have some significance; this structure will be treated of together with the other formations in the third body cavity.

In the transparent animal pulsatory contractions can be seen in a vesicle lying on the dorsal side of the notochord, but owing to the imperfect transparency of the body walls in this region nothing more could be definitely affirmed as to the course of the blood. As will be seen in considering the internal structure, a large trunk appears (2—3 *g. s.*) in the dorsal mesentery; pulsations could also be observed in this structure, which seemed to pass from behind forwards, but occasionally an appearance was produced as of a reversal in the direction. But in consideration of the fact that the ventral wall of this vessel is by the nature of the case adherent to the splanchnopleura, while the dorsal wall was fixed in the somatopleura, no very certain importance can be attached to any observations of pulsation in the dorsal vessel, since any peristalsis in the gut might produce this appearance. The same applies to the ventral vessel, which is said to be contractile in B. minutus (Spengel). On the whole, however, the balance of evidence was distinctly in favour of postero-anteror pulsations in the dorsal vessel. No corpuscles were discovered in the blood which is colourless. In preserved specimens it appears as a homogeneous coagulum, which in specimens preserved in Perenyi's fluid shows a slight tendency to granulation.

Large amœboid-looking cells are visible, floating about in the body cavities, especially in the second.

After about seven to eight gill-slits are formed the general look of the animal changes, mainly owing to the fact that the walls of the body become more and more opaque. This seems to be

due to thickening of the ectoderm and the appearance of numerous mucous glands on the skin. The whole skin is uniformly ciliated from the time when the blastopore closes throughout life.

At about seven to eight gill-slits the suctorial tail disappears, probably atrophying rapidly, but as to this process nothing more can be predicated. It is to be found in some larvæ with eight gill-slits, while others in the same stage are without it. The anus is then terminal, circular, and permanently open.

At ten gill-slits the ovaries are first perceptible, but as yet are not marked enough to appear in a surface view. In older animals they form large yellowish-grey projections from the sides of the body. Their minute structure, together with that of the testis, will be given later.

The body of the adult is very highly coloured, the proboscis being of a yellowish-white tint. The collar is a brilliant red orange (especially in males), with a white line round the edge of the operculum, while the rest of the body is of an orange yellow, shading to pale green yellow in the intestinal region, which is semi-transparent throughout life. The distinction between the colour of the males and females is very well marked in B. Kowalevskii, the genital regions being grey in females and yellow in males. The sexes are of different colour in all the Enteropneusta, most prominently so in B. salmoneus (Giard), in which the males are chrome yellow and the females salmon coloured.

It may be well, before passing to the internal development, to mention the peculiar odour which the creatures possess. This odour is very penetrating and persistent, resembling that of chloride of lime with a fæcal admixture. All the species of Enteropneusta which I have examined alive possess more or less offensive odours. This peculiar property is most developed in B. Brooksii (new species), in which the smell is very distinct after the animals have been months in spirit, which has been often changed. The smell of this species is strongly suggestive of iodoform. It is so powerful as to be a considerable drawback to investigating the species.

Another feature which ought not to be overlooked in a general account of this species (B. Kowalevskii) is its extreme vitality. In a bucket of unaërated water in which all other animals had died some days before, in a hot climate, these creatures were able to carry on their existence, and parts of the body may be seen moving about by means of the ciliated skin to which a completely macerated skeleton of the branchiæ is attached. Lobes of the testis, torn off, will likewise swim about for days. To what extent the body is capable of regeneration I cannot say. Specimens were found in which there was at all events an appearance suggesting that the proboscis had grown again. Spengel has alluded to regeneration of tissues as occurring in B. minutus, and I have little doubt that it is also common in B. Kowalevskii.

With regard to the specific name of this form, it appears that the figure and description given by Agassiz of B. Kowalevskii identify it with the form which is the subject of this paper. The mode of development ascribed by him to the species is of course entirely different. Seeing, however, that he was unable to show the connection between the animals found by him in the beach and the Tornaria which he reared, it does not seem by any means certain that these Tornaria were the larvæ of B. Kowalevskii. On the whole, it is at least possible that they were the young of some other species, *e. g.* B. Brooksii, which occurs at least as far north as the Chesapeake, and probably higher still on the coast.

From a general survey of the group Enteropneusta, which I hope subsequently to attempt, I think it will appear likely that B. Kowalevskii stands, in many respects, in a group differing in several features from the other members, which agree with one another in these points, *e. g.* short proboscis, complicated branchial skeleton, operculum small, liver saccules present, eggs minute, &c. It is to the latter division I am inclined to believe that Tornaria alone belongs.

In studying the anatomy of Balanoglossus by means of sections difficulty arises owing to the variable amount of con-

traction which the body may undergo in preservation. The trouble chiefly occurs in the case of the proboscis-stalk and folds of the collar. By comparing figs. 3 and 4, which were drawn from living specimens in the extended state, with fig. 5, which is taken from a preserved specimen, these relations will be understood. In the contracted animal the gill-slits are always more or less obscure, owing to the great shortening which takes place in the branchial region, together with the protrusion of the valves (fig. 5, *vlv.*).

In older animals this contraction is not nearly so great, probably owing to the increased firmness of the branchial skeleton.

Internal Structure.

Stages F and G—Skin.—The ectoderm is composed of long fusiform cells arranged two to three deep over the body, except in the dorsal side of the collar groove, where they are columnar and one layer thick. Also in the posterior dorsal region the skin is thinner than that of the rest of the body. The whole surface is ciliated. Beyond the fact that the cells are more compressed, and closely arranged, the structure is similar to that of the previous stage.

Nervous System.—The solid cord which began to separate from the skin in the middle dorsal line at Stage F continues to sink inwards. No lumen is as yet present in it. Throughout its length it still remains in contact with the skin, fusing with it at both ends (figs. 10 and 20—24).

Towards the end of Stage G a differentiation begins between the upper and lower parts of this cord, the upper being formed of cells, while the lower part consists of lightly stained substance, which in older animals is distinctly made up of fibres. Its fibrous nature cannot in this condition be certainly affirmed, probably owing to defect in preservation. The formation of a nervous network over the whole body, which afterwards occurs, is not yet begun.

Hypoblast.—The mouth is ventrally directed (fig. 7), and

the anterior wall of the gut (Stage E to F) runs at right angles to the long axis of the body. As will afterwards appear this feature is of importance. In longitudinal section, a commencing differentiation between the branchial and digestive region is perceptible. The cells of the former are columnar, while those of the latter have irregular amœboid processes which give the inner wall of the gut an irregular contour. The anus is not yet formed.

In the front end of the third segment of the larva (Stage F), anterior to the ring of cilia, the sides of the gut give rise to a pair of dorso-lateral evaginations; these pouches are the first indications of the gills. No change has occurred in the skin covering them. Subsequently they come in contact with the skin, the walls fuse and then a perforation is formed through the fused portion, apparently occurring by a process of degeneration of the tissue. Fig. 29 is from a section taken through the side of one of these evaginations. The subsequent appearances are shown in figs. 42 and 43, which are from an older larva.

Notochord. — In the later stages of F and G in the anterior dorsal wall of the gut, arises a most remarkable structure. For reasons which will appear when its later development and fate is considered, I propose to compare this organ with the notochord of the Chordata, and by this name it will be subsequently spoken of.

In Stage E it was stated that the anterior wall of the hypoblast came vertically to join the skin at the mouth (figs. 7 and 10). As, however, development proceeds, the dorsal wall of the pharynx becomes partly constricted from the remainder (figs. 20 and 22). As this process of separation of the dorsal wall proceeds, the part so separated grows forwards so that it comes to project slightly in front of the anterior end of the gut (fig. 30). By this means a hypoblastic tube is formed dorsal to the gut, with a lumen which opens into the archenteric cavity (figs. 21, 22, and 30).

Mesoblast.—The lining of the anterior body cavity in Stages E and F is composed of rounded cells arranged in con-

tact with the ectoderm. These cells are in some parts only one layer thick (anterior and posterior walls) (fig. 8), while in others (ventrally) they proliferate rapidly (fig. 9), forming loose masses of cells, the outer elements of which are elongated, with rounded heads from which the spherical cells which form the inner portion are budded. This proliferation continues until the proboscis cavity is partially filled up. But in the later phases of Stage F elements are formed other than the rounded cells above mentioned, in the shape of fibres (fig. 13) which appear to arise in a curious way, as is shown in figs. 9, 10, 12, &c. The elongated cells gradually become pyriform, the round ends being for the most part central, while the fine ends are drawn out into peripheral fibres. The round heads then appear to separate from the fibres, so that in examining the mesoblastic structure lining this cavity in late larvæ of Stage G, the elements are arranged in the following order: centrally, a small empty cavity surrounded by a ring of spherical granular cells; next a layer of pear-shaped cells continued into peripheral fibres, and externally a layer, composed almost, and later in life entirely, of radial fibres. Some of these are inserted into the lower layer of the skin, and are probably a peculiar form of connective tissue. In the heads of the pyriform cells brightly refractive granules may be seen; whether these are food or waste products cannot be affirmed.

Such then is the lining of the proboscis-cavity. In the anterior third it is evenly distributed over the inner surface, but at the back of the posterior third, where the proboscis tapers abruptly to its stalk, the layer of mesoblastic tissue is much thinner dorsally than laterally and ventrally (compare fig. 13 which is through this region with figs. 12 and 11 which are anterior to it). On passing further backwards, the cavity in Stage G is divided into two by a great proliferation of mesoblast from the dorsal surface, which grows downwards until it meets the ventral mesoblast. The position occupied by this structure at first coincides with the point at which the anterior mesoblastic pouch closed off from the archenteron. In that stage the anterior body cavity was a simple sac con-

tinued backwards into two lateral horns. The mass of cells which now arise at the original point of separation, therefore, constitutes a continuation of the division between these two horns into the anterior simple cavity (fig. 14). As will shortly be seen, this division of the back of the anterior cavity into two is correlated to the forward growth of the notochord.

In this septum, which is composed of roundish, hexagonal cells containing many granules (fig. 16), appears the first rudiment of a peculiar organ which subsequently is a conspicuous and characteristic structure in the proboscis of all the Enteropneusta, viz. the proboscis-gland ("heart" of Spengel [1]). This gland has at first the relations shown in fig. 10, *gl.* It consists (Stage F) of a triangular mass of loose tissue containing nuclei in which but few cell-outlines can be seen, and would appear to be formed by a sort of degeneration of the mesoblast of the septum. As yet it contains no cavity. Immediately behind and ventral to it is the anterior end of the notochord (fig. 17).

Fig. 18 is taken through the posterior apices of the mesoblastic horns behind the gland.

Middle Body Cavities.—These are (Stage E) a pair of simple cavities divided by a dorsal and a ventral mesentery, completely closed from both the anterior and posterior cavities, as they remain throughout life. They are lined by round or crescentic mesoblast cells. As the proboscis-stalk is constricted, the anterior parts of these cavities are compressed into two forwardly directed horns. The horn of the left side is shown in fig. 18. From an early period it projects in front of that of the right side.

In Stage F a histological differentiation occurs in the splanchnopleure at the place of union between that part of the hypoblast which is destined to form the notochord and the lower section of the pharynx. This appearance is shown in fig. 25, *x*). It consists in the prolongation of the ends of the

[1] For reasons which will subsequently appear this term is somewhat inappropriate.

mesoblast cells which are in contact with the hypoblast into curious tails, which are refractive.

I was at first led to suppose that these tails were muscular, and this undoubtedly is the fate of many of the mesoblastic elements of this region, but no direct evidence of the contractile nature of these pear-shaped cells was attained beyond the general suggestion of their shape; on the other hand, when the notochordal sheath is developed in this region, an appearance is presented which suggests the possibility of their having taken part in its formation. Their histological characters are, however, strikingly similar to those of the cells which occur at the sides of the notochord in the same region in Amphioxus at the time when it has eleven pairs of mesoblastic pouches (Hatschek, No. 4, figs. 124, 126, &c.). These cells are stated by Hatschek to be muscle-fibres; by analogy it seems, therefore, likely that this may be the real nature of the same cells in Balanoglossus.

As development proceeds, proliferations of mesoblast are formed in the ventral region of the middle body cavities. From each side in the posterior region of these cavities a tubular portion is separated off from the rest (fig. 28).

The fate of these parts is not quite certain. It seems, however, likely that they unite with two forward growths from the posterior body cavities to form the tissue space in which the dorsal blood-vessel is ultimately enclosed throughout the collar region. This tissue space I propose to call the perihæmal cavity (fig. 60, &c., *Ph. c.*).

The posterior body cavities are simple cavities, similar to the middle pair. The dorsal and ventral mesenteries persist throughout life. As yet they contain no special differentiations.

Period between the Formation of the First and Second Pair of Gill-Slits.

Skin and Nervous System.—The histology of the skin in the proboscis and collar regions has not undergone material

alteration since the last stage. The epiblastic cells are somewhat smaller and more closely packed. In the posterior regions, however, the ectoderm is thinner than in the younger animals, owing to the rapid growth which occurs at this time in the trunk region (fig. 45). Unicellular mucous glands occur at rare intervals in it. But in the lower layer of the skin at the posterior surface of the proboscis is formed the beginning of that network of nerve-fibres which is such a prominent feature in these regions of the body in later life. Though a network of this kind eventually is formed on the inner surface of the skin all over the body to a greater or less extent, it is as yet only to be seen in the base of the proboscis. The exact process by which this layer is deposited is not certain, but it would appear that cells of the inner layer elongate and form multipolar cells with long, thread-like, anastomosing tails (fig. 32).

At this stage nuclei are still visible in this fibrous layer, though in later stages they have almost entirely disappeared from it (fig. 54, &c.). From this point in development onwards these fibres constitute a perfectly defined layer of tissue. Projecting into it may be seen some of the fibres which were described as being formed from the mesoblastic lining of the proboscis cavity. These fibres are presumably supporting structures. Occasionally an appearance is presented as of an anastomosis occurring between them and the tails of the nerve-fibres. Whether this is really the case or not, it can scarcely be doubted that the muscle-fibres, which are now forming in the proboscis cavity, receive their innervation from the fibrous layer of the skin, to which many of them are attached, and thus such an anastomosis is not *à priori* improbable. On the other hand, the structure of the mesoblastic fibres is rather indicative of a supporting than of a contractile function. But, as will afterwards appear, there are eventually present in the mesoblastic elements of these animals cells which present almost every shade of variety between undoubted contractile fibres and obvious connective tissue, so that it is by no means easy to determine the nature of these fibres with precision.

On the whole I am inclined to regard them as supporting structures.

The separation of the dorsal nervous system is much more marked during this period than it was before it. It is now completely separate from the point of junction of the proboscis with the trunk to almost the level of the first pair of gill-slits. At its anterior end (fig. 36) may be seen the beginning of the process by which the anterior lumen is formed. This is effected by a forward growth of the collar, together with a continual sinking and horizontal invagination of the nerve-cord.

This lumen, thus formed, never extends for more than a short distance into the cord, which, however, in its middle and posterior regions in older animals, contains remarkable spaces lined by columnar cells, more or less separated from each other by strands of tissue, which will be described, together with the later development and histology of the nervous system.

The nerve-cord, as always, joins with the skin at both ends, but from its posterior point of junction the rudiment of its dorsal continuation in the skin may already be seen in section as a small area of fibrous tissue in the base of the skin in the middle dorsal line (fig. 42). A similar strand (fig. 42) may also be seen on the ventral side, beginning a little in front of the first gill-slits. The two cords are still quite unconnected.

The Proboscis Pore.—The first appearance of this structure is a thickening on the inner surface of the epiblast in the proboscis stalk, which soon becomes hollow while still attached to the skin (fig. 34, *p. pr.*). This epiblastic sac is from the first asymmetrical, being on the dorso-lateral aspect of the left side. From the first, the cells of which it is formed are columnar, and it has no communication as yet (two gill-slits) with the exterior or with the body cavity.

Hypoblastic Structures.

Branchial Region and Notochord.—The process by which the mouth comes to be forwardly directed has already been described. In larvæ with one to two gill-slits it has already

begun (fig. 45). The walls of the branchial region are distinctly differentiated from those of the rest of the gut, consisting of long, solid-looking cells arranged in layers of one to two deep. The lumen of the gut is very small anteriorly and has an irregular outline in preserved specimens (figs. 36 and 37), but in the middle of the collar region as at present marked out, its lumen is continuous dorsally with that of the notochord (fig. 39).

This notochord, which is now a very prominent feature in sections of the anterior end of the body, arose in the first instance, as already stated, by a forward growth of the anterior dorsal wall of the pharynx, which thus shuts off a short diverticulum of hypoblast (fig. 30). The part of the pharynx with which the walls of this diverticulum are continuous, then separates itself from the rest by longitudinal constriction, which at first causes the lumen of the gut to take an 8-shaped figure, the separation of the dorsal part of the 8 becoming finally complete from before backwards. This process gives rise to the appearance seen in fig. 37. The part thus constricted off becomes then entirely separated except at its posterior end, where throughout life its lumen opens into the pharynx.

In its anterior region the lumen of the notochord is always suppressed at this stage, owing to the compression of the ventral against the dorsal wall. Moreover, in larvæ of this, as of all subsequent stages, the lumen is altogether obliterated in part of its course. This obliteration does not appear to occur progressively from before backwards, but more or less irregularly, so that, as in fig. 38, the lumen may have already disappeared while still present in a region anterior to this (fig. 36). As, however, in older animals the lumen is always continued far into the notochord of the proboscis cavity (namely, to a point anterior to that where it is already obliterated in two-gill larvæ), it is almost certain that the subsequent increase in the length of the notochord is due to a growth from behind forwards, and that all the notochord which is as yet formed (two gill-slits) is pushed bodily forwards by a proliferation, probably occurring at the point of union with the gut. The

alternatives, that the growth occurs at the apex or at any point intermediate between the two ends is unlikely, from the fact that almost immediately after two gill-slits the tissue of which it is composed becomes vacuolated and irregular, undergoing the "degeneration" characteristic of notochordal substance, presenting therefore by no means the appearance of a growing tissue.

The length and proportions of the notochord at this stage are indicated in fig. 45, which is, however, not a truly median section. Its anterior end already projects far into the anterior body cavity, pushing in the mesoblastic lining. Fig. 45 is from a specimen slightly older than that from which figs. 37, &c., are taken, and the commencing degeneration of the notochord tisssue is already begun.

To recapitulate: the growth of the notochord is due to:

1. A forward growth of the dorsal anterior portion of the archenteron (fig. 30). This is supplemented by—

2. A longitudinal constriction of the dorsal region of the pharynx, which gradually travels backwards (cp. figs. 21 and 22 with figs. 38 and 39), separating a hollow hypoblastic tube which remains open to the gut behind.

3. A forward growth from the point of junction with the gut.

In connection with the notochord must be mentioned the skeletal rods, which now just appear, though it cannot be positively affirmed that they are of hypoblastic origin. When first visible, they are two short rods of a deeply-stained, structureless substance, which lie in the angles between the notochord and the dorsal wall of the pharynx. As first seen in this position they appear to be formed externally to the hypoblast cells, against the ends of which they lie. Their posterior ends are enclosed in the hypoblast (fig. 39), and it is difficult to understand how this can have been brought about if they were secreted by the mesoblast cells. This would involve an outward growth of the hypoblast to inclose them, of which there is no appearance. As will afterwards be seen the view that they are of hypoblastic origin is supported by the fact that they continue growing with the growth of the animal, and that their

thickness is, so to speak, inversely proportional to that of the cellular tissue of the notochord, which becomes thinnest in the region where they attain their maximum size. This, therefore, suggests that they are formed at the expense of the notochord. An analogy moreover at once occurs of the secretion of such a substance from notochordal tissue in the case of Amphioxus, in which discs are deposited of very similar histological character to these masses in Balanoglossus. These two rods posteriorly bend downwards and then slightly forwards lying in the hypoblast. They are therefore each cut twice in sections through this part of the body.

Behind the end of the notochord are the gill-slits, which are still only circular pores leading to the exterior (fig. 43). The lumen of the gut in the branchial region is (in contracted specimens) much suppressed, and its ventral side, however, always is grooved, and in this groove the cilia which line the branchial region are well developed.

When the animal contracts, the branchial region of the gut posteriorly projects over the front of the digestive region on the dorsal side (fig. 44).

The digestive region is quite distinct from this point in development onwards. Its cells have the appearance shown in fig. 45, being large cells with amœboid processes containing large granules. From the back of the digestive region the intestinal region is now marked out. Its walls are thinner, and the cells composing it are long and ciliated at their inner ends. The anus is a large aperture, permanently open when the animal is extended, situated dorsal to the tail. There is no epiblastic proctodæum.

Mesoblastic Structures.

Anterior Body Cavity. The mesoblast of this tract may now be divided into two parts—(1) a peripheral portion which lines the body walls of the proboscis, and (2) a central portion which is pushed in by the forward growth of the notochord; between these two portions there is a body cavity which retains a clear central space throughout life.

(1) The peripheral part consists of cells and fibres. The cells are mostly pyriform similar to those of one-gill larvæ. They contain few granules and are much reduced in number. The fibres are more numerous. They are of several kinds: thick strands that are obviously muscular, running as yet only in a longitudinal direction, having their ends inserted in the skin: and also thinner fibres, which are arranged circularly, as in the periphery of the middle third; longitudinally, only occurring as a sort of sheath separating the circular fibres from the general connective tissue, and occasionally in the central portions; radially, as over the whole periphery of the proboscis cavity, and finally, very fine fibres forming a loose network connecting all the mesoblastic elements together. As may be seen in figs. 31, &c., there is as yet no suggestion of the peculiar concentric arrangement of the longitudinal fibres, which gives a section of the adult proboscis its curious appearance. The radial fibres are mostly inserted into the skin (fig. 32), their possible connection with the epiblast has been already discussed.

There are still present a few of the large spherical cells which formed the inner layer of mesoblast in one-gill-slit larvæ, but they contain fewer granules, and are only found in the posterior parts of the cavity.

(2) The central portion in the one-gill-slit larva was made up of a mass of spherical and polygonal cells surrounding a central part filled with loose tissue (fig. 16). As the notochord grows forward, this structure, which, from its subsequent fate may now be termed the proboscis gland, is prolonged with it. As this forward growth occurs, a basement membrane forms between the mesoblast outside the proboscis gland and the loose tissue contained in it. Fig. 47 shows a section anterior to the part where this basement membrane is formed. The mesoblastic cells are here large and granular, having irregular shapes. The proboscis gland now is hollowed out, so that it contains a space which extends from the proboscis stalk to the front end of the notochord. This space contains a few mesoblastic cells, which are as yet not obviously differentiated.

The position of this space is shown in fig. 45. It will be seen in the sequel that the mass of the secreting tissue of the gland is formed from the cells covering this space, and forming the sides of this central portion of the mesoblast. The space itself contains eventually but few of these secreting cells, and will be spoken of as the sac of the proboscis gland (fig. 52, *gl. s.*).

The heart is as yet not represented.

Middle Body Cavities.—The tissue lining these cavities does not exhibit more than a general progress of differentiation. Owing to the increased narrowing of the proboscis stalk the two anterior horns of the cavities are more distinct. In the dorsal and ventral mesenteries basement membranes occur. The cells lining these cavities are generally pyramidal or crescentic, some of them being radially directed and fusiform.

Posterior Body Cavities.—The tissue of the posterior mesoblastic pouches has undergone the same proliferation and progressive differentiation as that of the middle cavities. These processes are not, however, quite so far advanced. The two horns (peri-hæmal cavities) which began to grow forwards above the gut in the one-gill larva are now much more developed. They now extend into the back of the collar region, in front of the first gill-slit. They are filled more or less with loose mesoblastic tissue containing a few fibres. As before stated, in the mesentery between them is formed the dorsal blood-vessel. This structure appears as a split in the mesentery, and is as yet quite empty in preserved animals. This split extends already through the whole course of the perihæmal cavities. On the ventral side also a split is formed in the lower mesentery of the posterior body cavity to form the ventral blood-vessel. There is as yet no connection between the dorsal and ventral vessels.

From this point the details of the subsequent development and anatomy of the parts will be given in the section dealing with the separate organs; but, before doing so, it may be well to describe briefly the general course of the later history of the internal structures.

Notochord.—As will be seen, this structure increases

greatly in size, and assumes a vacuolated appearance closely resembling that of the notochord of young Lampreys and Elasmobranchs.

The skeletal rods attain a considerable size. Their anterior ends unite, forming a single bar, while their posterior ends diverge, partially enclosing the gut. This whole structure forms the support of the proboscis.

From its development, position, relations to surrounding parts, histology and function it appears to me to be comparable with the notochord of the Chordata, and this name is strictly appropriate to it. Even if the suggestions which will be made hereafter as to its phylogenetic significance be not accepted, this rejection would in no way militate against the fact that this structure is to all intents and purposes a notochord, which can only be designated as a longitudinal dorsal supporting rod, derived from the hypoblast.

The nervous system afterwards attains a great development (fig. 60). The dorsal cord in the collar sinks further and further from the skin, being (in B. Kowalevskii) connected to it by a mesentery. The lumen is in this form less developed than in B. minutus, &c. The ventral cord is the next to appear, and almost simultaneously with it arises the deposit of nervous tissue in the skin at the base of the proboscis. This deposit afterwards attains a great extent, forming a thick band round the proboscis stalk. It may be noticed that this nerve-ring has practically the same relation to the proboscis that the ring of ganglia in Nemertines presents, the proboscis of Balanoglossus being, however, permanently protruded, and the nerve-ring still in the skin. Both these nerve-rings agree in being traversed in Nemertines by two and in Enteropneusta by one pore communicating from the exterior to sacs which were originally archenteric diverticula.

Body Cavities.—As before mentioned, the left horn of the anterior body cavity comes to open by the proboscis pore to the exterior. This opening is median and dorsal in other species, but in B. Kowalevskii it is on the left side throughout life. In all species it perforates the nerve-ring of the stalk.

Nearly all the proboscis cavity is eventually filled up with loose tissue. This is composed of a number of concentric rings of longitudinal fibres and connective tissue. These rings attain the maximum number of eight. In fig. 51 the general appearance of an older proboscis cavity is shown. The concentric arrangement is, however, not yet attained in the stage there figured.

A free space is always present between these rings of tissue and the central structures in preserved specimens.

The proboscis gland becomes a large mass of tissue composed of anastomosing blood-vessels covered with conical cells fixed on the vessels by their apices. Many of these cells contain remarkable yellow granules, which are also to be found outside the cells, sometimes presenting a conglomerate arrangement. They would seem to be formed in the cells and thrown out. They are also to be found in the sac of the proboscis gland. This sac is blind posteriorly, but anteriorly the loose tissue which it contains passes into unbroken connection with the remarkable cellular layers covering the blood-vessels. Hence the sac is in communication with the central body cavity through the tissue spaces of the gland. The function of this gland is quite unknown. Spengel suggests that it is an "internal gill." It does not seem probable to me that an animal with some sixty pairs of true branchial clefts would also possess another large and complicated organ of entirely different structure also for respiratory purposes. The presence of the brown granules suggests that it may be excretory. If this were so, the excreta might be expected to pass out by the proboscis pore which opens into the cavity in which the gland lies. No direct evidence was obtained as to the normal direction of the flow through this pore. Spengel and other observers state with regard to B. minutus that water is taken into the body cavity at the proboscis pore, but my own observations do not confirm this statement. On the contrary, particles of Indian ink or carmine held in suspension in the water in which the animals have lived for days, cannot be found to enter the proboscis cavity, while similar particles,

if placed in the tissue spaces of the proboscis, are certainly expelled by the pore. This evidence does not of course demonstrate, beyond doubt, that inwardly directed currents never enter the pore but only gives a presumption against them.

Spengel's statement of the absence of the pore described by Kowalevsky and Agassiz, at the apex of the proboscis, is true for all the species which I have examined.

The Heart.—As mentioned by previous observers, a large vesicle may be seen pulsating above the water-vessel in Tornaria; such a pulsation may be observed in the dorsal side of the base of the proboscis in B. Kowalevskii at the stage of two to three gill-slits. Spengel states that this contractile sac is the upper of the two cavities lying above the notochord (figs. 51, 53, &c.); this he calls the "heart." In consideration of the fact (which he also admits) that it contains no blood, gives off no vessels, and has no muscular walls, this name seems open to misconstruction. As this so-called "heart" is merely a space filled with loose tissue which is part of the proboscis gland I have preferred to call it the sac of the proboscis gland, and to reserve the name "heart" for the sac which lies between this space and the notochord (figs. 50 and 51, *ht.*). That this is the actual heart can I think hardly be doubted. It arises at about three gill-slits as a single horizontal split in mesoblast between the notochord and the sac of the proboscis gland. It acquires muscular walls and is always nearly full of a coagulum similar to that which is found in the remaining blood-vessels of the body, which can all be traced into connection with it.

These peripheral vessels are (1) a longitudinal dorsal one, running from the heart to the tail in the dorsal mesentery from the back of the collar, and in the collar as a blood-space surrounded by the perihæmal cavities (fig. 60); (2) a ventral longitudinal vessel running from the back of the collar to the tail in the ventral mesentery. These two are connected by blood sinuses in the skin and in the wall of the gut. I have not seen the definite circular vessel which other observers state

surrounds the gut anteriorly. The principal skin sinuses are a pair of large ones which extend on each side of the dorso-lateral regions of the proboscis (fig. 51).

The Collar Pores.—On the outer wall of each atrial cavity appears a thickening at about eight gill-slits. This thickening acquires a perforation which leads from the collar body cavity to the atrial cavity. These perforations acquire a curious folded lumen and become ciliated constituting the collar pores. Their opening into the atrial cavity is continuous with that of the first gill-slit. From analogy it may be expected that these pores are of an excretory character. With regard, however, to the direction of the flow through them, the evidence is as unreliable as that as to the currents in the proboscis pore. Spengel states that water is taken into the body cavity at these points, while I was unable to find that coloured particles ever entered it. Similarly, however, such particles placed artificially in the collar body cavity were washed out at these points.

The Middle and Posterior Body Cavities.—These cavities become in adult life more or less filled with connective tissue, &c. The cavity of the middle pair becomes practically obliterated owing to the great development of loose tissue in it. But in the posterior cavity this proliferation is never so great. The middle pair of body cavities is far more choked up in B. Kowalevskii than in B. minutus, but in B. Brooksii the amount of connective tissue is even greater. This fact is interesting in the present state of views as to the morphological meaning of "cœlom," as presenting an example of a body cavity arising in a most typical "mesodermic" manner, assuming ontogenetically precisely such an appearance as is presented by the "mesenchyme" of Platyhelminths, &c.

A statement as to the origin of the generative organs is reserved for the present, as some doubt exists as to the layer from which they are derived. The general appearance is, however, suggestive that they are of epiblastic origin.

Before beginning a detailed account of the later development

it may be desirable to discuss briefly the new light which these facts throw upon the affinities of the Enteropneusta.

In 1881 Metschnikoff published a detailed comparison of Balanoglossus with the Echinoderms, comparing Tornaria with Bipinnaria, showing that the resemblance is close, and concluding with the suggestion that Balanoglossus should be included among the Echinodermata in a separate division, "Bilateralia." The branchial structures he compared to the openings from the body cavities of Echinoderms. This view, as thus expressed, receives no support from further observations, and would now appear to be untenable.

As mentioned above, all the Enteropneusta possess a supporting structure which is comparable with the notochord in every way, except in extent and in the persistence of its connection with the alimentary canal. Its resemblance to that of Amphioxus is especially striking, for in Amphioxus the notochord projects a long way in front of the mouth. It moreover possesses gill-slits which are not only without parallel, except among the Chordata, but also in structure, position, and development, agree exactly with those of Amphioxus, in which the slits acquire the same U-shaped form.

The agreement in the position of the blood-vessels and skeleton of the gill bars is also very close. The fact of their gradual increase in number from before backwards throughout life is another common feature.

The position and mode of origin of the central nervous system is also similar in both forms; the invagination of the dorsal cord in Balanoglossus being, however, only partial, while that of Amphioxus is complete.

The mesoblastic pouches suggest the same resemblance, differing only from those of Amphioxus in number, being one median and four lateral, while those of Amphioxus are one median and twenty-eight lateral. As I have already pointed out, the fate of this anterior pouch is in the two animals closely similar. In both it is divided into two as the notochord grows forward. In Amphioxus the division is complete, while in Balanoglossus it is partial. In both, the backwardly-projecting

horn upon the left side becomes lined by ciliated columnar cells, and opens to the exterior. Moreover, in both animals this opening has a definite relation to the nervous system. In Amphioxus it becoms the "olfactory" pit (Hatschek), while in Balanoglossus it is surrounded by a mass of nervous tissue. Finally, the collar folds, especially of B. Kowalevskii, would appear to be comparable with the commencing atrial folds of Amphioxus, for the most anterior gill-slits open into the cavity which is thus enclosed.

The pair of ciliated funnels opening from the collar body cavities to the atrium has been compared above to the excretory tube mentioned by Hatschek in a similar position in Amphioxus.

A pair of tubes has been described by Lankester in Amphioxus opening into the back of the atrial cavity, communicating with the dorsal body cavities. It may be remarked that if the collar fold of B. Kowalevskii were prolonged backwards, as the atrial folds are in Amphioxus, the two collar funnels would then be carried backwards, and have relation similar to that of these tubes, which, as suggested by Lankester, may be excretory.

To recapitulate: striking resemblances to the Chordata, and especially to the Cephalochord type, are to be found in the following structures:

(1) The notochord.

(2) The gills and branchial skeleton and blood supply.

(3) The central nervous system.

(4) The origin of the mesoblast.

(5) The peculiar fate and remarkable asymmetry of the anterior pouch.

(6) The atria.

(7) The excretory funnels.

In each of these cases, excepting that of the branchial structures and the excretory funnels, the condition is that which would be produced by a partial or arrested development of the corresponding structure in Amphioxus.

The above considerations appear to justify us in including

the Enteropneusta among the Chordata. I would, therefore, tentatively suggest the following table :

Chordata.—Hemichordata (Enteropneusta).
Urochorda (Ascidians).
Cephalochorda (Amphioxus).
Vertebrata.

It is not now proposed to enter into a more detailed discussion of the morphology of the group, or of the light which an acceptance of this suggestion throws on the origin of the Chordata. A fuller examination of these points is reserved for a subsequent occasion.

It may nevertheless be advisable to point out that since, according to Spengel, the tissue of the "water vessel" of Tornaria forms the lining of the proboscis cavity of B. minutus, this "water vessel" is therefore the same structure as the anterior body cavity in the form just described. If, then, the "water vessel" of Tornaria is comparable to the "water vessel" of Bipinnaria, which has a similarly asymmetrical development upon the left hand side of the body, which view has been held by all previous observers, it would therefore appear to follow that the water vessel of Bipinnaria is *primâ facie* comparable with the asymmetrical anterior body cavity of Amphioxus.

Later Development and Comparative Account of the Organs in the various Species.

The species which I have examined are the following :

B. Kowalevskii (Alex. Agassiz.) (Coast of North America).

B. Brooksii, n. sp. (ditto).

B. minutus (Kowalevsky.) (Bay of Naples).

B. salmoneus (Giard.) (Iles de Glenans, off South Coast of Britany).

B. Robinii (Giard.) (ditto).

B. Kowalevskii differs from all the others in having—

(1) a relatively long proboscis ;

(2) no hepatic sacculations ;

(3) a simple branchial skeleton, not connected by longitudinal bars, as in the other forms;

(4) very short collar funnels, the external opening of which is directed transversely instead of posteriorly, as in the others, in consequence of

(5) the greater extent of the backwardly-directed atrial fold.

As far as can be determined from Agassiz' account, his species agrees in these points with the one which is the subject of this paper.

The Notochord and Axial Skeletal Rods.

B. Kowalevskii.—The general course of development of the notochord has been already described. Fig. 47 shows the histological characters of the cells at two gill-slits, when they are still large full-looking cells with large nuclei.

Fig. 48 is from a section of the proboscis stalk in the region of the pore of a larva with three gill-slits. It exhibits the commencing degeneration of the notochordal tissue and the increase in size of the structureless deposit which constitutes the skeletal rods.

In this region the skeletal rods unite to form a single median rod, which is continuous with the notochordal sheath.

In figs. 49—53 the appearance of the notochord at four gill-slits is illustrated. The degeneration is now far advanced. Nuclei are rare in the notochord, and the cells are vacuolated, as shown by the fact that the nuclei occur in the nodes of the cell outlines. The protoplasm of the cells merely forms a kind of network containing a few nuclei. The remainder of the space is probably occupied by some homogeneous non-protoplasmic substance, such as may be supposed to fill up the notochordal tissues of other forms.

Figs. 49—52 are from sections taken in front of the lumen (cp. fig. 57). In fig. 53 the lumen is reached. It will be observed that the lumen at this point still ends as a fine tube. In later life a great thickening of the notochord takes place at this point, and the lumen then acquires a downward extension

(figs. 57 and 56). Immediately behind this downward extension lies the anterior end of the united skeletal rods, which here attains its greatest thickness, almost filling the sheath of the notochord, the tissue of which is here almost suppressed.

In old specimens the shape of the anterior parts of the notochord becomes rather irregular in section.

In that part of its course which lies behind the proboscis the notochord in the adult is more or less elliptical in section, containing a large and somewhat irregular lumen. Its tissue is here greatly reduced, and this reduction appears to progress regularly as the animal grows older. In fig. 60 the appearance of the notochord in such an old adult is shown. Degeneration has progressed far, leaving the notochord as a space surrounded by vacuolated cells enclosed in a sheath. With this sheath are connected the skeletal rods, which attain a great size. Centrally, on the dorsal side, between the notochord and the gut, lies the principal rod; this is formed by the uniting of the two rods (figs. 37, 38, &c.), whose development has already been described. This fused portion is now diamond-shaped in section; its lower angle causes a dorsal ridge to project into the mouth cavity. Laterally are placed two long rods, which are continued into the central rod and notochordal sheath anteriorly. To these lateral rods are attached large bunches of longitudinal muscles, by which, doubtless, the notochord may be pulled backwards, and the proboscis retracted so as to shut the mouth (fig. 60).

Posteriorly the median rod divides into two, and the opening from the notochordal lumen into the gut lies in the angle formed by the separation of these two diverging rods (fig. 57).

A considerable deposit of "structureless" substance takes place, filling up the spaces in the proboscis stalk, and forming a partial sheath around the perihæmal cavity. Whether this substance is chitinous or of some other material I am unable to say. The ensheathing parts of it have exactly the histological appearance presented by the "structureless" substance, which in Amphioxus is continued from the notochordal sheath,

&c., between the myotomes. The rods, however, are seen in adult specimens to have concentric markings in section, suggesting that they are formed by a deposit around a central core. In that part of the rod which lies just in front of the point of divergence of the two horns there are two such cores. The cores stain more deeply than the rest of the rods.

In B. minutus the position and general appearance of the notochord is similar to that in B. Kowalevskii, with the exception that the lateral rods are not developed to the same extent. The histology, however, is somewhat different. In figs. 61—63 the appearance of the tissue is shown. Spengel has stated that he is unable to find, in B. minutus, any structure resembling the notochord of the Chordata, figuring that organ as though composed of columnar cells. My own observations give no support to this statement. No specimen of notochord of any of the species which have been examined by me present the appearance indicated by Spengel. On the contrary, specimens preserved severally in picric acid, corrosive sublimate and acetic acid, Perenyi's fluid and osmic acid, all equally show this body as made up of vacuolated tissue strongly suggestive of the notochord of Chordata. This is especially the case with regard to that of B. minutus. Fig. 61 is taken from a section of B. minutus in front of the lumen. The sheath is here very slightly developed. It will be observed that the nuclei are fewer in number than in B. Kowalevskii, and that they are gathered round the upper centre. The section shown in fig. 62 is from the same in the region of the proboscis stalk. It shows the diamond-shaped section of the skeletal rod and the concentric markings upon it. The notochord itself is here elliptical in section, and the strands of protoplasm running across it are well seen.

Fig. 63 shows its appearance in front of the point at which it opens. Dorsally its wall is very thick and solid, while ventrally it is comparatively thin. Behind this part the skeletal rod divides into two divaricating horns as in B. Kowalevskii.

In the other species the structure of the notochord is

essentially the same as in B. minutus. In the anterior region of the notochord of B. Brooksii there are hardly any nuclei at all. I hope to publish figures illustrative of the later development and anatomy of the other parts at no distant date.

Methods of Investigation.

From the characters of the unfertilized egg of B. Kowalevskii it was extremely improbable that the earliest stages of development could be passed anywhere else but in the mud which the parents inhabit. Though the examination of Agassiz had failed in the attempt to find any but adult Balanoglossus in this situation it seemed worth repeating. Accordingly a large quantity of mud inhabited by Balanoglossus was placed in a glass vessel of water and worked up, avoiding rotatory currents, until the whole was in suspension. A number of Balanoglossus which had previously been minced very finely were then thrown in and the whole was then left to settle for a few minutes. I then siphoned off the water and lighter particles in suspension, which consisted chiefly of vegetable *débris*, stopping the siphon when the layer of chopped Balanoglossus was reached, which could easily be seen by the bright orange colour of the fragments. This portion was drawn off and examined separately. It was found to contain great numbers of larvæ and embryos of Balanoglossus, minute Nemertines, free Nematodes, &c. In this manner all the animals living in several hundredweight of mud may, in an hour or two, be collected into about a pint of water and sorted with a simple microscope. This was generally performed by rotating a little of the water in a shallow saucer with a slight peripheral groove. The larvæ then all lie in the groove which may be passed under the lens by rotation. After a little practice it becomes unnecessary to discolour the water with fragments of the animal required, as of course the right layer can easily be detected by the size and character of the particles composing it. It appeared to be worth mentioning this mode of obtaining mud larvæ as its application on a large scale does not seem to be generally employed. It should be remarked that small

Nemertines, &c., are usually found still suspended long after most of the mud is precipitated. The water should then of course be poured off and left to settle separately. The degree to which any particular animals required may thus be easily separated off from the rest is very great. The disadvantage of such a method is that a certain number of larvæ are sure to be broken in the stirring necessary to effect suspension. I have little doubt that the larvæ which were observed free-swimming earlier than stage F were thus liberated from the egg-shell.

Preservation.—All my specimens of B. minutus were obtained from Naples, being very kindly prepared for me by Mr. Weldon with picric acid.

Most of the larvæ of B. Kowalevskii were placed for less than a minute in corrosive sublimate sat. sol. two parts, mixed with one part glacial acetic acid, washed with water and successively passed through 30 per cent., 50 per cent., 70 per cent., and 90 per cent. spirit. On the whole the results given by this reagent were the best. The softer parts, however, are best preserved in those specimens which were treated with Perenyi's fluid one hour, then 90 per cent. spirit for twelve hours, the 90 per cent. spirit being then changed. In the case of adults preserved with Perenyi's fluid, the fluid was changed once or twice.

Osmic acid did not give good results, but probably this was due to bad manipulation. The sections were cut in continuous series with Caldwell's automatic microtome.

On the

Morphology of the Enteropneusta.

PART II.

By

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With Plates VII—XII.

THE following paper is descriptive of the figures which illustrate my concluding account of the morphology of the Enteropneusta.

As an abstract of some of these facts was given with the first part a certain amount of repetition has become unavoidable.

Since the publication of Part I, I have been able to make some further observations on the histology of the fresh tissues of the Brittany species (B. salmoneus and Robinii). For this opportunity I am indebted to the kindness of the directors of the Zoological Laboratory stationed at Concarneau, Finistère. And especially my thanks are due to Dr. Chabry for affording me these facilities.

The Skin and Nervous System.

The skin of all the species is entirely ciliated.

In the fresh condition I have chiefly studied it in B.

Robinii, and it will be better first to describe its features in this form. Its structure is best seen by killing the tissue in a mixture of one part of 1 per cent. osmic acid and one of sea-water, then washing with sea-water, and staining with picrocarmine. This tissue on being teased out in glycerine shows the structure figured in figs. 76 and 77. The cells are very long, and most, if not all of them, extend the whole length of the skin (cf. fig. 75). The heads of these cells in the natural living state are closely in contact with each other, but on pressing out the tissue both in living and also in preserved specimens these heads may be stretched away from each other, but each remains attached to its neighbours' by more or less regular anastomoses. It thus is brought about that the surface of the skin is made up of a sort of honeycomb of tissue, each of the nodes being the outer end of an ectoderm cell. The cells are very difficult to separate finely, but the skin may easily be broken up into small rectangular pieces. On separation each cell is very thin; its outer end is slightly pyramidal, and is continued into a thin fibre which gives off anastomoses with adjacent cells and dilates at intervals. In one of these dilatations, generally the last, the nucleus is placed. Below this point the cell is continued into a very fine filament which may be traced for some distance. Many of these filaments terminate in small round knobs, which are possibly due to reagents.

In sections of hardened specimens these filaments may be followed into the layer of nerve-fibre, which is always more or less developed at the base of the ectoderm cells over the whole body. These cells compose the larger part of the skin of the proboscis and collar. Amongst them are distributed cells which probably secrete mucus, &c. These cells are of several kinds. First, in the skin of the proboscis are large goblet cells whose nucleus alone stains (fig. 75, *mu'*). Next, in the skin of the back of the collar and of nearly all the rest of the body excepting those parts in which concentrations of nervous tissue are found, almost the whole tissue is made up of large cells full of some substance probably lubricating also, which

does not stain. These cells are sufficiently represented in figs. 72 and 72A, which are, however, from B. minutus. In parts of the skin which are of this kind the long cells of the ectoderm are comparatively few in number, and thus the skin has a spongy consistency which is very characteristic. This is true of the skin behind the collar in B. minutus, B. salmoneus, and B. Robinii. There is a general similarity between the skins of all these forms, and probably their structure is the same as in B. Robinii. This statement, however, only rests on the evidence of sections, as no teased preparations were made of B. minutus. In the skin of the collar and proboscis especially a small number of nuclei may be seen in the higher layers of the skin. Whether these belong to young cells of the tailed series or of the secreting type was not determined. Another set of small, generally bifid secreting cells, are found in the proboscis skin; the contents of these cells are granular.

There is one other point of importance in treating of the skins of these forms, viz. the constant presence in teased preparations of large spindle-shaped cells (fig. 77, *c*). As the result of many observations it appeared nearly certain that these had really been broken off from the ends of the long ectoderm cells. Unless care was taken in the preparation this frequently happened, many of the ectoderm cells being broken and therefore without nuclei, and hence the probability that this was the origin of the spindle-shaped cells. Since these fusiform cells are generally most abundant at that level of the skin at which the nuclei of the long cells are placed, the appearance is suggested that they form a second layer of ectoderm cells; but for the reasons above stated it seems likely that this is erroneous, and that there is no such definite second layer.

The resemblance between this skin and that of some Nemertines, e.g. Monopora vivipara (Salensky, 'Arch de Biologie,' 1884), is very close. In this animal the same spongy appearance is produced, and it is possible that the deeper layer of ectoderm may be capable of the same explanation.

The skin of B. Kowalevskii differs in some ways from that of B. minutus, &c., especially that of the trunk, in which the large goblet cells are comparatively rare. In all parts of the skin round, unicellular glands are more or less frequent, but their contents stain more or less deeply with hæmatoxylin, &c. These cells often fall out, leaving empty spaces. In the collar of B. Kowalevskii the skin is very thick and is full of very long cells (figs. 80 and 81) containing granular contents, which stain very deeply.

Fig. 79 shows a section of part of the proboscis skin in which the layer of nerve-fibre is very thick. In the upper part of this kind of skin there is a definite row of long nuclei which with some reagents assume a dice-box shape, probably due to preservation. To what extent these cells reach the whole depth of the skin cannot be affirmed, but many of them can be traced into fibres which run into the layer of nerve-fibres.

Nervous Concentrations in the Skin.—As has been already mentioned, in all the parts of the skin a greater or less quantity of unstained substance may be found in the base of the skin. The substance contains no nuclei (excepting a few in the nerve-sheath of the base of the proboscis), and may oe seen, especially in fresh osmic acid preparations, to consist of fine fibres. Into it run the tails of ectoderm cells. In the next place fibres may frequently be seen running out of it through the basement membrane, and losing themselves amongst the mesoblastic tissues. The question as to the nature of these fibres is one of great interest. They may either be mesoblastic fibres penetrating into the ectoderm as supporting structures, or they may be epiblastic fibres leaving the skin, in which latter case they are in all probability nervous.

Somewhat similar fibres have been described by Ludwig in the similar tissues of Asterias, and he is of opinion that they are connective tissue. The possibility, however, that these fibres in Balanoglossus are nervous is supported—firstly, by the fact that they always taper inwards and not out-

wards; secondly, that as a matter of fact, in B. Robinii at all events, the ectoderm cells may themselves be traced into tails of this kind; thirdly, the general absence of nuclei in the "punktsubstanz," for if these fibres are supporting cells, nuclei might be expected to be found in their course; fourthly, there is an à priori difficulty as to the nerve supply to the muscles in these animals, for, though the body of some of the species is very thick, no definite nerve-cords are to be found crossing the body cavities, with the exception of the "dorsal roots" mentioned hereafter. How, then, are the muscles innervated? It seems, then, at least possible that the nerve supply is derived directly from the skin, in which case the fibres leaving the "punktsubstanz" naturally suggest themselves as the transmitting agents. Finally, the view that these fibres are ectodermic is rendered likely from the fact that their origin may occasionally be traced from a very high level in the skin, though the appearance which is sometimes produced in sections as of their actual continuity with the undoubted ectoderm cells may not be quite reliable. In a few instances these fibres appear to anastomose with mesoblastic elements, though this cannot be quite definitely affirmed. On the whole, the balance of evidence seems in favour of the view that they are ectodermic. If this be correct the skin of Balanoglossus is to be regarded as a collection of sensory cells ending in long fibres, which may either be connected to the central nervous system, probably by the longitudinal fibres of the "punktsubstanz," or may pass directly through this as motor fibres into the muscles.

The next point relates to the question as to the intervention of some third cell in their course functioning as a ganglion cell. In B. Robinii, in which the examination of this subject is most complete, as stated above, the occurrence of such cells could not be shown; but this is, of course, by no means conclusive in face of the antecedent probability of their occurrence. The "punktsubstanz," then, would mainly consist of afferent fibres passing to the central nervous system, and the motor fibres

probably pass directly through it. As will be shown in the next paragraph its distribution agrees with this view.

In the account of the general development the central nervous system was shown to have arisen chiefly by a solid delamination from the skin, added to which its anterior, and to some degree its posterior, ends are being continually invaginated as growth continues, so that each end is tubular. This tubular form results not so much from the longitudinal closure of a tube as from a forward and backward growth of skin at the extremities of the delaminated cord. Soon after delamination histological differentiation occurred between the upper cellular and lower fibrous parts of the cord. While this was proceeding (2, *g. s.*) fibrous tissue was deposited to form the ventral cord at the point of this structure, which was most anterior (viz. the back of the collar). While this is proceeding the deposition of similar tissue in the region of the dorsal cord commences at the posterior attached end of the central nervous system. Next, the deposition of fibrous tissue extends itself forwards on to the proboscis, being first laid down in the dorsal middle line of the proboscis stalk (*v.* figs. 34 and 35, *pkt.*). On the appearance of the atrial fold the ventral and dorsal cords become united by a fibrous ring in the inner angle of the fold. This ring, therefore, may be supposed to bring up the fibres from the ventral cord to the central nervous system, which it enters at its posterior end, together with the dorsal cord (*v.* diagram, fig. 65).

The greatest concentration following upon these occurs in the skin of the base of the proboscis. In the larva with four gill-slits (fig. 99, *P. rg.*) it is already well marked. Concentrations are formed in the line of the gill-slits (figs. 72A and 104), and slight fibrous anastomosing tracts run irregularly, following the line of the wrinkles from both the dorsal and ventral cords. These wrinkles taper towards both the cords and are permanent, being, in fact, limiting lines between patches of glandular cells.

Now, all these tracts of fibres are thickened as they approach the central nervous system, and dwindle peripherally. If this

diminution were due to the continual separating of efferent fibres from the cords it would reasonably be anticipated that it would be greatest in the case of those parts of the body which lie behind the collar (i. e. behind the central nervous system) ; for these cords have almost the whole body to supply, but, on the contrary, it is the nervous sheath of the proboscis which presents the greatest concentration, and this continually thickens on approaching the collar, though the proboscis is conical and its base is towards the collar. This may be taken to show that this sheath of nerve-fibre is afferent, and is continually increasing in thickness owing to the incoming of sensory fibres from the ectoderm cells lying above it. Its sudden increase on the proboscis stalk is due to the sudden tapering of the base. This feature is particularly well seen in B. minutus. On any other hypothesis it would seem unlikely that this great deposition of nerve-fibre should occur in a region which is generally covered up by the anterior folds of the collar.

The Central Nervous System.—The changes occurring in this structure in B. Kowalevskii after its separation consist in an increase in size and in histological differentiation. As the result of these changes its anterior end comes to have the structure shown in fig. 60. Among the cells lining the anterior end of the lumen are always some few gland-cells. The cellular part of this cord is continuous, of course, with the cellular part of the skin, and the fibrous part or white matter, as we may call it, with the fibrous layer of the skin. Behind the lumen it has the appearance shown in fig. 78. The white matter does not enclose the upper part of the cord. Above it are a number of pyriform cells, probably ganglionic, whose tails project into the white matter. Central to these the cells are more or less irregularly grouped into strands enclosing spaces. The histology of this central part of the cord is very difficult, and I have not been able to determine how these spaces are filled. In B. minutus (*v.* fig. 67) they are so definite as to make it certain that they are not due to reagents.

Among this loose tissue of the centre of the cord are remarkable stellate groups of cells (fig. 78, *stel.*) whose heads are thus placed radiating from a small lumen, which is generally sharply defined on three sides and usually irregularly bounded at some part of its margin. The nature of these stellate groups did not appear. They are commonest in the sides of the "grey" tracts, viz. at the points where the white matter is bent up (*v.* fig. 74, *b*). It is possible that the spaces thus enclosed may in some indirect manner communicate with the neural tube.

The histology of the cord is nearly the same in all the species. In B. salmoneus and B. Brooksii, however, there is always a quantity of yellowish granules embedded in the central substance (on the analogy of Nemertines this substance may function like hæmoglobin). The shape of the cord in section varies in the different forms and in different parts of its course (*v.* fig. 74).

From the lower surface of the white matter of all species many fibres may be seen leaving the cord and losing themselves among the subjacent muscular tissues. In B. Kowalevskii alone no connection exists between the dorsal side of the cord and the skin. In B. minutus this is accomplished by three cords of skin substance. Their outsides are covered with a fibrous sheath (Spengel), and this is in connection with the fibrous layer of the skin. As Spengel has stated, these cords contain a more or less distinct lumen. I have not been able to trace these out upon the skin, though they occasionally appear to lead to the cavities enclosed by the radiating cells. These cords I propose to term the dorsal roots. They occur in B. minutus, Robinii, salmoneus, and Brooksii. Their homology will be discussed when the other morphological questions arising out of these facts are treated of.

The histology of the rest of the nervous system has been sufficiently described.

The relations of the parts are explained by figs. 60, 64, 65, 67, 73, &c.

There are no special sense organs.

As the "dorsal roots" do not occur in B. Kowalevskii their development has not, unfortunately, been observed.

The Hypoblastic Structures.

The notochord has been described already, as also the mode by which the mouth comes to be anteriorly directed.

The cavity into which the mouth leads is lined by very thick walls (figs. 90, 67, &c.), composed of long cells supported by some intracellular substance, probably the same as that of the notochord. In B. Kowalevskii it leads continuously into the branchial chamber, but in the other species, in which the branchial chamber is separated by longitudinal ridges (fig. 91), from the lower cavity of the branchial region (which thus has the well-known figure-of-8 shaped cavity). The anterior end of the branchial cavity comes to be almost enclosed in the pharyngeal cavity. As the result of this on either side the branchial cavity projects as two blind horns, which are enclosed in the pharyngeal cavity.

The structure of the gill-slits has been sufficiently described by Kowalevsky, Agassiz, and Spengel.

To these accounts there is little to add. The figures 84 and 85 illustrate the mode by which their final structure is attained. It is practically impossible to follow their structure by means of transverse sections, but longitudinal sections and surface-views make them easily intelligible. Each gill-slit of B. Kowalevskii is U-shaped and surrounded by a skeletal secreted structure, as shown in fig. 85. In my last paper I stated that, though the origin of these structures was uncertain, the balance of evidence favoured the view that they were hypoblastic. Since the above was written I have been led to regard them as more probably mesoblastic, owing to some of the appearances since observed. It should be noticed that the body cavity is continued into the valves always, but never into the bars separating adjacent gill-slits in which the bordering bars are in contact. This is due to obliteration of the cavity by the skeletal bars. This feature is very useful in distinguishing these parts in sections.

The atrial cavity must be described in this connection. As stated in the general account, its origin is due to the backward growth of the collar-fold to form an operculum. In B. Kowalevskii (*v.* fig. 88) it is more marked than in B. minutus, but in B. salmoneus the collar-fold does not reach as far as the first gill-slit, which consequently opens directly to the exterior (fig. 107). In B. Kowalevskii it covers about three gill-slits. (In fig. 88 only one gill-slit is thus shown; this is owing to the slight obliquity of the section.)

The relation of the opercular fold in B. minutus is shown in figs. 73 and 104.

The dorsal wall of the branchial chamber is thickened in the middle line to form a ridge (figs. 89 and 92). This ridge contains a groove in its posterior part. It is no doubt a supporting structure, and may conceivably be homologous with part of the backward extension of the notochord in other Chordata.

The digestive tract follows upon the branchial region. The branchial chamber ends in a short blind sac above it, and it is in this sac that the new gills are added after three pairs are formed (*v.* fig. 44). The walls of the digestive tract in B. Kowalevskii are thrown into an irregular spiral fold (*v.* figs. 82 and 108), which is not continued into the intestinal region as a definite feature.

The cells of the digestive region are arranged (fig. 82) in a single layer for the most part. They contain large granules and bear a few long cilia. In the walls of the gut in this region are numerous blood-vessels. The lumen of the gut in this region varies greatly in size, probably with the digestive processes (cf. Salensky, loc. cit.), the liver being in B. Kowalevskii occasionally obliterated.

In B. Kowalevskii there is no distinct sacculation to form the liver, but in B. minutus the dorso-lateral walls of the digestive region are pushed out to form the characteristic liver outgrowths. These structures are not regularly paired. Their walls are full of secondary foldings (*v.* fig. 93). The

cells lining these folds are similar to those of the digestive tract, containing large granules and fluid-looking vacuoles.

The skin covering these liver-saccules is very thin, and in B. salmoneus it may often be seen fused with the hypoblast, forming openings which place the cavity of the liver diverticula into actual connection with the exterior. The histological appearances are such as to leave no doubt that an actual fusion occurs. When the extreme softness of the tissue is remembered, it seems likely that these perforations may, in the first instance, be due to wounds which have healed so as to form fistulæ. [In a single case of B. minutus a fistula of this kind was found forming a perforation from the intestine to the body cavity. In this animal the fusion between hypoblast and mesoblast was quite complete.]

The liver of B. salmoneus is dark green in colour, and this colour is due to minute round granules or drops in the hypoblast. In B. Robinii the tint is generally dark brown.

The histology of the intestine, which is usually more or less diamond-shape, two of the angles being dorsal and ventral, is in no way remarkable. From the first the wall is formed of a single layer of cells, ciliated, and smaller than those of the digestive region (*v.* fig. 83). The anus opens immediately above the tail until this structure disappears, and then it opens widely in a terminal position (*v.* figs. 83 and 6).

The Tail and Anal Lappets.

The tail is present in the period between one and eight pairs of gill-slits. Its skin is full of unicellular glands. The third pair of body cavities are prolonged into it, and the mesentery between them remains. The anal lappets (fig. 3, *a*) also disappear with the tail.

Mesoblastic Structures.

Muscles.—The muscle-fibres of the proboscis are not gathered into bundles. They consist of circular, radial, and longitudinal fibres. The circular fibres are few in number, and chiefly occur in the external parts of the middle third of the proboscis.

The radial fibres are very few in B. Kowalevskii, but in B. salmoneus and B. Robinii they are common, and have a very characteristic appearance (*v.* fig. 94, *a*). Their peripheral ends are very long and fine, occasionally branching. Their central ends taper suddenly from a thick part containing a nucleus to a very fine fibre. These fibres are always plain fibres. Probably the peripheral ends are inserted into the skin, and the central end into the meshes of connective tissue which permeate the body cavity (*v.* fig. 79).

The longitudinal fibres of B. Kowalevskii are arranged in concentric rings, and united to each other by a peculiar connective tissue, which contains stellate cells with large nuclei. These concentric rings seem to be more numerous in old than in young animals, reaching the observed maximum of eight. This concentric arrangement is not a distinct feature until adult life is nearly reached. These fibres appear in section to have the same structure as those shown in fig. 94, *b*, which is taken from B. Robinii. The muscles of B. Kowalevskii were unfortunately not examined in the fresh state.

In B. minutus the longitudinal muscles do not form such definite concentric rings as in B. Kowalevskii, but all the mesoblastic tissues filling the proboscis cavity are broken up in preserved specimens into radial segments. This is not the case in living B. Robinii, and hence is probably due to reagents in B. minutus; as, however, I have never had an opportunity of seeing the latter in the fresh state this cannot be affirmed.

In passing inwards from the outside to the centre of the proboscis the structures are thus arranged:

1. Ectoderm.—Ciliated tailed cells.
   Glandular cells.
   Nerve-fibres as a layer.
   Basement membrane.

2. Narrow tissue space crossed by ingoing fibres from ectoderm, and by supporting fibres in all directions, together with a very few circular fibres (*v.* fig. 51).

3. Tract densely filled with radial and longitudinal muscles (in B. Kowalevskii concentrically disposed in rings) and connective tissue.

4. The tissue space into which the central organs project.

5. The central organs:
   (*a*) Proboscis gland with its sac.
   (*b*) Heart.
   (*c*) Notochord.

The muscles of the collar body cavity in B. Kowalevskii are not gathered into bundles or definitely arranged, excepting those which are attached to the lateral rods of the axial skeleton (fig. 60). These large muscles are inserted into the back of the collar. The whole cavity between the pharynx and the skin, being originally second pair of mesoblastic pouches, becomes obliterated, being filled with muscles and connective tissue.

In B. salmoneus, B. Robinii, and B. Brooksii this also occurs, but in B. salmoneus (fig. 106) the longitudinal muscles are grouped into bundles. These bundles form two series, the one on the somatic and the other on the splanchnic side, and in the narrower parts of the cavity the groups of the two series dovetail into each other (fig. 106), being each gathered around a connective tissue septum projecting into the cavity.

These fibres in B. Robinii occasionally, after osmic acid, show a slight striping (fig. 94, *c*).

In B. minutus the longitudinal muscles of the collar lie in a layer immediately under the skin and under the pharyngeal wall. The cavity is crossed by many radial fibres, upon which some cells are placed, but is not so much filled up as in the other species.

The muscles of the third body cavity are not markedly different from those of the collar. In B. Kowalevskii alone a large muscular band runs along each side of the ventral nerve-cord, forming a projection from the body (*v.* fig. 108).

The perihæmal cavities are similarly almost filled with tissue, and always contain more or less longitudinal muscle-fibre. These are gathered into two bundles, and are inserted into the notochord sheath in the proboscis stalk. They are most developed in B. minutus, &c. (*v.* figs. 67 and 68).

The Mesenteries.—The dorsal mesentery persists throughout life in B. Kowalevskii and B. salmoneus. In the other species it disappears in the collar region. The ventral mesentery persists in the trunk in all species, but is always obliterated in the collar.

In B. minutus the body cavity of the trunk in the hepatic region is again divided in consequence of an attachment between the lateral angles of the diamond-shaped intestine to the body wall (*v.* fig. 93). In this position two large lateral vessels run.

As Spengel has stated, strands of connective tissue run in B. minutus from the body wall between the follicles of the ovaries, forming a sort of radial septa. These septa are probably not of morphological importance, beyond indicating the "accidental" way in which such septa may arise (cf. Polygordius, &c.).

All the body cavities are full of corpusculated fluid, as Spengel has observed. These corpuscles, when living, are full of bright granules and vacuoles, and exhibit amœboid movements.

The Proboscis Gland.—In B. Kowalevskii (fig. 47, *gls.*), at about the age of two gill-slits, a space appears in the proliferation of mesoblast lying dorsal to the anterior end of the notochord, when the latter is pushed forwards into the anterior body cavity. This space is the first rudiment of the sac of the proboscis gland. Soon after its appearance it becomes enclosed in a membrane, which is added first at the posterior part of the sac (cp. figs. 45, 31, and 47). Its cavity is therefore a tissue space arising in the wall of the body cavity,

and it is in communication with the body cavity by means of the interstices between the cells bounding its anterior end.

Its further development is involved with that of the heart which had better be now described. The heart arises in animals with three pairs of gill-slits, as a horizontal split in the tissue between the notochord and the sac of the proboscis gland. Its walls are very thin (*v.* fig. 52). From the first it appears to contain blood, which is apparently non corpusculated, and can be coagulated by reagents. Whether the heart is originally in connection with the dorsal vessel or not could not be determined. Its walls soon become slightly muscular (*v.* figs. 67 and 97), and the pulsations, which can be dimly discerned through the skin in the living state, are doubtless occurring in this vesicle.

After the formation of the heart a plexus of vessels in connection with it is formed among the mesoblastic cells covering the tip of the notochord (fig. 50). As this occurs the cells standing on the capillaries assume a pyriform shape, the sharp ends being fixed to the vessels and the wide ends free. These wide ends acquire a very transparent appearance, as though filled with fluid (fig. 49). These bunches of capillaries eventually acquire a great development and communicate with two larger blood-vessels (fig. 53, *b. v.*), and with a sinus in the periphery of the gland.

The sac of the proboscis gland anteriorly becomes filled up with a quantity of loose tissue, in which some granules of a yellowish colour are embedded.

In B. minutus these yellow granules are of much commoner occurrence (*v.* fig. 98). The capillaries of the gland are more regularly arranged.

In B. salmoneus the capillaries are still more regular, running parallel to each other to the periphery of the gland, where they are united in a plexus of larger vessels (cp., figs. 95—97). The outer cells of the gland are modified to form a peculiar tissue (fig. 97). They are large cells, which stain deeply and have a nucleus usually on their outline. The cells standing on the capillaries contain some yellow granules, and

larger granules or even masses of them are to be found in the spaces surrounding them.

The gland of the living B. salmoneus is light green in colour.

The nature of these glands is entirely obscure. These yellow granules occur amongst nearly all the mesoblastic tissues. In B. Robinii (collar) they may be found in the fresh state, presenting the appearance shown in fig. 100. They are never crystalline.

An attempt was made to investigate the chemical nature of these bodies, but with only negative results. They may, perhaps, be excretory, and it is possible that they are more or less removed by the proboscis pore and collar funnels respectively. This does not explain their presence in large masses in the trunk body cavity (*v.* fig. 93, *a*), from which no pore has been observed to open. Occasionally granules of this character occur in the ectodermic structures, suggesting that they are a product of the activity of all the tissues.

The proboscis pore was shown to arise at two gill-slits as a small vesicle in the skin of the proboscis stalk upon the left side (*v.* fig. 34); at three gill-slits it acquires an opening to the exterior, and at four gill-slits its tissue fuses with the lining of the left posterior horn of the anterior body cavity (*v.* fig. 99), placing this cavity in communication with the exterior.

In B. Kowalevskii this pore is permanently on the left side of the body; in B. minutus, &c., it is median.

The collar funnels arise as thickenings in the outer wall of the arterial cavity opposite the opening of the first gill-slit (*v.* fig. 101). These thickenings soon become perforated (8, *g. s.*). At their origin they are simple conical funnels, but they soon acquire a crescentic lumen owing to a thickened inward folding of their outer wall. This is not conspicuous in B. Kowalevskii (cp. figs. 88 and 104). Their histology is sufficiently indicated in the figures.

As previously mentioned, the blood-vessels consist of (1) a dorsal vessel leading from the heart to the tail; (2) a ventral vessel running from the back of the collar to the tail;

(3) in B. minutus a pair of large lateral vessels (*v.* fig. 93) in the digestive region. These are connected by plexuses in the skin and under the epithelium of the gut. In the operculum this capillary system of the skin forms a more or less definite circular vessel. In parts of their course these vessels are always more or less filled with a fibrous-looking substance, apparently cellular, which lines the walls (fig. 71). The generative organs lie in blood-sinuses derived from the subcutaneous plexus.

I stated (Part I) that the branchial blood-supply resembled that of Amphioxus. From further observation I have come to the conclusion that this is a mistake, and that the vessels supplying the gills are all derived from the dorsal vessel, as Spengel has stated, being, in fact, merely the skin capillaries of the dorso-lateral regions. The main vascular trunks are all formed from the mesoblast of the first cavity and of the third pair of cavities. The capillaries under the skin and round the gut are formed in sitû in the mesoblastic walls in which they occur.

The Generative Organs.

The Ovaries.—The animals are all diœcious. The origin of the ovaries is not certain, but there is very strong evidence that they are epiblastic. At all events, from almost their earliest appearance, they are connected with the skin in the dorso-lateral regions (*ov.* fig. 110). It is almost impossible to believe that an attachment of this kind is secondary, and I have never seen an ovarian follicle entirely separate in the body cavity.

Soon after its appearance it consists of a mass of loose round cells. A cavity next appears in its interior, as though due to a disintegration, and after the appearance of this cavity the cells bounding it develope into ova (figs. 111 and 112).

The egg-shell appears soon as a close-fitting membrane. The germinal spot is enclosed in a remarkably tough membrane in all the species examined. Though the ovaries are connected

with the skin by ducts the ova are dehisced by the breaking away of whole follicles, which then disintegrate. In the branchial region of B. minutus there is a general correspondence between these ducts and the gill-slits, as Spengel has observed.

The testes are lobed masses placed in the same situation as the ovaries. The outer zone of each testicular follicle is made up of spherical cells (figs. 108 and 109, *a*), which contain several (? eight) deeply-stained dots. These cells are young spermatoblasts, and the dots, which increase in size in the spermatoblasts of the inner zone, are the heads of spermatozoa which are finally set free into the central cavity. Here they are arranged in curious strings, which wave above parallel to each other in preserved specimens (fig. 108). The testes, when mature, break up in B. Kowalevskii as masses, but in B. Robinii they exude from the skin as a yellow slime.

Mucus.—All the species secrete vast quantities of mucus when irritated. That of B. Robinii sets to form a mass of tough consistency, which collecting grains of sand forms a sort of tube. In this the animal can move slightly. The body of this species is very flat in the generative region, and is naturally folded up dorsalwards within the tube. The mucus of this form, which comes out after prolonged irritation, turns to a reddish-violet colour on exposure to the air, which is very characteristic.

In B. Brooksii, Robinii, and salmoneus the sides of the body are produced dorsalwards into flaps which nearly meet in the branchial region, and thus cover the gill-slits and dorsal nervous system.

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EXPLANATION OF PLATES I—VI,

Illustrating Part I of Mr. Bateson's Memoir on the "Morphology of the Enteropneusta."

Complete List of Reference Letters.

a. Anus. *Ac.* Archenteron. *Al.* Alimentary canal. *a. l.* Anal lappets. *bc.*[1], [2], [3]. The anterior, middle, and posterior body cavities respectively. The letters *l* and *r* affixed to these letterings denote that the parts are of the left or right side. *br.* Branchial chamber. *C.* Apical tuft of cilia. *C. N. S.* Central nervous system. *c. g.* Groove between the collar and the trunk. *cil.* Transverse band of cilia. *Circ.* Circular muscle-fibres. *Cl.* Collar. *Cl'.* The posterior fold of the collar, which eventually forms the operculum. *Cl. sk.* Skin of collar. *D. b. v.* Dorsal blood-vessel. *D. mes.* Dorsal mesentery. *D. n. s.* Dorsal nervous system. *dig.* Digestive tract of alimentary canal. *E.* Ectoderm. *ex. g.* Refractive granules in mesoblastic cells. *f.* Mesoblastic fibres. *g. s.* Gill-slit. *g. s. r.* Branchial supporting rod. *gl.* Proboscis gland. *gl. s.* Sac of proboscis gland. *gn.* Ganglion (?) cells. *H.* Hypoblast. *ht.* Heart. *Int.* Intestine. *l. r.* Lateral rods of the skeleton. *lu.* Lumen of notochord. *M'. M''. M'''.* Mesoblast derived from the anterior, middle, and posterior pouches respectively. *Mo.* Mouth. *msc.* Muscle-fibres. *mu.* Mucous gland. *n. s.* Nervous system. *n. cnl.* Neural canal. *Nc. pr.* Pore by which the notochord lumen opens into the pharynx. *Nch.* Notochord. *N. pr.* Neural pore. *Op.* Operculum. *pkt.* Fibrous substance of the nervous system. *P. pr.* Proboscis pore. *P. h. b. c.* Perihæmal body cavity. *P. sk.* Skin of proboscis. *Sep.* Septum between the horns of the anterior body cavity. *Sh.* Sheath of notochord. *Skr.* Sucker. *Sk.* Skin. *S. r.* Supporting rod. *Sp.* Tissue-space in the proboscis cavity. *ts.* Testis. *V. vs.* Ventral vessel. *V. n. s.* Ventral nervous system. *Vlv.* Valve of gill-slit. *x.* Pyriform cells of splanchnopleure of middle body cavity.

With the exception of Figs. 5 and 6, the outlines were all drawn with Zeiss's camera lucida. I have to thank Mr. Edwin Wilson of the Lithographic Department of the Cambridge Scientific Instrument Company for drawing for me two beautiful figures (Figs. 5 and 6) of the whole animal from preserved specimens, and also for Figs. 1, 2, 3, and 4, which he has prepared for me from my own sketches, the original outlines of which were traced from living specimens.

Fig. 1.—Whole animal seen from the side, immediately after the appearance of the second pair of gill-slits. (Obj. A, oc. 2.)

FIG. 2.—Similar view of an older animal, with two gill-slits. (Obj. A, oc. 2.)

FIG. 3.—Similar view of a three-gill-slit larva. (Obj. A, oc. 2.)

FIG. 3*a*.—Posterior end of the same in a retracted state, seen in profile (from a preserved specimen). (Obj. A, oc. 2.)

FIG. 4.—Side view of larva with five pairs of gill-slits. The fold of the operculum covering part of the first gill-slit is semi-transparent. (Obj. A, oc. 2.)

FIG. 5.—Side view of preserved specimen with nine pairs of gill-slits Owing to the contraction of the body and the protrusion of some of the valves, few only of these are visible. (Obj. AA, oc. 2.)

FIG. 6.—The adult animal (♂). (× 2 diameters.)

FIG. 7.—Longitudinal vertical section (not quite median) of a larva in Stage F. (Obj. C, oc. 2.)

FIG. 8.—Cells of mesoblast of anterior pouch, from a transverse section of a larva shortly after this pouch is closed off from the hypoblast. (Obj. F, oc. 2.)

FIG. 9.—The same tissue from the posterior third of the proboscis of a rather older larva, showing the proliferation and commencing differentiation of the mesoblastic elements. (Obj. D, oc. 2.)

FIG. 10.—Section similar to Fig. 7, from a rather older larva to show anterior, dorsal structures (Stage G—H). (Obj. D, oc. 2.)

Figs. 11—20 represent transverse sections of a larva in Stage G—H, with the exception of Figs. 12 and 16, which were drawn under Obj. F, oc. 2. All these figures were drawn under Obj. CC, oc. 2. In most of these figures the ectoderm is only indicated on a short arc of the circle of the whole section. They are numbered from before backwards.

FIG. 11.—Transverse section of proboscis cavity. Mesoblastic elements distributed nearly uniformly all round the interior.

FIG. 12.—Portion of these mesoblastic elements more highly magnified, to exhibit the differentiations.

FIG. 13.—Section taken behind Fig. 11. The mesoblastic layer is thinner dorsally than elsewhere.

FIG. 14.—In this section the septum separating the two horns of the cavity is reached.

FIG. 15.—Still further back the first rudiment of the proboscis gland is reached (cp. Fig. 29).

FIG. 16.—The rudiment of the gland lying in the septum more highly magnified.

FIG. 17.—Section across the proboscis stalk. The anterior end of the notochord is reached. The back of the gland is nearly passed. The mesoblastic horn of the left side is apparently divided into two parts, this appearance is due to shrinking.

FIG. 18.—The lumen of the notochord is reached. The extreme ends of the two anterior mesoblastic horns and the posterior apex of the left horn from

the first cavity are all cut in this section. (The irregular folds of skin are due to the contractions of the body; it will be understood by comparing the figures of the whole animal that only the central part within the folds is the real stalk of the proboscis.)

Fig. 19.—The section crosses the end of the archenteron, lying in front of the mouth. (The whole of this part of the archenteron becomes eventually pushed forward to form the notochord.)

Fig. 20.—The mouth is here traversed, as also the anterior end of the nervous system. (A space, due probably to shrinking, is visible in the dorsal mesentery.)

Figs. 21—29 are transverse sections of a larva, slightly older than the foregoing. Fig. 25 was drawn under Zeiss's Immersion 2 and oc. 2; the others were drawn under Obj. D and oc. 2. They are numbered from before backwards.

Fig. 21.—Section taken just behind the mouth. The lumen of the notochord is here shut off from the archenteron.

Fig. 22.—The notochord still open to the archenteron.

Fig. 23.—The nervous system is attached to the skin.

Fig. 24.—The nervous system is already nearly separated from the skin.

Fig. 25.—Part of the foregoing enlarged, to show the peculiar pyriform cells of the splanchnopleure (x).

Fig. 26.—Nervous system still in the skin.

Fig. 27.—The nerve-cord is separated from the skin.

Fig. 28.—Two parts of the middle body cavities may be here seen separating from the rest, probably forming part of the perihæmal cavities.

Fig. 29.—The anterior ends of the third pair of body cavities are here cut as a solid mass of mesoblast on each side.

Fig. 30.—A longitudinal vertical, nearly median, section of a larva, in the same stage as that shown in Figs. 21—29. The differentiation of the walls of the digestive tract may be here seen.

Figs. 31—44 are from transverse sections of a larva which has just acquired the second pair of gill-slits. They are numbered from before backwards.

Fig. 31.—Transverse section of the proboscis cavity. The loose tissue in the sac of the gland is shown. The membrane which is deposited round it is visible (cp. Fig. 47). (Obj. D, oc. 2.)

Fig. 32.—Small portion of skin and mesoblast on a larger scale, to show structure of the nervous layer. (Obj. F, oc. 2.)

Fig. 33.—Region behind that shown in Fig. 31. (Obj. D, oc. 2.)

Fig. 34.—Through the proboscis stalk. The anterior horns of the middle body cavities are here cut.

Fig. 35.—Through the proboscis stalk and the anterior phlange of the collar which forms the lower lip.

Fig. 36.—Through the anterior end of the nervous system (in the region of the mouth), showing the manner in which the lumen arises in the nerve-cord.

Fig. 37.—Through the anterior part of the collar, showing the nearly complete separation of the notochord with the hypoblast.

Fig. 38.—Section taken behind the previous one. The nervous system is here separated from the skin.

Fig. 39. Through the junction of the lumen of the notochord with that of the gut. The skeletal rods, which here bend downwards, backwards, and then slightly forwards, are therefore cut twice on each side.

Fig. 40 is taken rather in front of the branchial sacs; it shows the two anterior horns of the posterior body cavities, which form the perihæmal cavities.

Fig. 41.—Section through the gill-sacs in front of the clefts. The nervous system here is fused to the skin dorsally.

Fig. 42.—Through the extreme posterior end of the second body cavities, and the anterior end of the ventral blood-vessel and nervous cord.

Fig. 43.—(Section not quite transverse.) Through the left gill-slit.

Fig. 44.—Section taken through the extreme posterior end of the branchial region of the gut, showing how this overlaps the digestive region. The dilatation in the sides of the branchial region here shown are parts of the second pair of gill-slits.

Fig. 45.—A longitudinal vertical section of the whole animal. (Two gill-slits.) (The section is not truly vertical, as it cuts the gill-slit.) It exhibits the relation of the notochord and other parts. (Obj. CC, oc. 2.)

Fig. 46.—By an oversight no figure bearing this number appears in the plates. The figure referred to as such in the text at pages 85, 98, and 103, is Fig. 45.

Fig. 47.—A transverse section of the extreme tip of the notochord, &c., in the larva from which Figs. 31—45 were taken. It shows the histology of the notochord and the arrangement upon it of the mesoblastic tissues, which are pushed in by it. (Obj. D, oc. 2.)

Fig. 48.—Transverse section across the proboscis pore of a larva with three gill-slits. The two skeletal rods are here fused and have attained a considerable size. (Obj. F, oc. 2.)

Figs. 49—53 represent transverse sections taken from B. Kowalevskii, at the stage of four pairs of gill-slits. (When the remaining structures are dealt with a fuller explanation will be given of these and of the subsequent figures; on the present occasion they are only introduced to explain the account of the notochord given in the text.) Figs. 49—53 are numbered from before backwards.

Figs. 49 and 50 illustrate the histology of the anterior end of the notochord. (Obj. F, oc. 2.) These sections are in front of the heart.

Fig. 51 shows a section of the whole proboscis, to illustrate the relations of the parts. The body cavity may be seen to be nearly filled up with muscle-fibres and connective tissue, with the exception of a small central space, in which lies the notochord bearing the central mesoblastic structures, viz. the heart and proboscis gland with its sac. (Obj. C, oc. 2.)

Fig. 52 exhibits the central structures of Fig. 51. (Obj. F, oc. 2.)

Fig. 53.—Here the lumen of the notochord is reached. At this point the sac of the proboscis gland is attached dorsally to the skin of the proboscis. (Obj. F, oc. 2.)

Fig. 54.—Transverse section of proboscis stalk of an older animal, taken behind the epiblast sac of the proboscis pore, the back of which appears in section. The skeletal rod has still in this region two central "cores." (Obj. D, oc. 2.)

Fig. 55.—A transverse section of the notochord of an adult B. Kowalevskii, taken in front of its lumen (cp. Fig. 57). (Obj. CC, oc. 2.)

Fig. 56.—Transverse section of the same as foregoing, through the dilated lumen in front of the skeletal rod.

Fig. 57.—Longitudinal vertical median section of the back of the proboscis and the front of the collar of B. Kowalevskii, to show the relation of the notochord. The other parts are diagrammatic. (Obj. A, oc. 2.)

Fig. 58.—Transverse section of the junction of the proboscis stalk with the collar. (Semi-diagrammatic.) Exhibits relations of the notochord and its sheath to the skeletal rods, nervous system, &c. (Obj. A, oc. 2.)

Fig. 59.—A diagram of a transverse section taken through the anterior region of the collar, to show the relation of the parts figured in Fig. 60. (Obj. A, oc. 2.)

Fig. 60.—The dorsal structures of the anterior part of the collar shown in Fig. 59, viz. the nervous system, notochord, perihæmal cavities, &c. (The gut is torn away from the skeletal rod, presumably by shrinking.) The lateral rods are here connected to the notochordal sheath. (Obj. D, oc. 2.)

Figs. 61—63 show transverse sections of parts of B. minutus.

Fig. 61.—The notochord in transverse section in front of its lumen. (Obj. CC, oc. 2.)

Fig. 62.—The same in the region of the proboscis stalk.

Fig. 63.—Through the notochord, &c., in the front of the collar, showing the thickened dorsal half of the organ. (Obj. CC, oc. 2.)

EXPLANATION OF PLATES VII—XII.

Figs. 64—112,

Illustrating Part II of Mr. Bateson's Paper on "The Morphology of the Enteropneusta."

Complete List of Reference Letters.

a. Anus. *al.* Alimentary canal. *at.* Atrial cavity. *bc.* ^{1}, ^{2}, ^{3}. The anterior, middle, and posterior body cavities respectively. *bg.* Rods bordering the gill-slits. *br. cls.* Border cells of proboscis gland (B. salmoneus). *b. v.* Blood-vessel. *C. N. S.* Central nervous system (*i.e.* the cord of the collar region). *Cap.* Capillaries of proboscis gland. *Circ.* Circular muscle-fibre. *Cl. f.* Collar funnel. *C. rg.* Ring of nervous tissue round the collar. *D. b. v.* Dorsal blood-vessel. *D. mes.* Dorsal mesentery. *D. n. s.* Dorsal nervous cord. *D. r.* Cords connecting central nervous system with the skin. *D. rdg.* Dorsal ridge of hypoblast in branchial region. *dig.* Digestive region of alimentary canal. *E.* Ectoderm. *fl.* Fold in wall of collar funnels. *g. s.* Gill-slit. *g. s.* ^{1}, ^{2}. First and second gill-slits respectively. *g. sc.* Lining of gill-sac. *g. sr.* Supporting rods of gills. *g. vs.* Germinal vesicle. *g. sp.* Germinal spot. *gl.* Proboscis gland. *gl. s.* Sac of proboscis gland. *gnl.* Granules in central nervous system of B. salmoneus. *gr.* Granules, probably excretory. *ht.* Heart. *int.* Intestine. *l. b. v.* Lateral blood-vessel. *l. rdg.* Lateral ridges separating the branchial chamber from the lower cavity of the gut in the branchial region. *l. msc.* Longitudinal muscle-fibres. *Lv.* Liver. *m. spz.* Spermatoblast cells. *Mo.* Mouth. *msc.* Muscle-fibres. *mu.* Mucous glands of skin. *mu'.* Goblet cells of skin. *mu''.* Long glands of collar skin of B Kowalevskii. *n. cnl.* Neural canal. *Nch.* Notochord. *N. pr.* Neural pore. *n. sh.* Nervous sheath of proboscis. *O.* Opening of collar pores. *Op.* Operculum. *ov.?* Ingrowth of skin, probably an ovary. *ov.* Ovarian follicle. *ph.* Pharyngeal region of gut with thick walls. *per.* Perforation into liver saccule. *pkt.* Fibrous substance of the nervous system. *P. rg.* Ring of nervous tissue round proboscis. *ph. c.* Perihæmal body cavity. *Scl.* Liver saccule. *Sf.* Surface of skin with anastomoses of ectoderm cells. *Sk.* Skin. *Skr.* Sucker. *S. r.* Supporting rod of notochord. *Sp. vlv.* Spiral fold in wall of gut in the digestive region. *St.* Stripes occasionally seen in preserved muscle-fibres. *Stel.* Stellate masses of cells in central nervous system. *t. pr.* Tube of proboscis pore. *ts.* Testis. *V. bd.* Ventral band of longitudinal muscle of B. Kowalevskii. *V. b. v.* Ventral blood-vessel.

V. g. Nervous concentration in the line of the gill-slits. *V. msc.* Ventral muscles. *Vlv.* Valve of gill-slit. *V. n. s.* Ventral nervous cord.

Fig. 64.—Diagrammatic longitudinal vertical section of B. minutus, to show the arrangement of the nervous system. [The openings of the gill-slits are indicated, though of course not visible in a section of this kind.]

Fig. 65.—Diagram of nervous system of B. Kowalevskii as seen from the dorsal surface. The ventral cord and the ring round the pharynx are indicated in broken lines. The sheath of nervous tissue covering the proboscis is indicated by shading, as though the tissues were transparent. The gill-slits are shown on one side only.

Figs. 66—73 illustrate the structure of the skin and nervous tissues of B. minutus.

Fig. 66. Nearly median longitudinal vertical section of the middle third of the central nervous system, showing origin of two of the cords connecting central nervous system with the skin. Their union with the skin is not here shown. (*v.* Fig. 68.) Obj. A, long tube, oc. 2.

Fig. 67. Longitudinal vertical section through the side of the central nervous system, showing the relation of the neural and proboscis pores to each other, &c. The wall of the heart is cut in this section. As the section is taken through the side of the central nervous system its continuation into the dorsal nerve-cord is not visible. Obj. A, oc. 2.

Fig. 68. Transverse section of the central nervous system at end of neural tube. Obj. A, long tube, oc. 2.

Fig. 69. Transverse section of the central nervous system behind the neural tube, showing attachment of dorsal cord to the skin. Obj. A, oc. 2.

Fig. 70. Longitudinal section of the anterior end of the ventral nerve-cord. Obj. D, oc. 2.

Fig. 71. Transverse section of ventral nerve-cord. Obj. A, oc. 2.

Fig. 72. Longitudinal section of skin in lateral region. Obj. A, oc. 2.

Fig. 72 A. Transverse section of skin in the space between the gill-slits. Obj. D, oc. 2.

Fig. 73. Longitudinal horizontal section through the back of the collar, showing the relations of the peripharyngeal nerve-ring.

Fig. 74 (*a*, *b*, *c*).—Three sections taken through the anterior, middle, and posterior thirds respectively of the central nervous system of B. salmoneus.

Fig. 75.—Section of a wrinkle of the skin of the middle third of the proboscis of B. salmoneus. Obj. D, oc. 2.

Fig. 76.—Teased out osmic acid preparation of the skin of the collar of B. Robinii. The cells remain attached to each other by their heads. The

network, *sf.*, is formed superficially by the anastomosing heads, each of the nodes being the head of a cell. Obj. D, oc. 2.

Fig. 77 (*a* and *b*).—Cells of preparation similar to Fig. 76, more separated. (*c*) Spindle-shaped cells from lower layer of skin, probably broken off from cells resembling *a* and *b*. Obj. F, oc. 2.

Fig. 78.—Transverse section through middle third of the central nervous system of B. Kowalevskii. Obj. D, oc. 2.

Fig. 79.—Longitudinal section of skin of posterior third of proboscis of B. Kowalevskii. Obj. D, oc. 2.

Fig. 80.—Horizontal section through the skin of the collar of B. Kowalevskii. Obj. D, oc. 2.

Fig. 81.—Vertical section of the above. Obj. D, oc. 2.

Fig. 82.—Section taken tangentially to the flexure of the body of young B. Kowalevskii (8, *g. s.*), showing the spinal folding in the digestive region of the gut. Obj. B, oc. 2.

Fig. 83.—Longitudinal section of the tail of young B. Kowalevskii (4, *g. s.*). Obj. D, oc. 2.

Fig. 84.—Longitudinal section through the wall of the posterior region of the branchial sac, showing the relations of the valves and skeletons of the gills (B. Kowalevskii, 10, *g. s.*). Obj. A, oc. 2.

Fig. 85.—Diagrams of successive stages in the development of the gill-slits of B. Kowalevskii.

Fig. 86.—Macerated preparation of the gill-skeleton of B. Kowalevskii. Obj. A, oc. 2.

Fig. 87.—Longitudinal section of adjacent valve and gill-bar of B. Kowalevskii. Obj. D, oc. 2.

Fig. 88.—Longitudinal horizontal section through atrial cavity of B. Kowalevskii in the plane of the opening into it of the collar funnel and first gill-slit. Obj. B, oc. 2.

Fig. 89.—Transverse section through the back of the branchial sac of B. Kowalevskii (10, *g. s.*). Obj. A, oc. 2.

Fig. 90.—Vertical section of pharyngeal wall of B. minutus. Obj. D, oc. 2.

Fig. 91.—Vertical section of one of the lateral ridges, separating the branchial sac from the lower part of the branchial region. Obj. A, oc. 2.

Fig. 92.—(*a*) Transverse section of the dorsal ridge of the branchial region of B. Kowalevskii. (*b*) The same of B. minutus. Obj. A, oc. 2.

Fig. 93.—Transverse section through the junction of a liver saccule with the gut, through the back of the adjacent saccule (B. minutus). Obj. A, oc. 2.

Fig. 93 A.—Longitudinal section of some liver saccules of B. salmoneus One of these is perforated at its end. Obj. A, oc. 2.

Fig. 94.—Muscle-fibres of B. Robinii (osmic acid preparations). Obj. F, oc. 2. (*a*) Three isolated radial muscle-fibres from the proboscis cavity. (*b*) Two adjacent fibres belonging to the longitudinal system of the collar. (*c*) Two fibres from the same region as (*b*), which on treatment with osmic acid show an appearance of striping.

Fig. 95.—Section of the central part of the proboscis gland of B. salmoneus, anterior to the notochord. Obj. D, oc. 2.

Fig. 96.—Outer part of the proboscis gland of B. salmoneus, anterior to the notochord, to show the arrangement of the border cells (*br. cls.*). Obj. C C, oc. 2.

Fig. 97.—A radial segment of the proboscis gland of B. salmoneus in the region of the notochord. (For the relations of this tissue *vide* "Later Stages," &c., Figs. 51 and 52.) Obj. D, oc. 2.

Fig. 98.—Group of cells from the interior of the proboscis sac of B. minutus. Obj. D, oc. 2.

Fig. 99.—Longitudinal vertical section through the left side of the proboscis stalk of B. Kowalevskii (4, *g. s.*), to show the internal opening of the tube of the proboscis pore. Obj. D, oc. 2.

Fig. 100.—Concretions from the living mesoblastic tissues of the 2nd body cavity of B. Robinii. Obj. D, oc. 2.

Fig. 101.—Transverse section through the collar funnels and first gill-slit of B. Kowalevskii (10, *g. s.*). Obj. A, oc. 2.

Fig. 102.—Transverse section through the collar funnels and upper end of the atrial cavity of B. Kowalevskii (young adult). Obj. B, oc. 2.

Fig. 103.—Transverse section of B. minutus, passing through the internal opening of one of the collar funnels. (Hypoblastic structures indicated roughly.) Obj. A, oc. 2.

Fig. 104.—Longitudinal horizontal section of collar funnel of B. minutus at the level of the opening of the first gill-slit. Obj. D, oc. 2.

Fig. 105.—Transverse section through middle of collar funnel of B. salmoneus. Obj. B, oc. 2.

Fig. 106.—Transverse section behind Fig. 105. Obj. A, oc. 2.

Fig. 107.—Transverse section through external opening of the collar funnel of B. salmoneus. Obj. A, oc. 2.

Fig. 108.—Half diagrammatic transverse section of generative region of male B. Kowalevskii.

Fig. 109.—(*a*) Spermatoblast cells, forming the outer zone of the testicular follicle. (*b*) Spermatoblast cells, forming the inner zone of the testicular follicle. (*c*) Spermatozoa in the interior of the follicle. Obj. F, oc. 2.

FIG. 110.—Transverse section of young ovary of B. Kowalevskii (young adult). Obj. D, oc. 2.

FIG. 111.—Group of ovarian follicles of B. Kowalevskii, older than the above. Obj. D, oc. 2.

FIG. 112.—Ripe ovarian follicle of B. Kowalevskii. Obj. A, oc. 2.

PLATE XII.

Diagrams.—Skin coloured light blue; nervous system, dark blue; hypoblast, light red; blood-vessels, dark red; mesoblast, green.

FIG. 1.—Blastosphere.

FIG. 2.—Gastrula.

FIG. 3.—Longitudinal vertical section through gastrula; blastopore being nearly closed.

FIG. 4.—Ditto; blastopore closed.

FIG. 5.—Ditto, later stage; mesoblast forming.

FIG. 6.—Longitudinal horizontal section through a somewhat later stage.

FIG. 7.—Longitudinal horizontal section through larva in Stage G.

FIG. 8.—Transverse section of collar of foregoing in plane of line *d d*.

FIG. 9.—Longitudinal vertical section of Stage H.

FIG. 10.—Transverse section of collar of foregoing in plane of line *d d*.

FIG. 11.—Longitudinal horizontal section of adult in plane of heart.. (This plane would not really take in the perihæmal cavities, but their relations are thus made clear.)

FIG. 12.—Transverse section of foregoing in plane of line *d d*.

FIG. 13.—Longitudinal horizontal section of junction of collar and trunk in a larva of about 4 gill-slits.

FIG. 14.—Similar section to foregoing of adult, showing formation of operculum, atrial cavity, and collar funnels.

Fig. 1

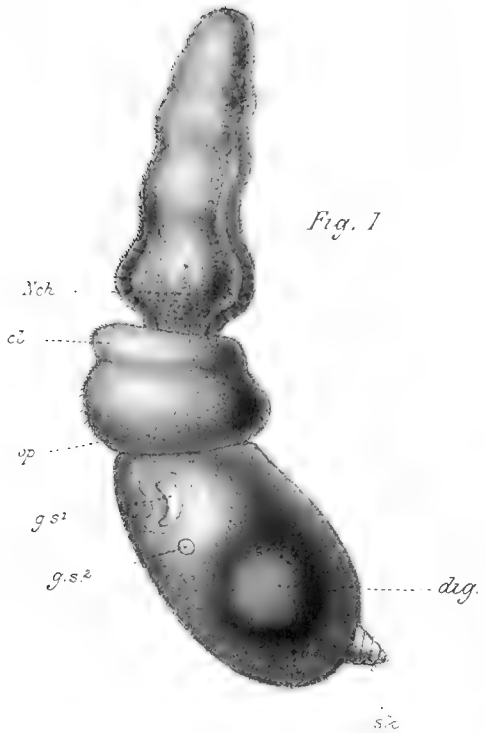
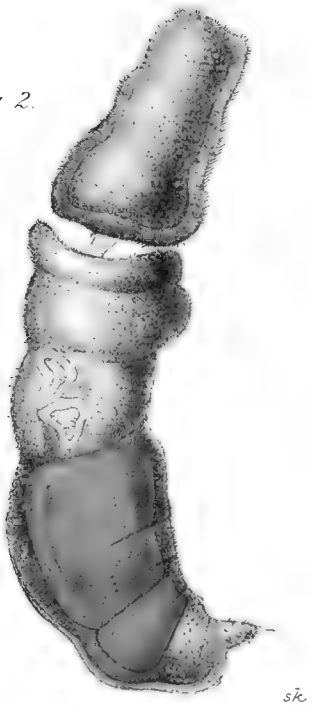


Fig 2.



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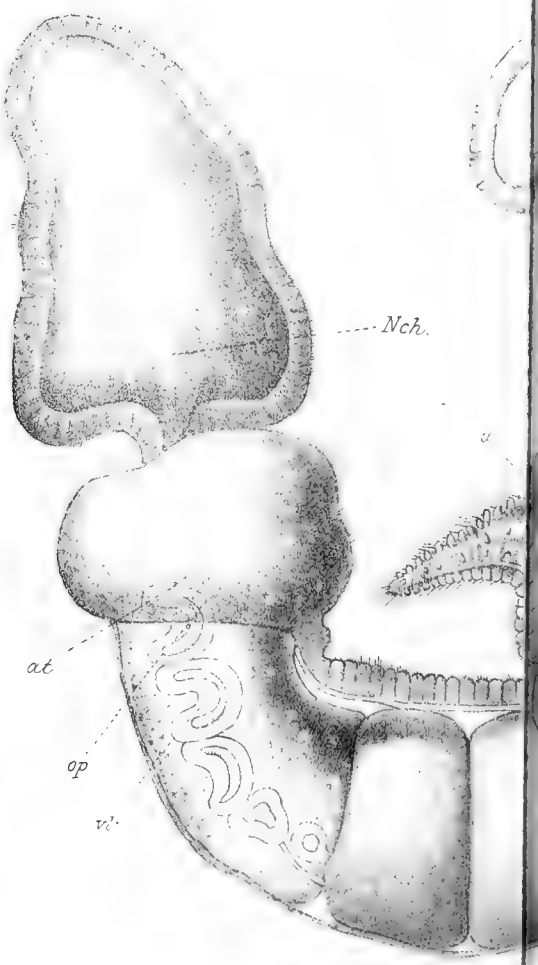

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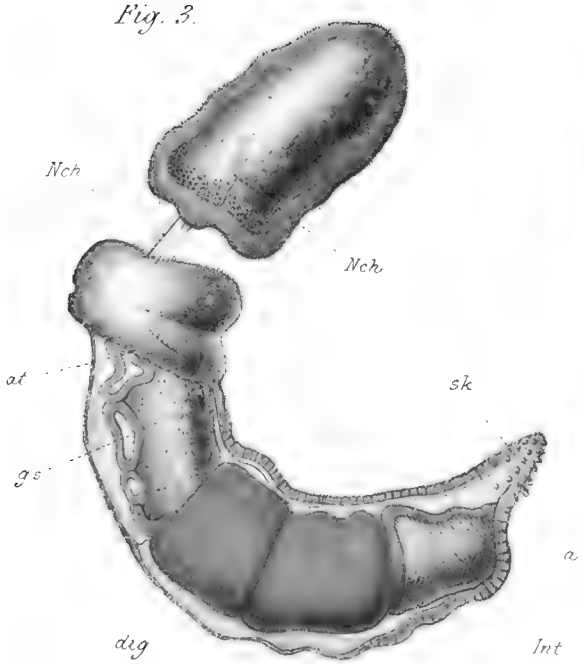

Fig. 3 a

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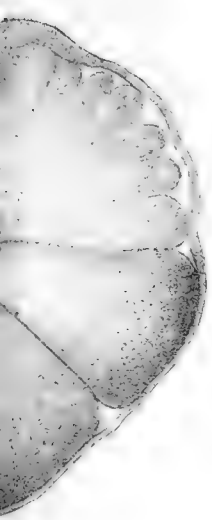

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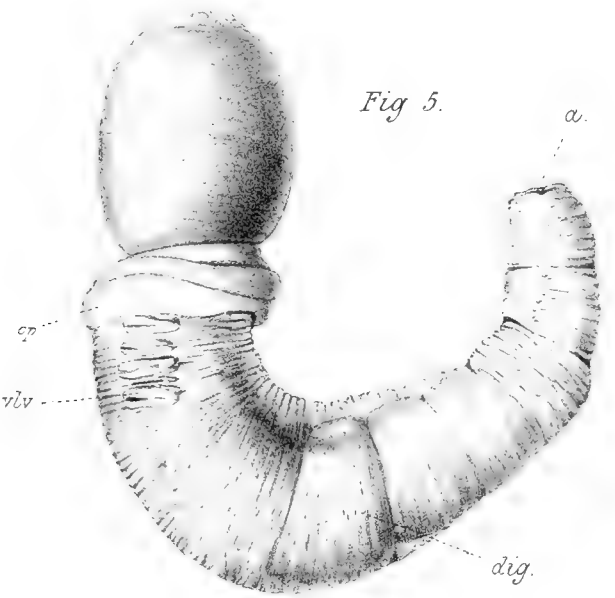

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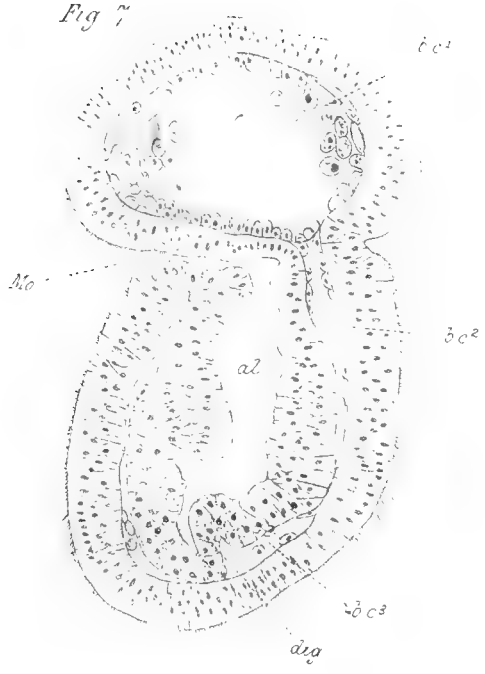

Fig 8.

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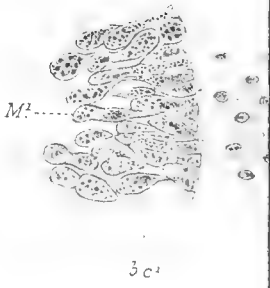

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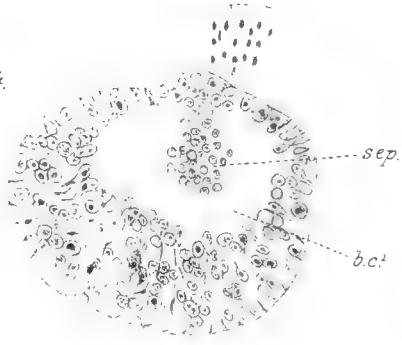

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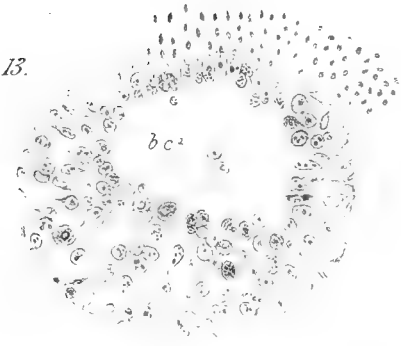

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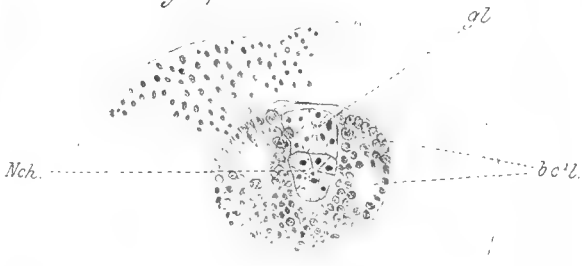

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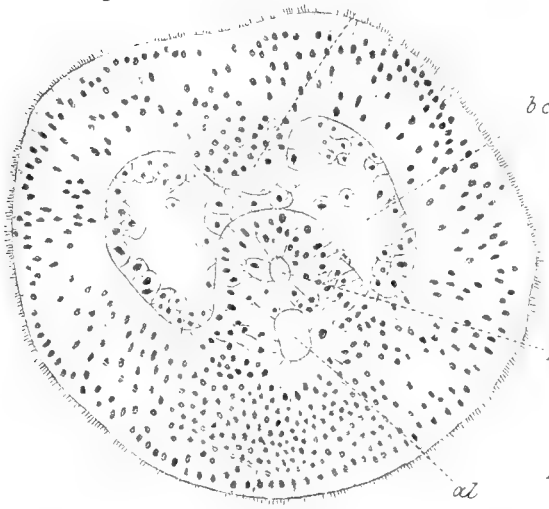

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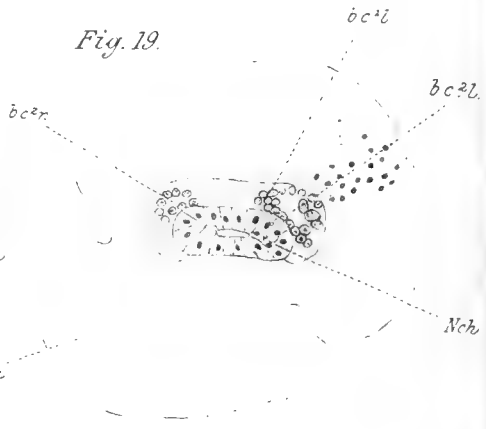
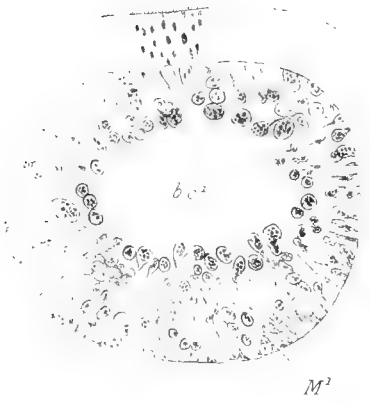


Fig. 10.

Fig. 11.



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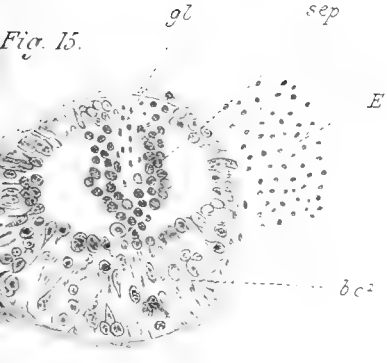

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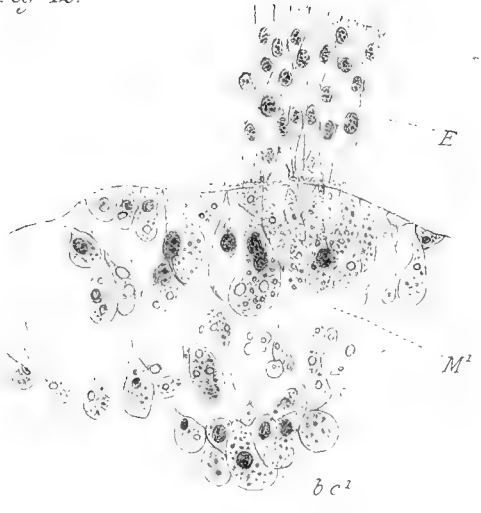

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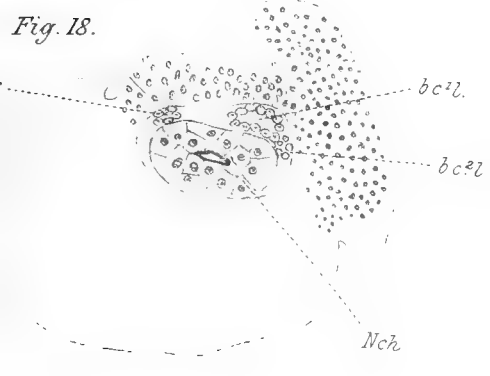

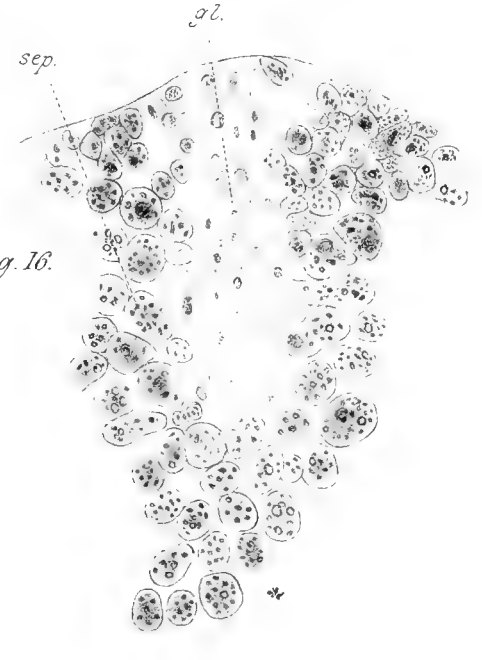

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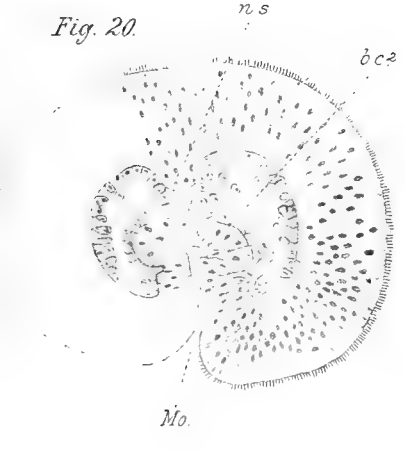

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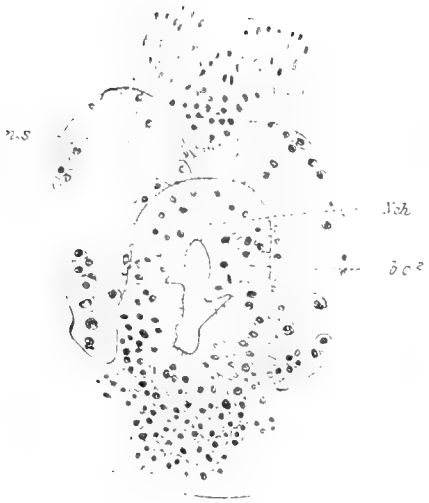

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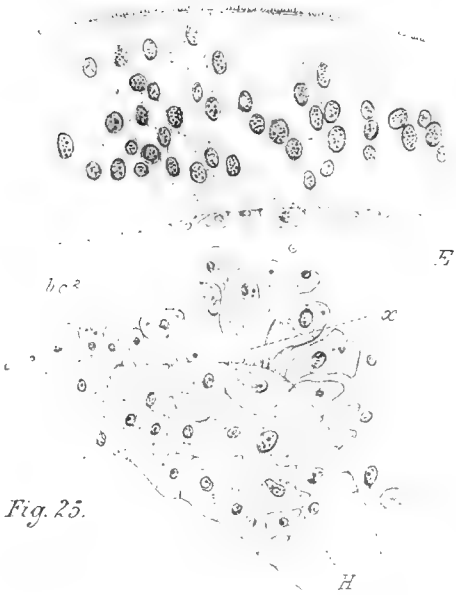

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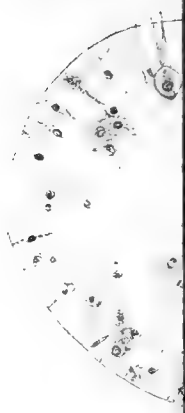

Fig. 29.

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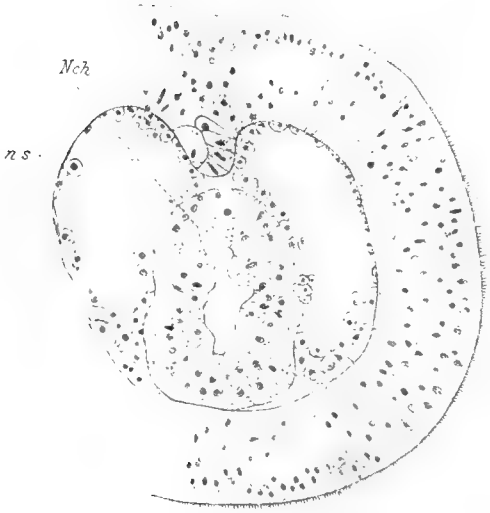

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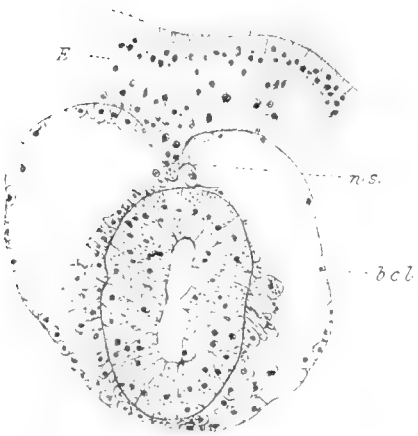

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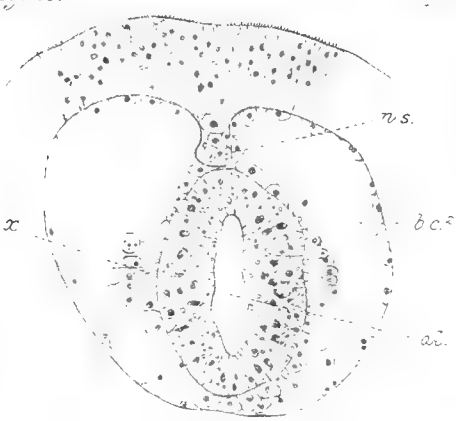

Fig. 27.

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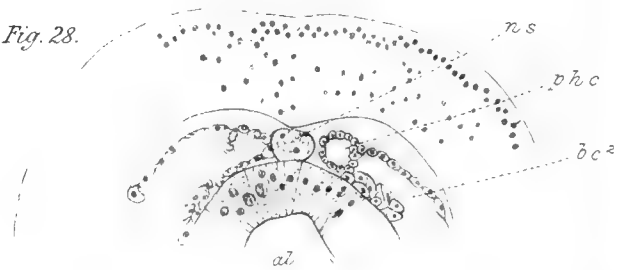

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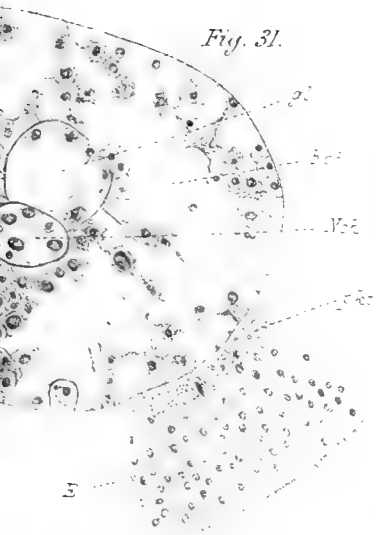

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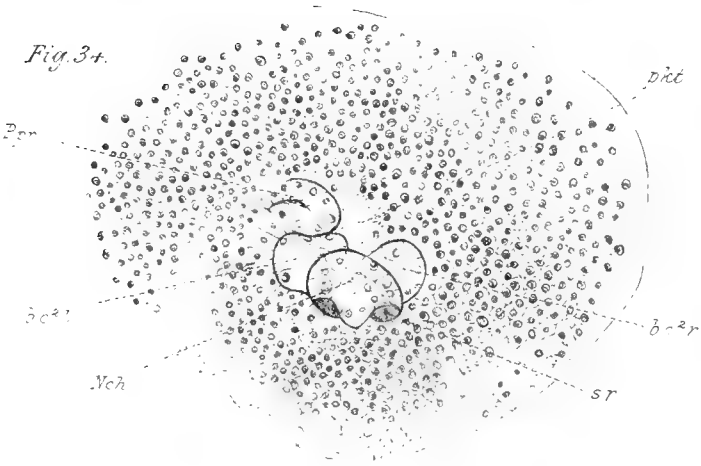

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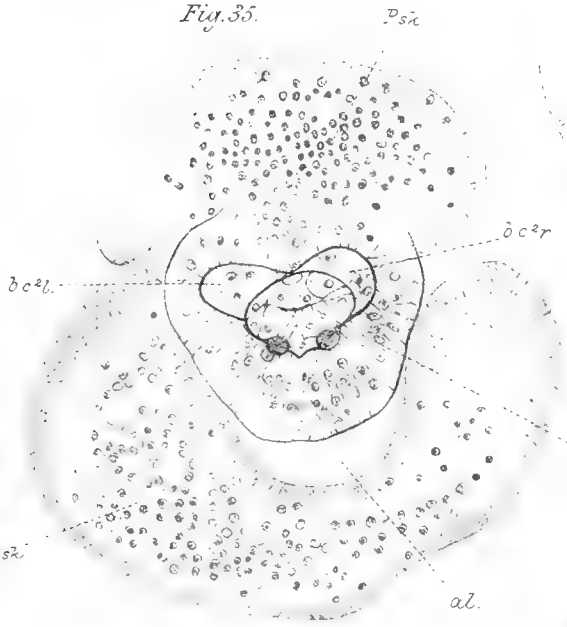

Fig. 32.

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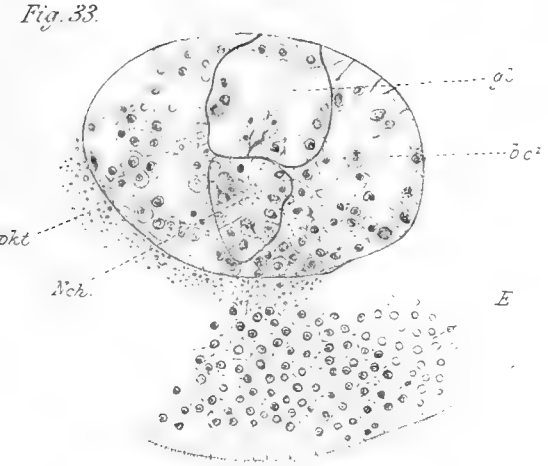

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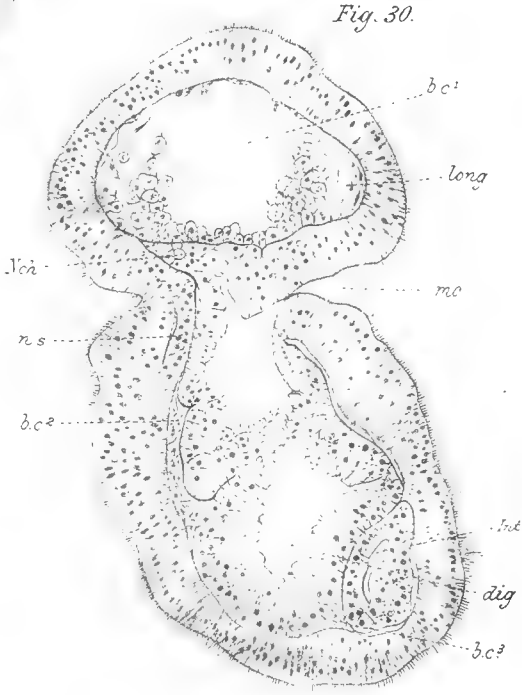

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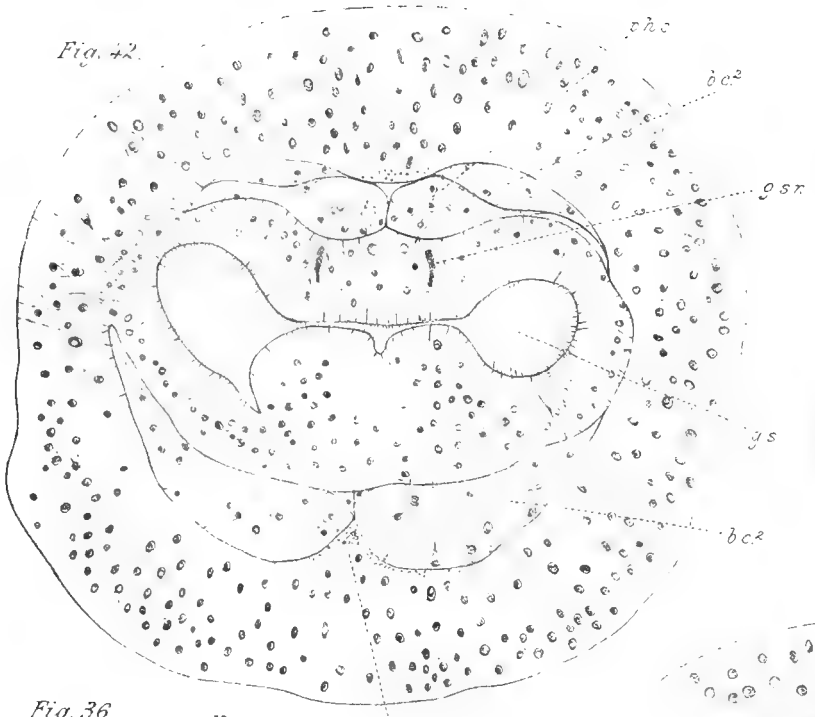

Fig. 39.

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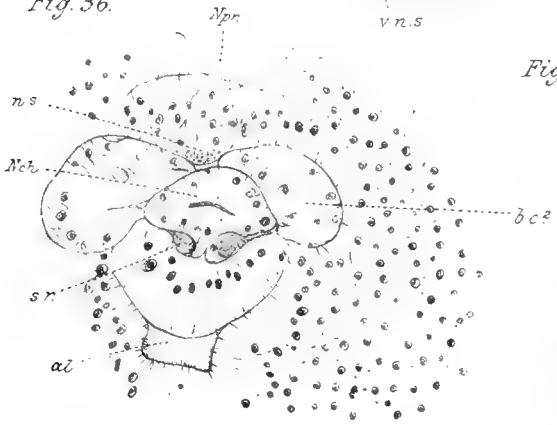

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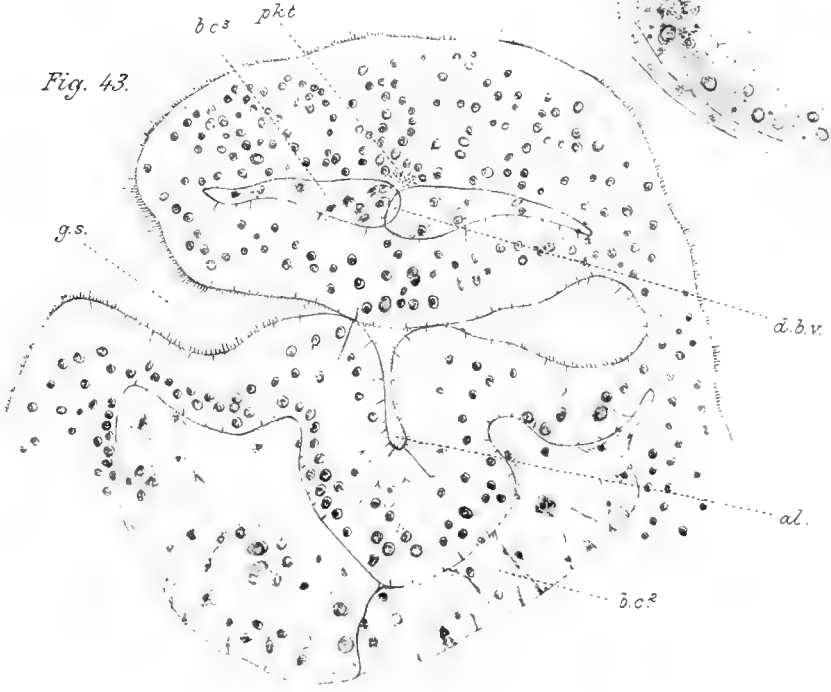

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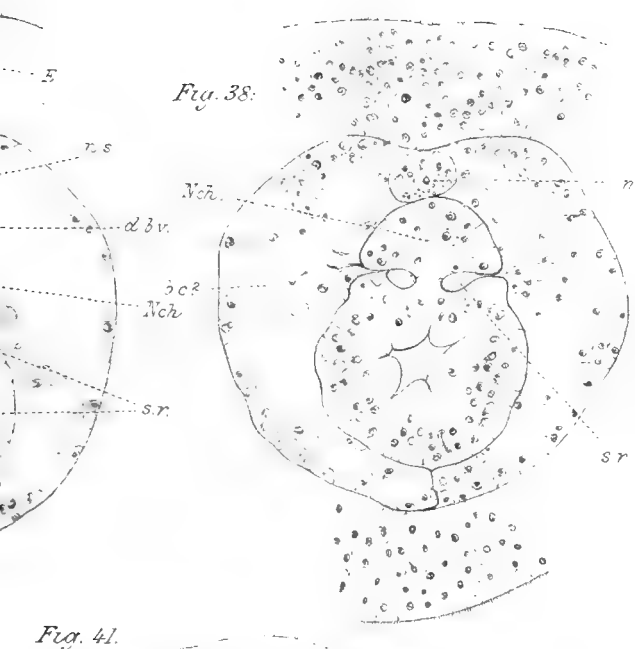

Fig. 38.

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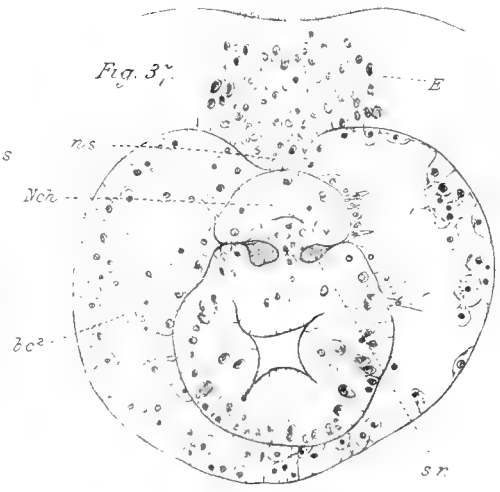

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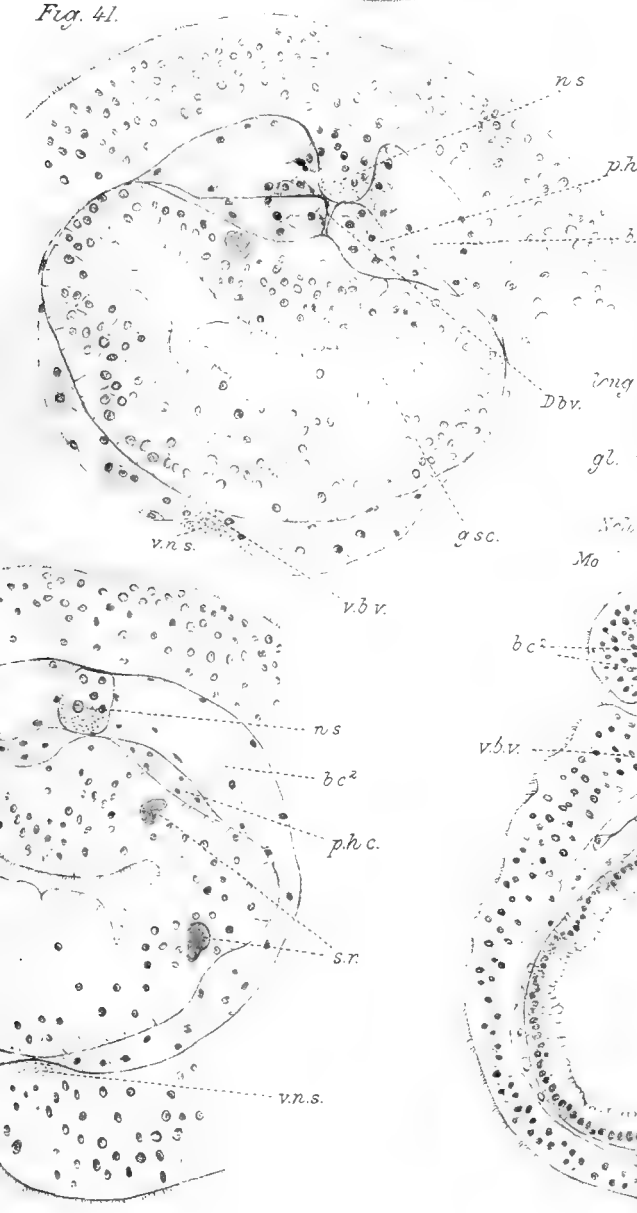

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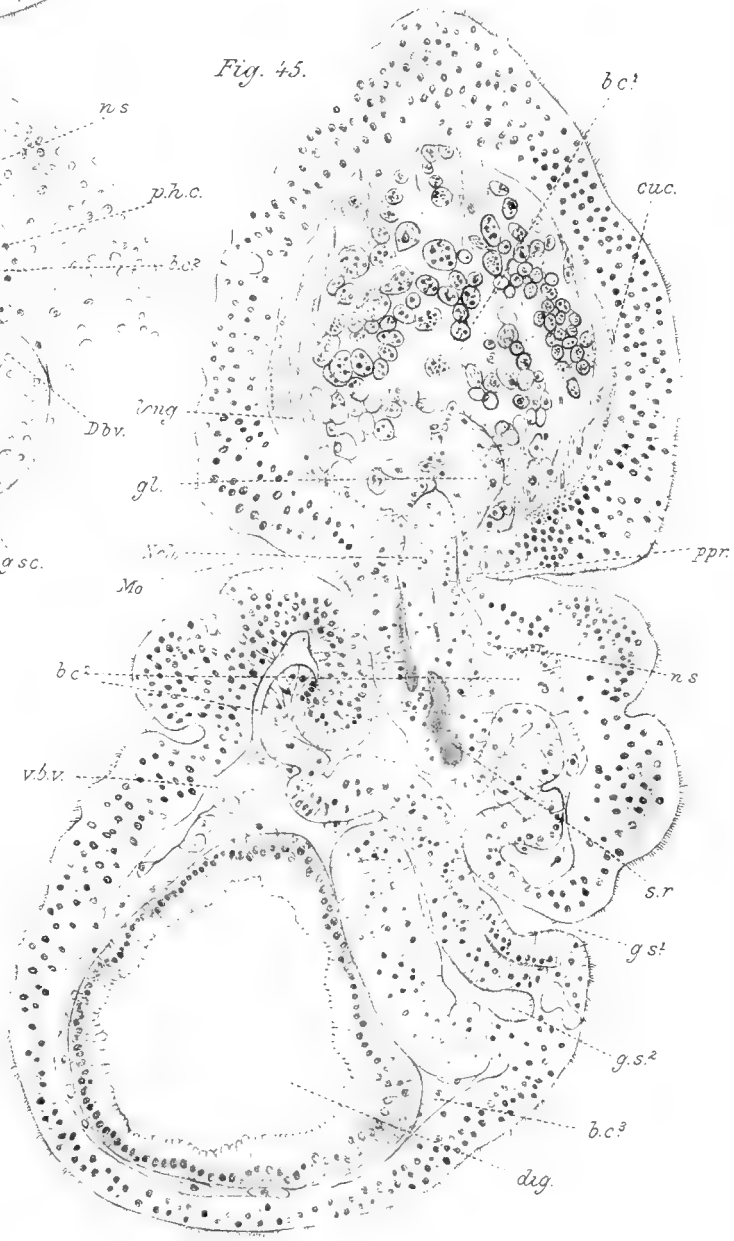

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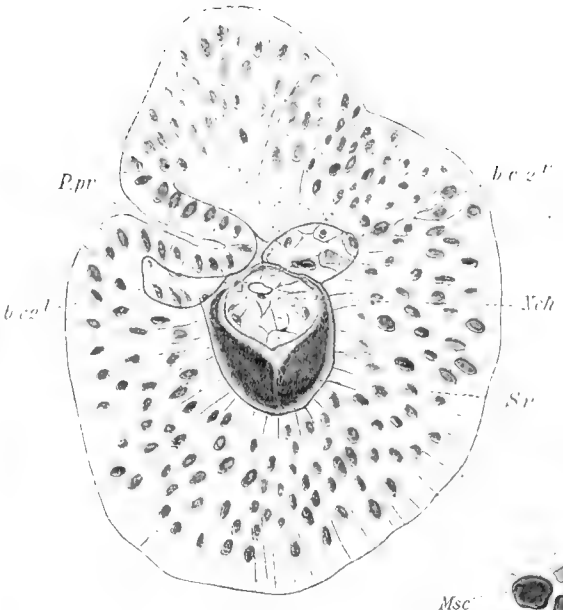

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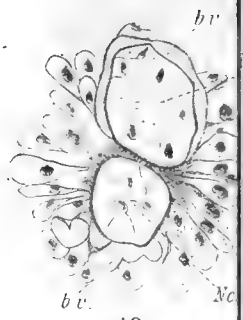

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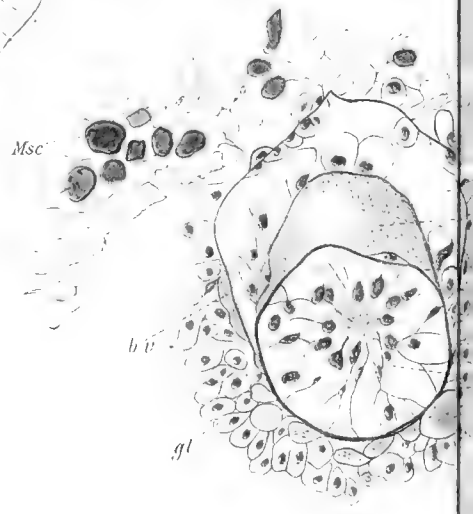

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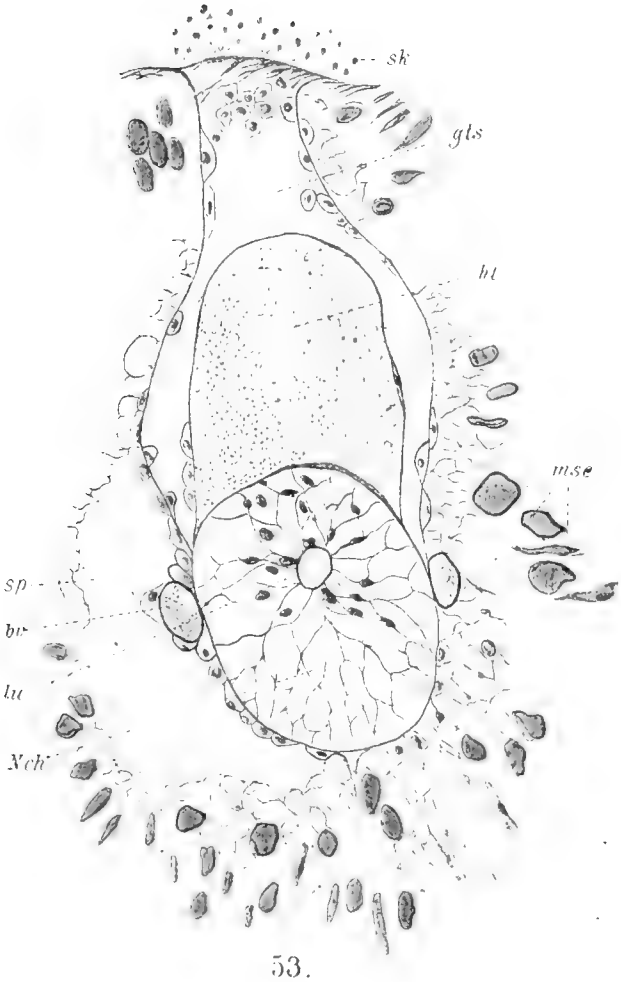

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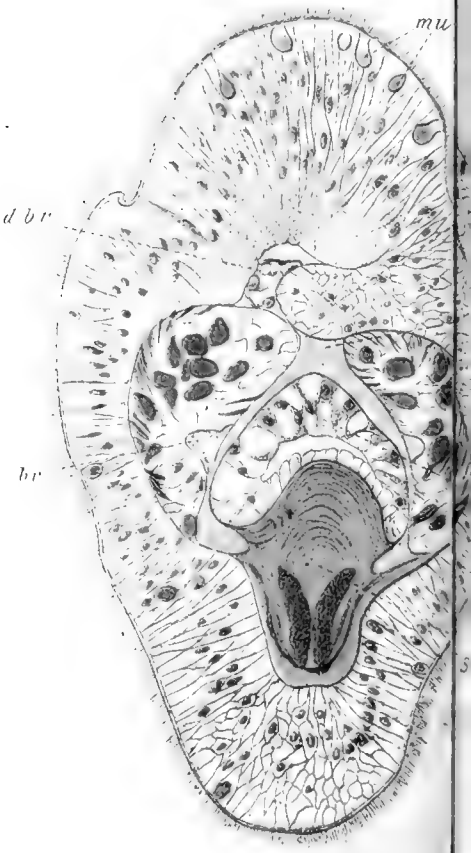

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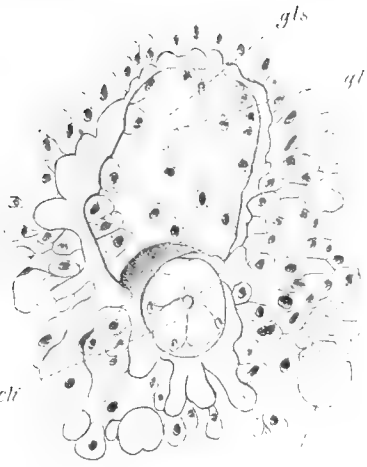

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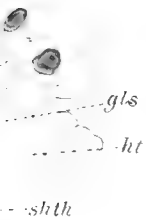

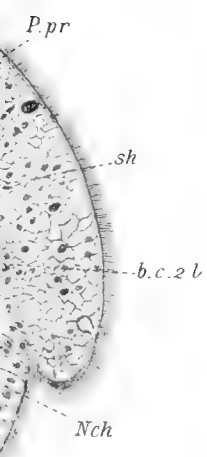

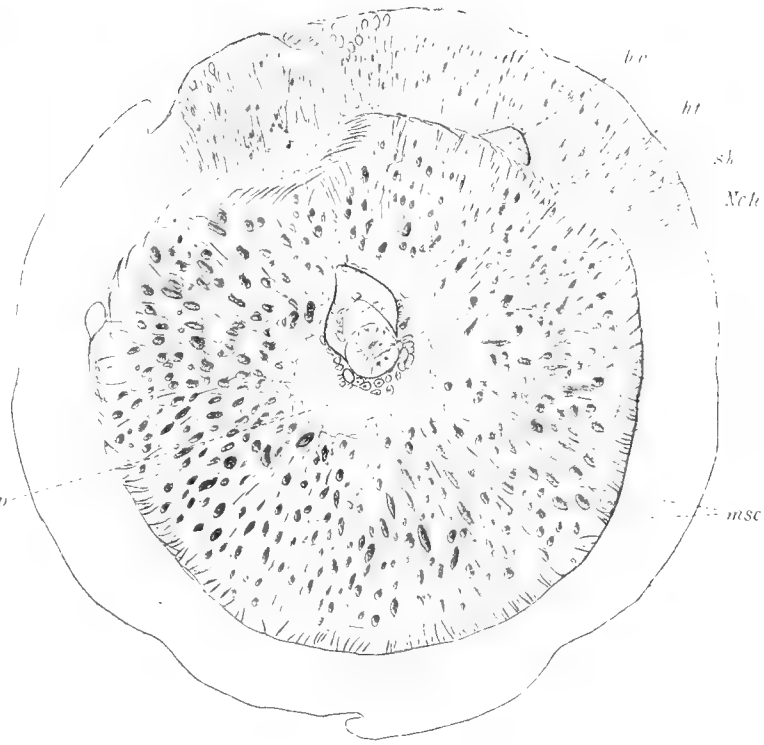

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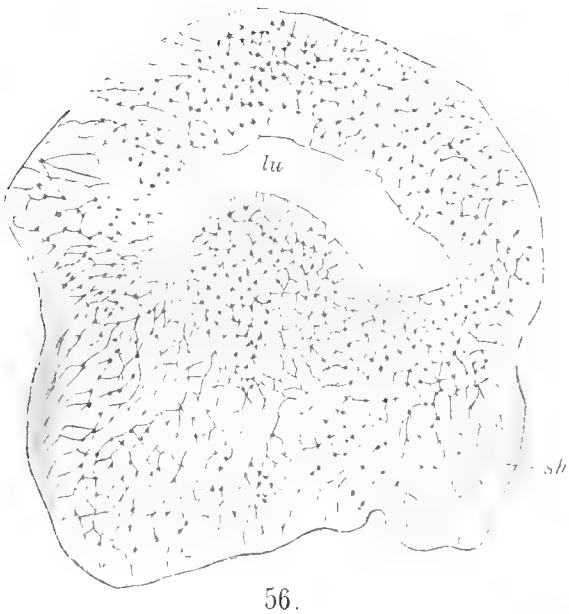

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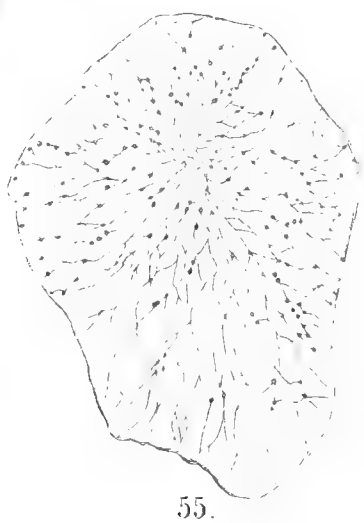

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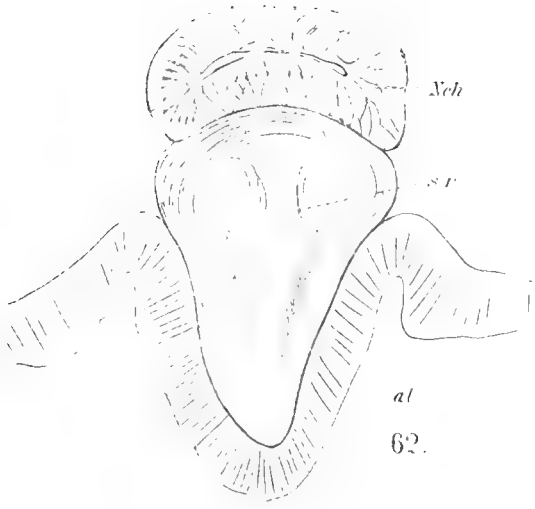
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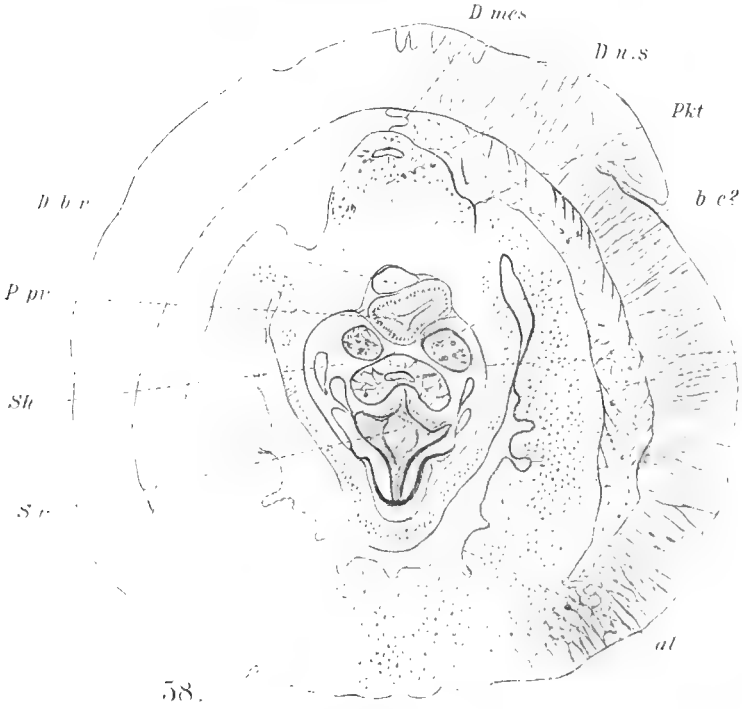
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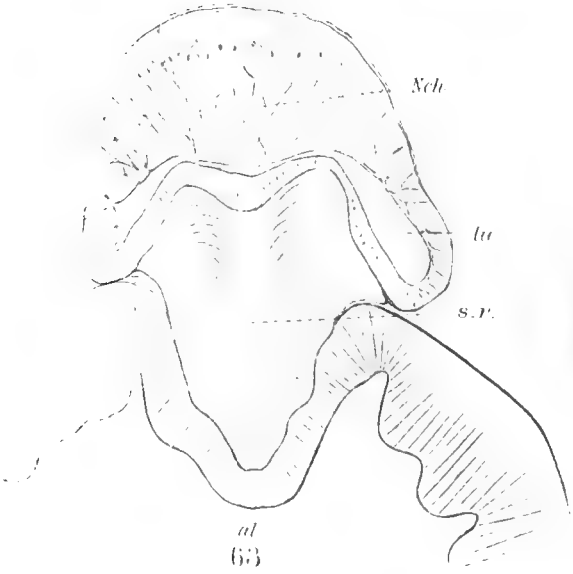
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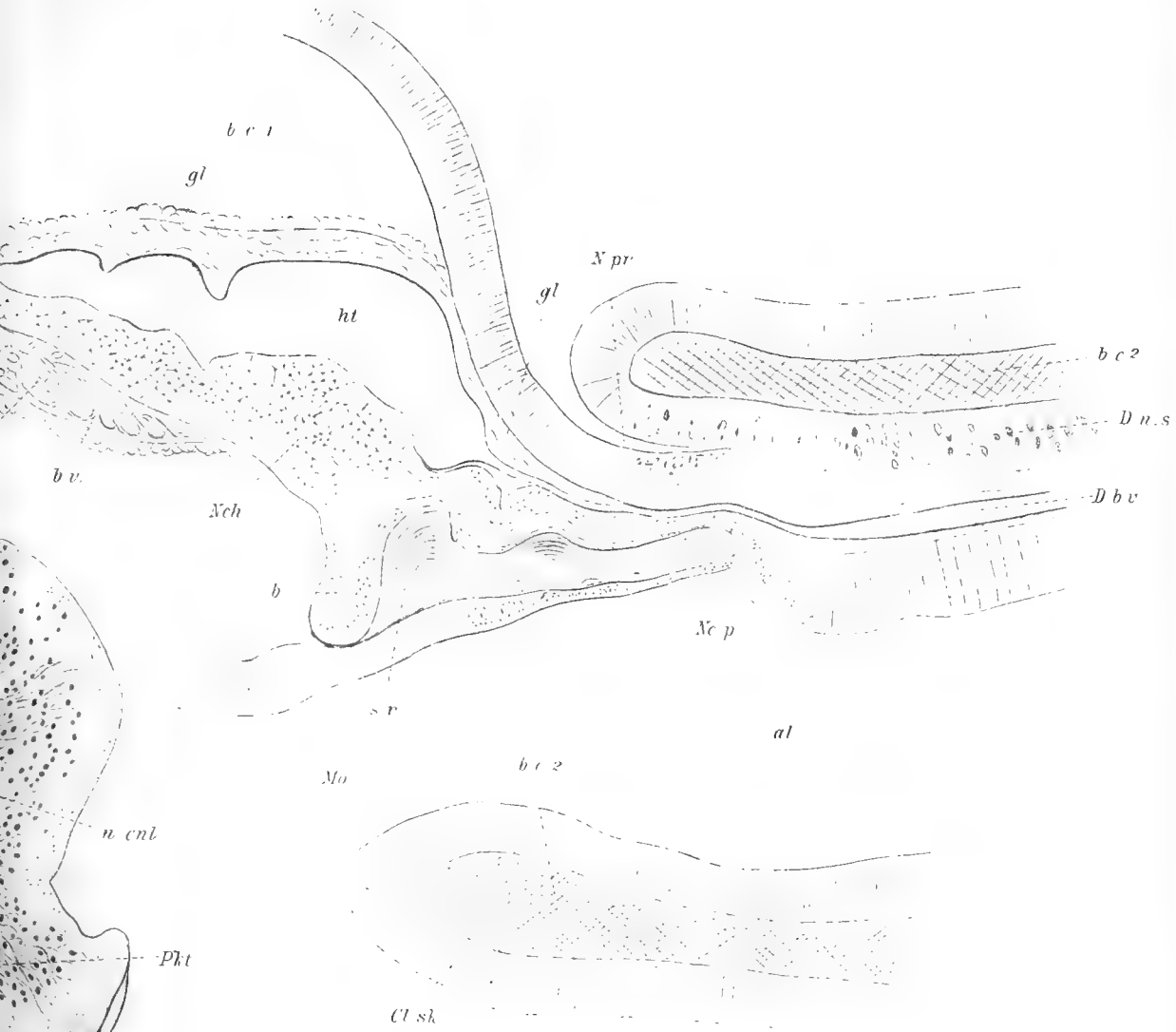
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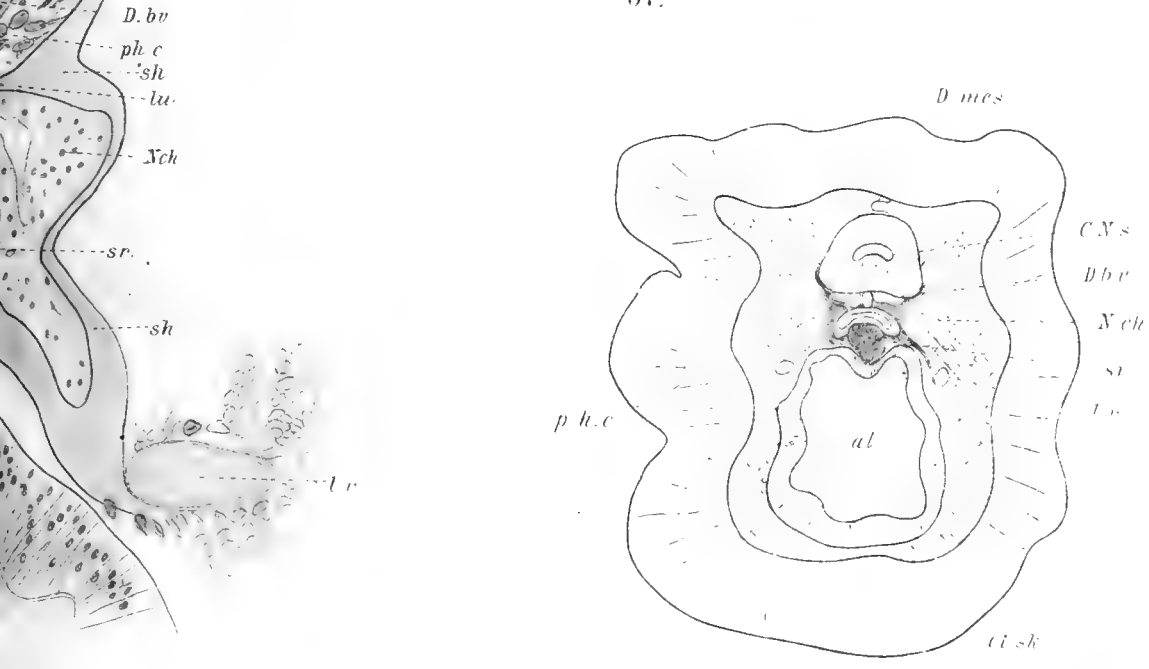
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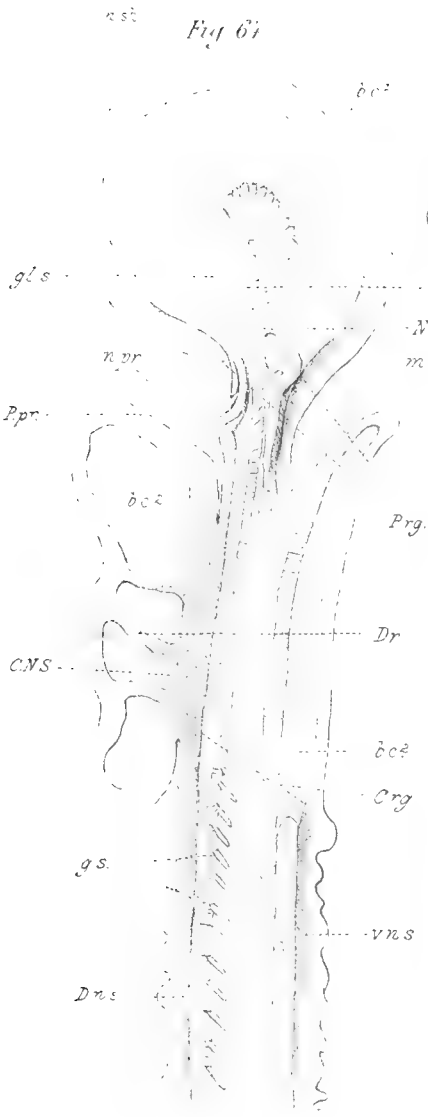
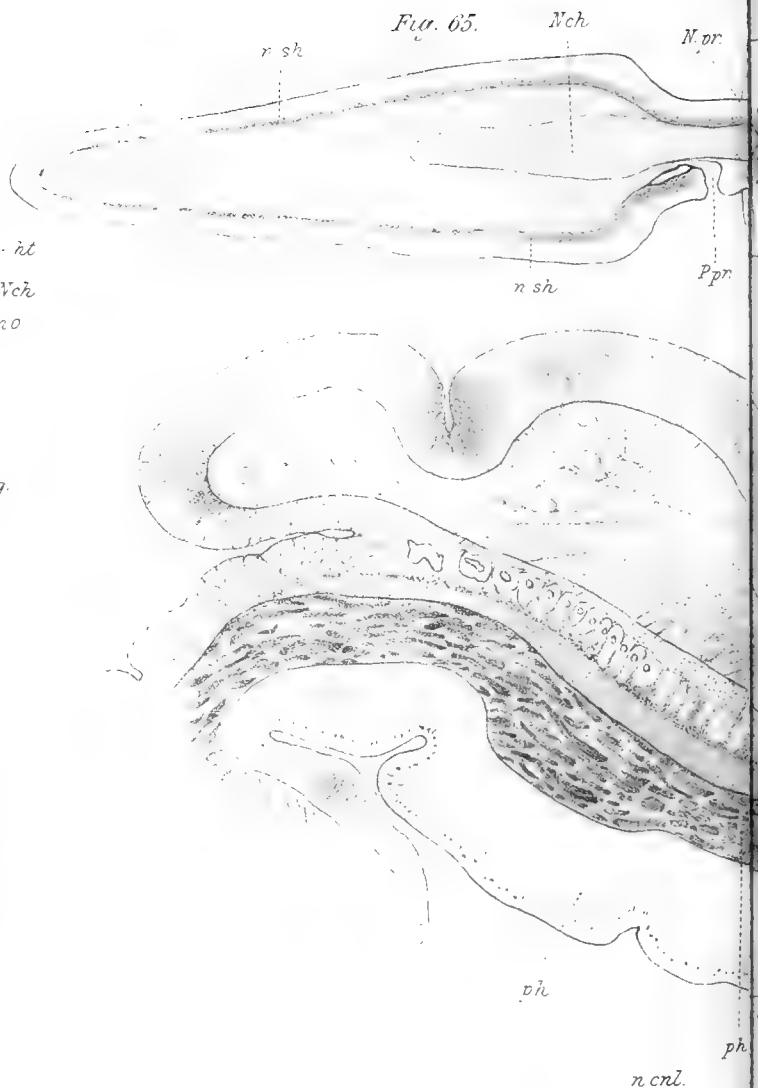


Fig. 67



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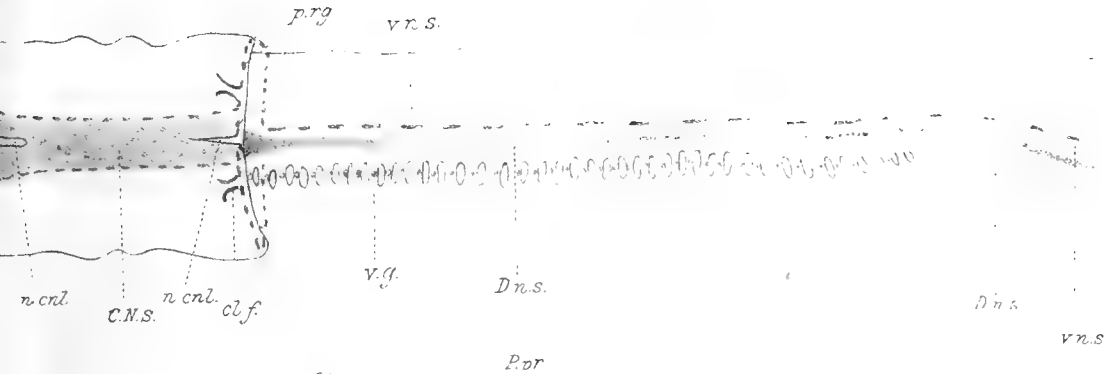

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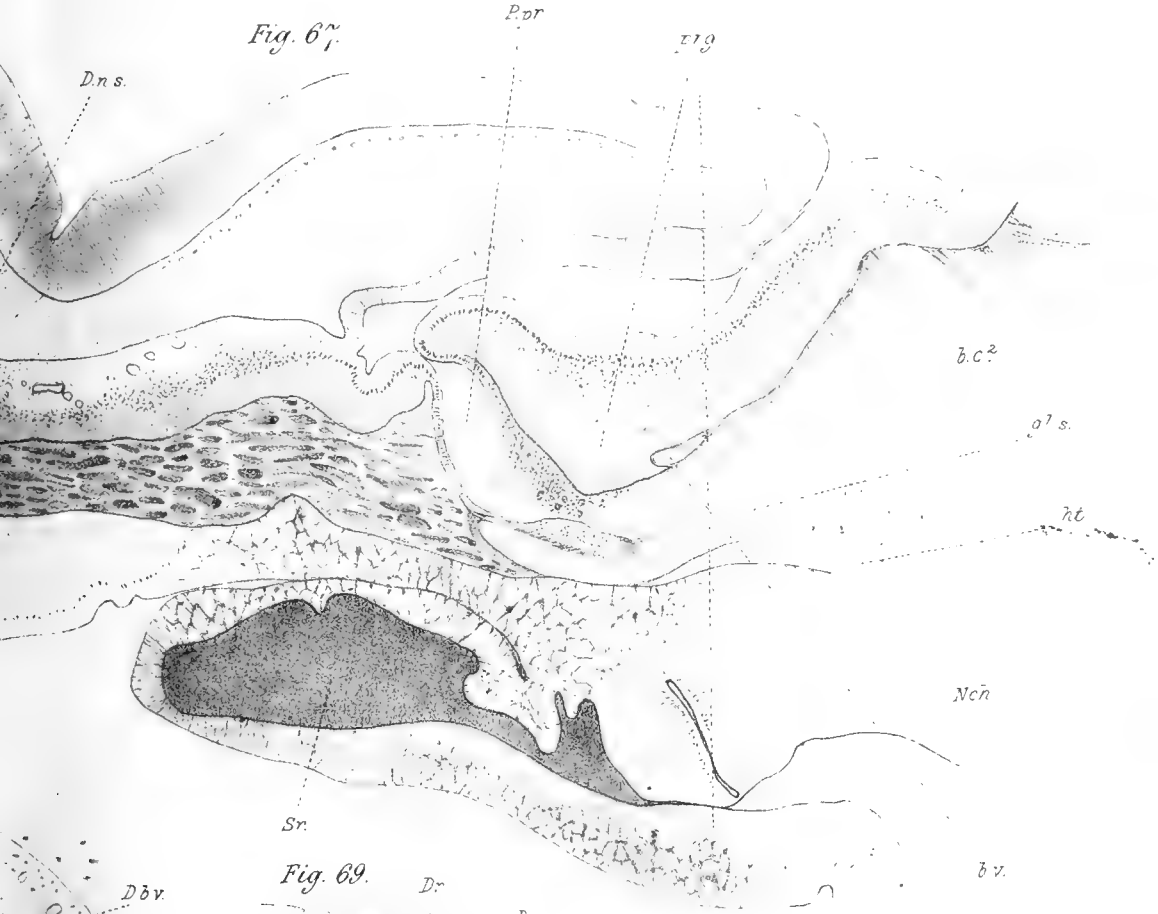

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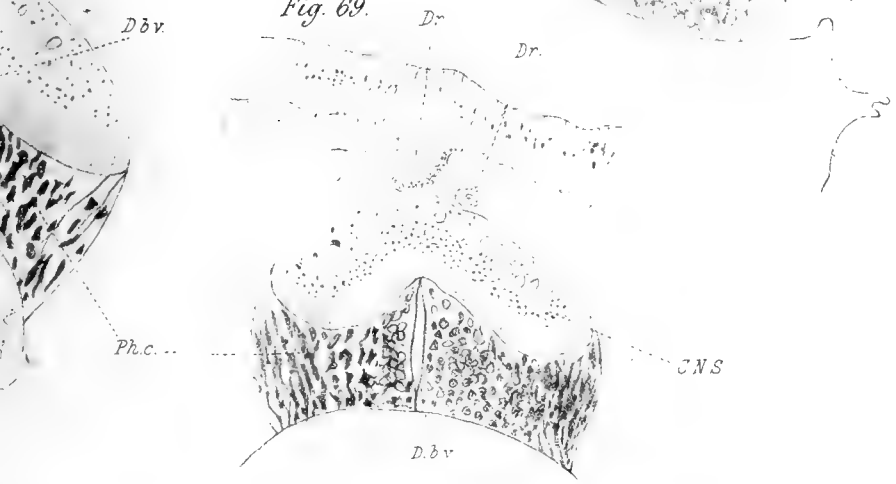

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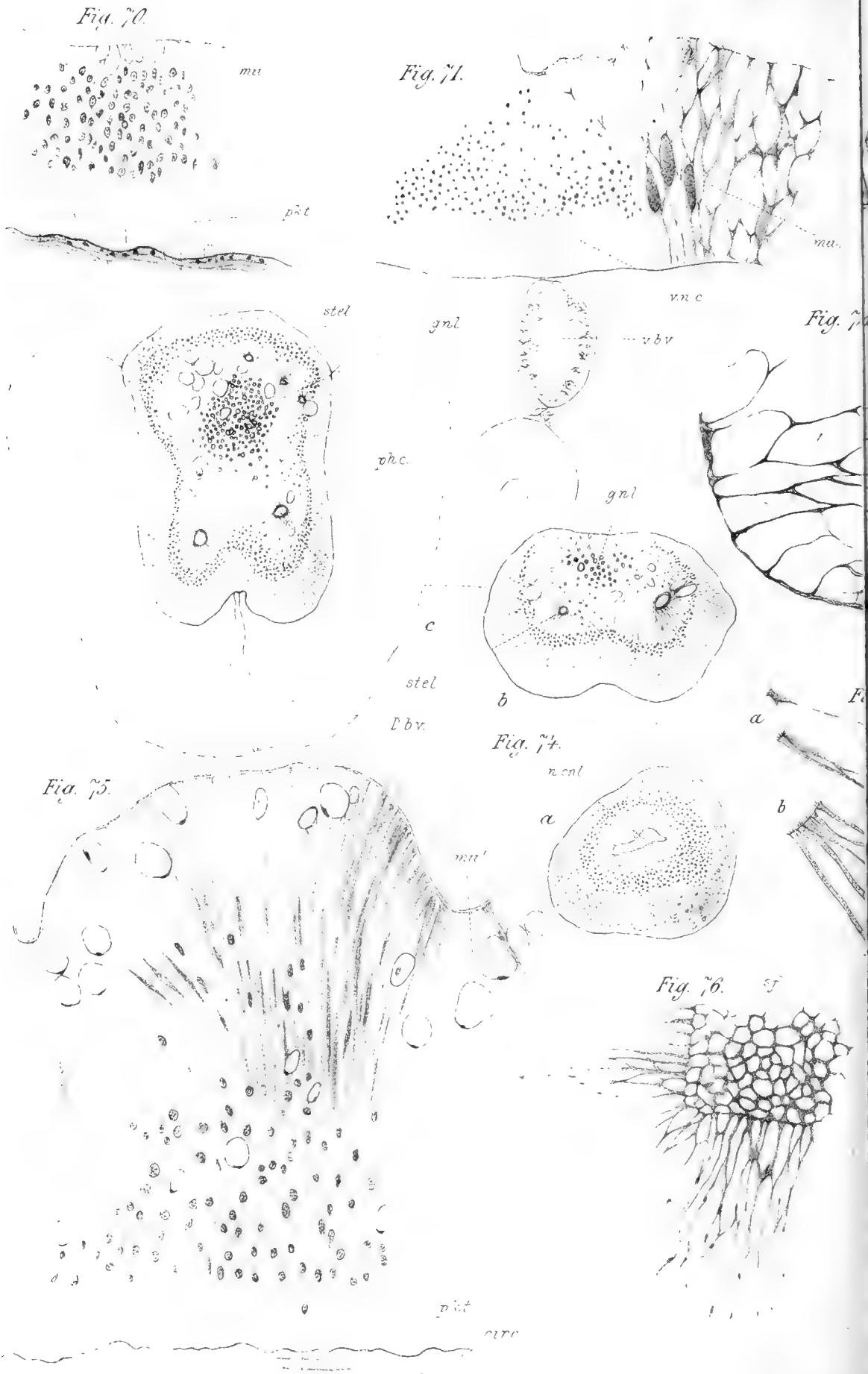

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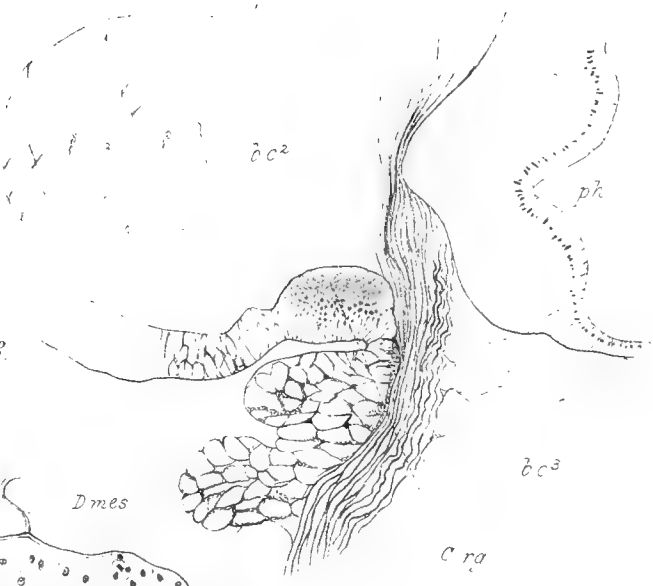

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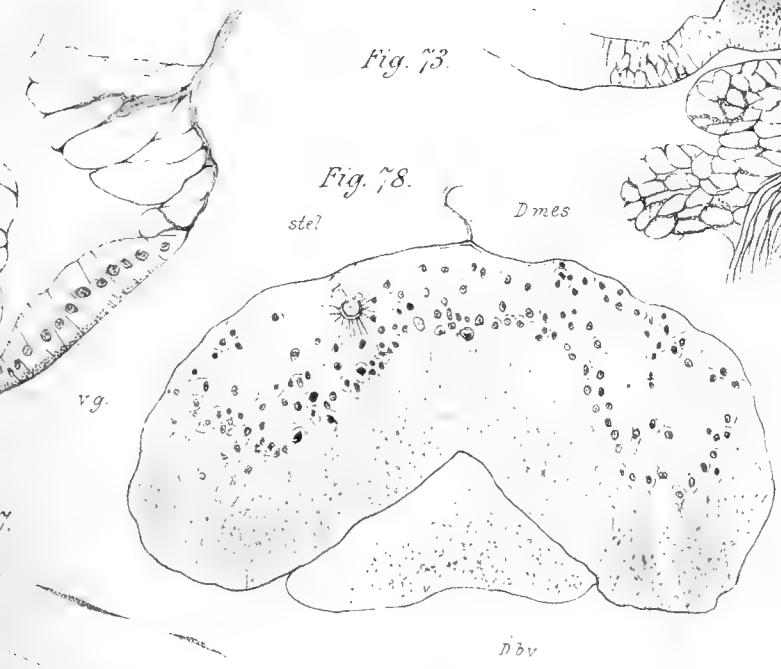

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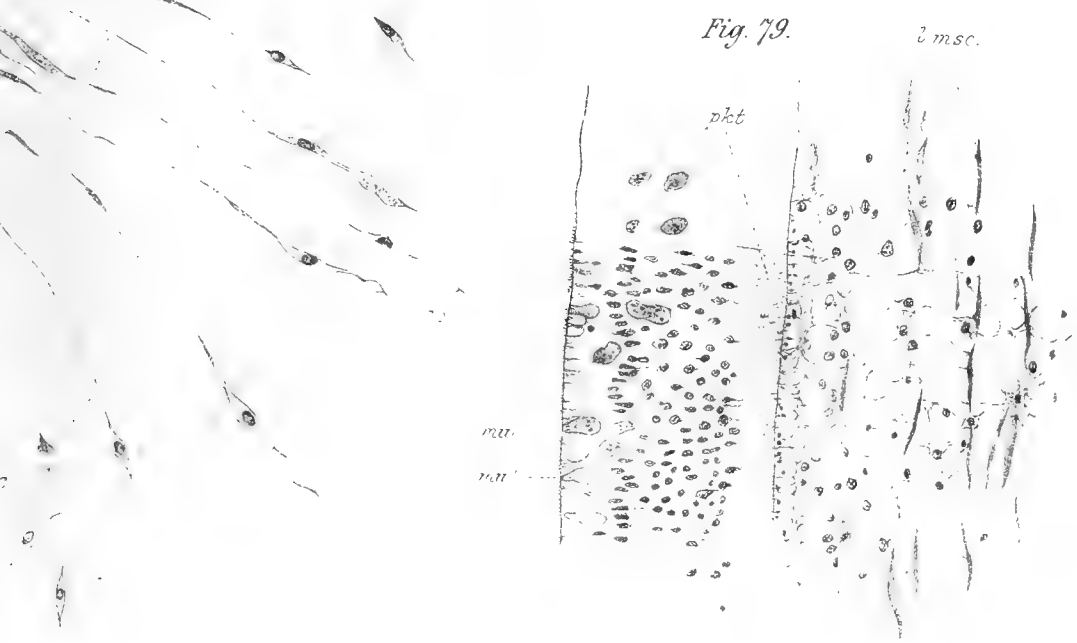

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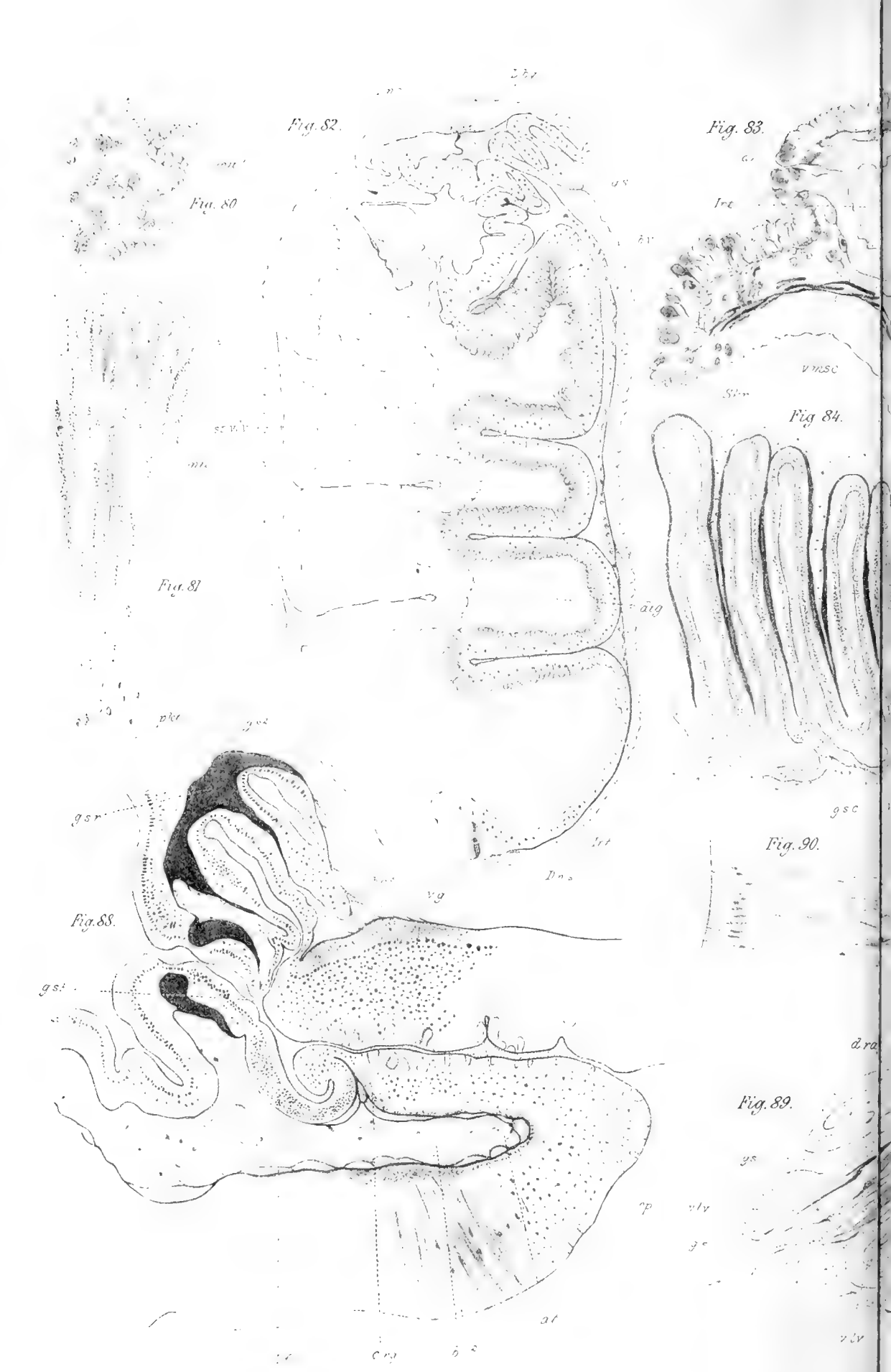
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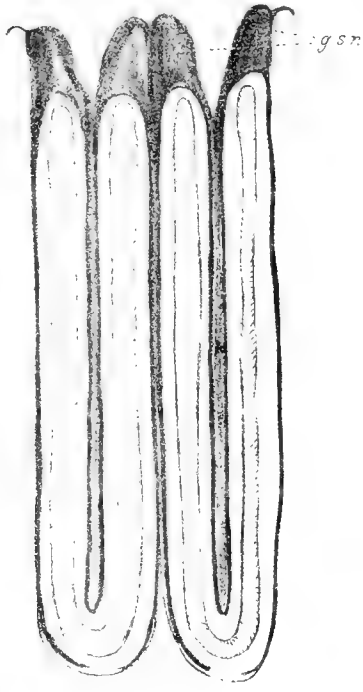

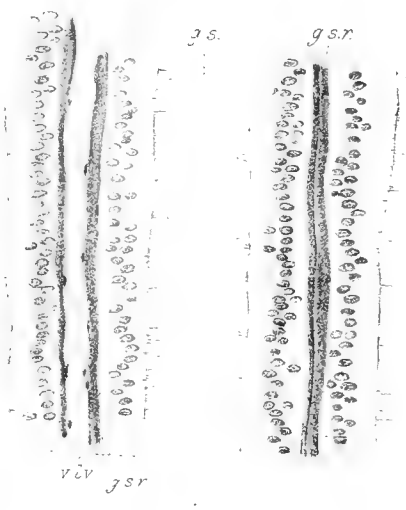

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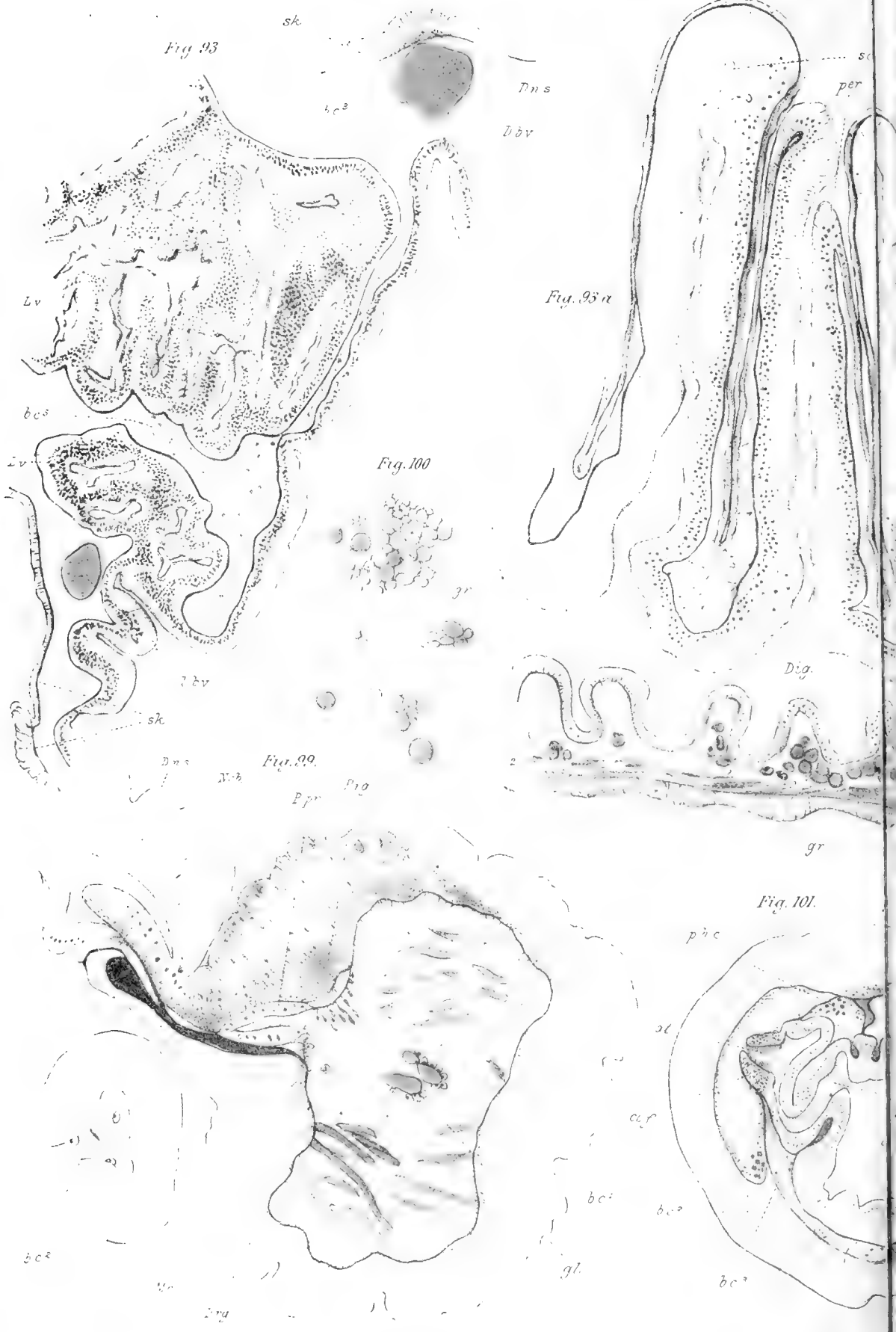

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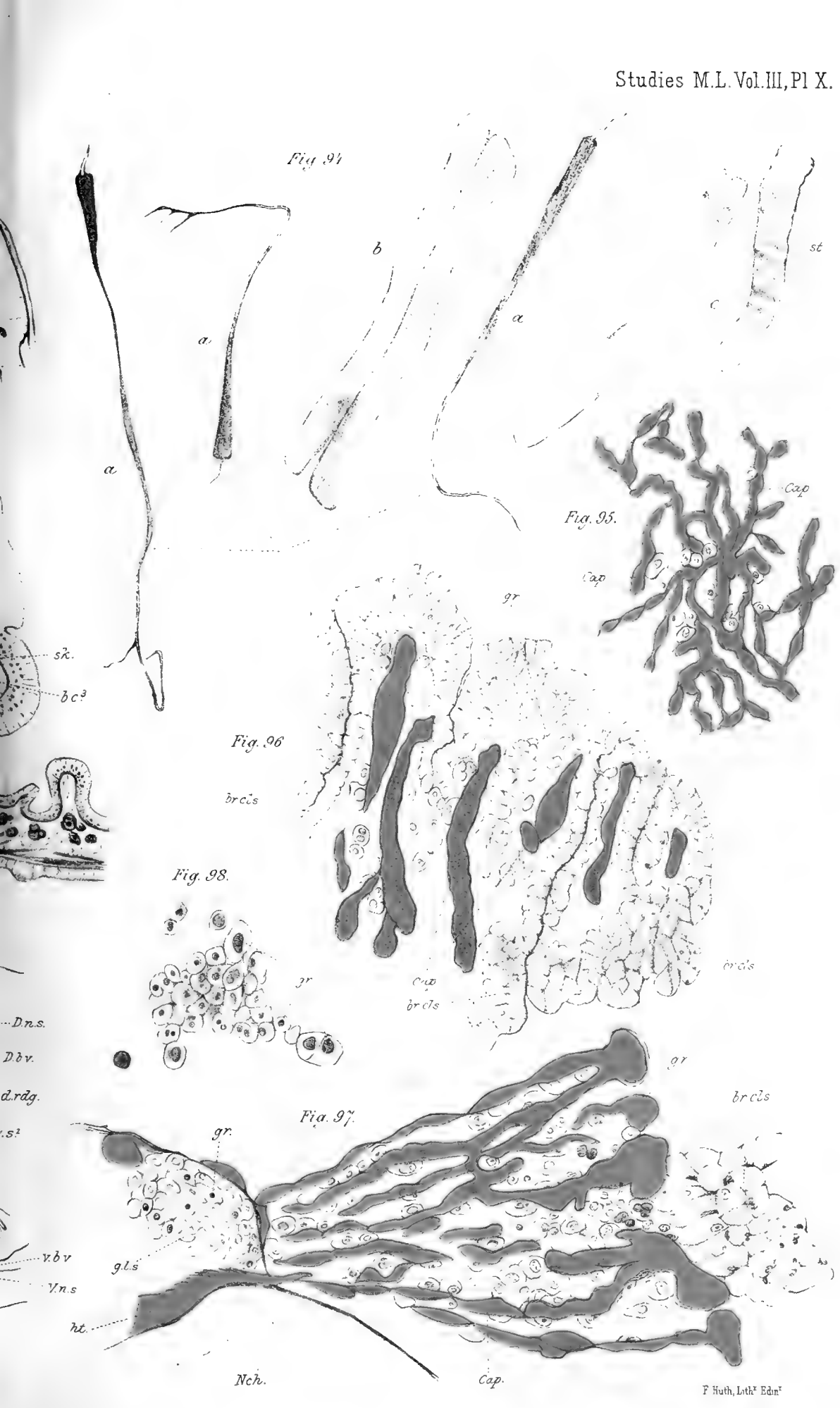
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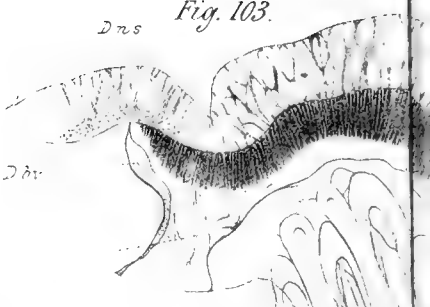

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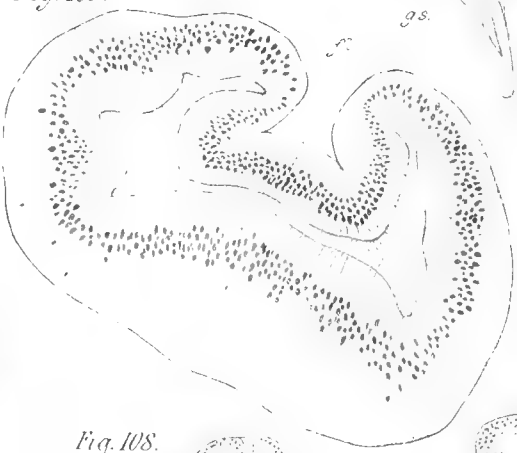

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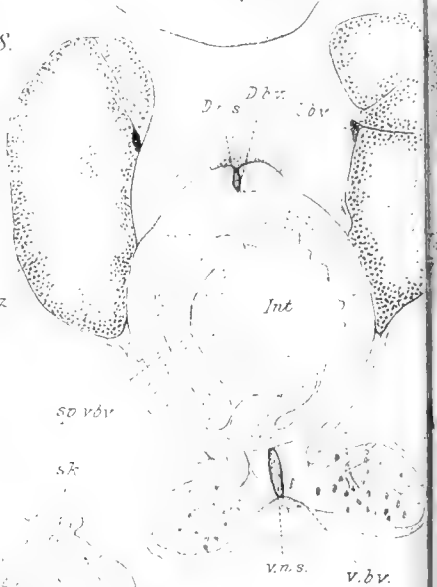

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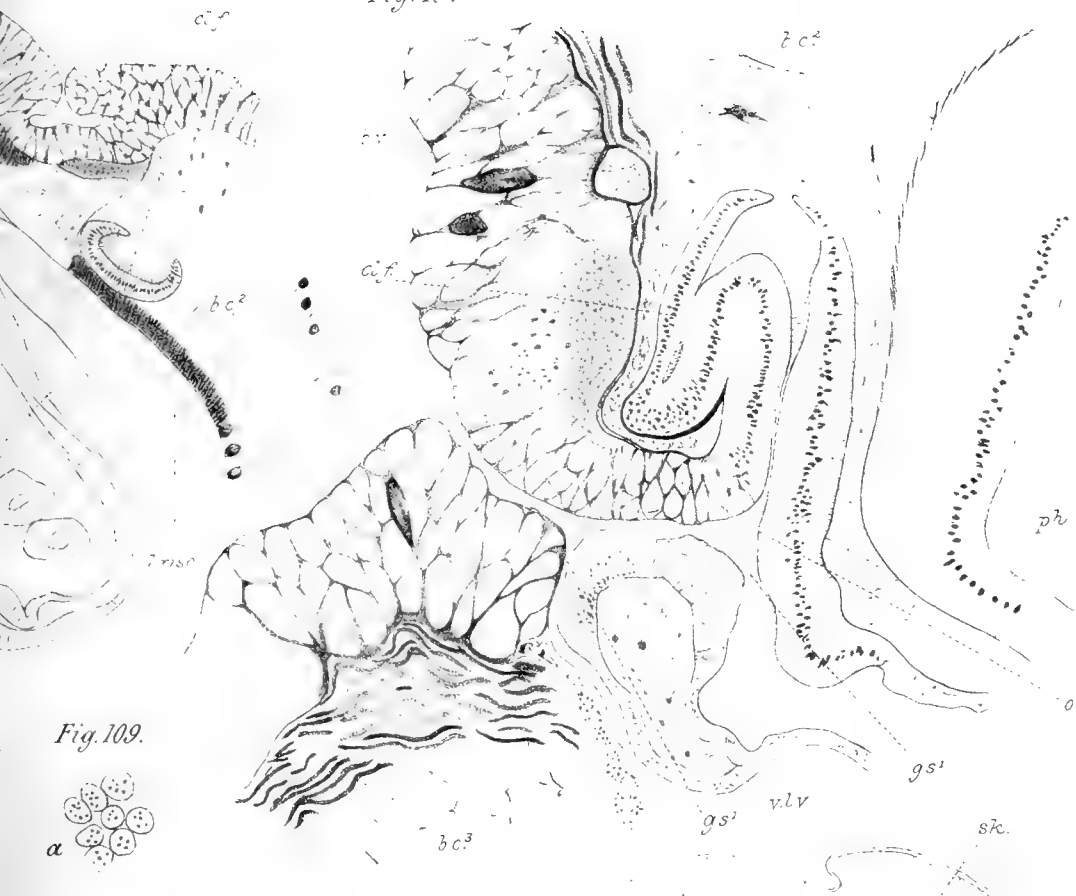

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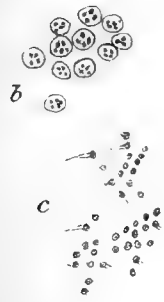

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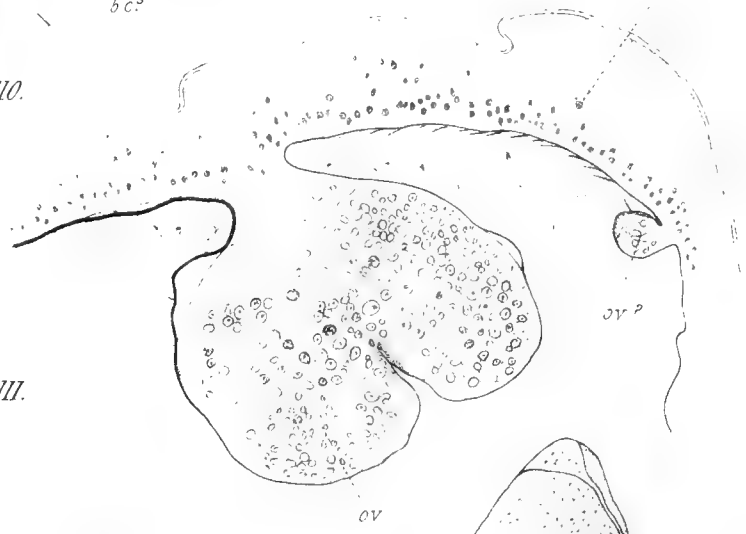

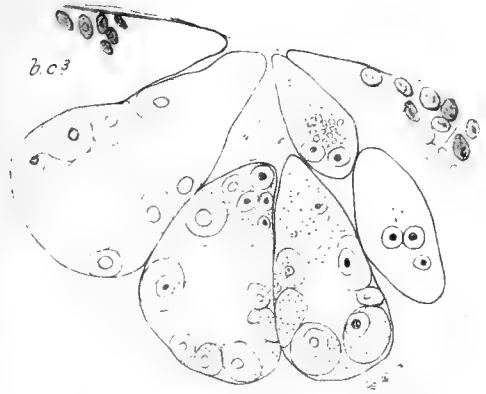

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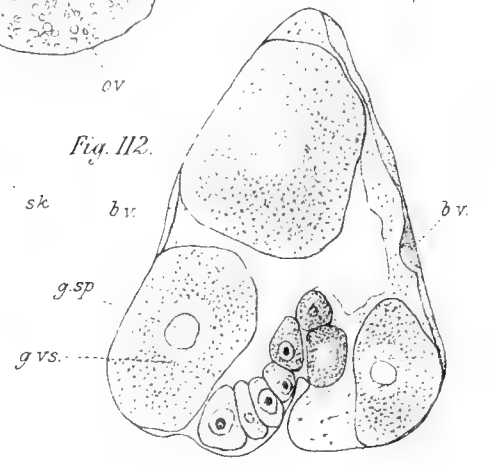

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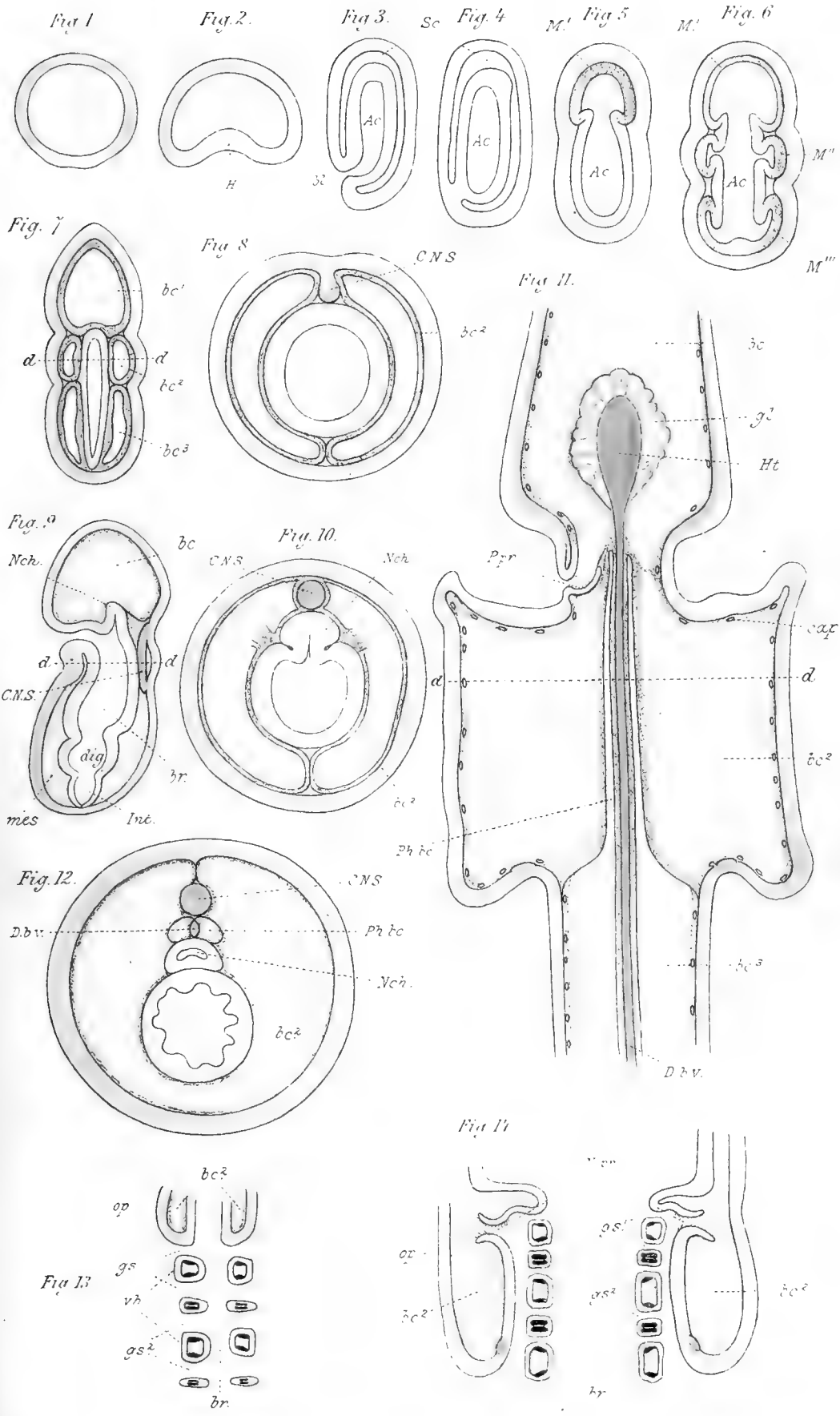

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The Ancestry of the Chordata.

By

William Bateson, M.A.,
Fellow of St. John's College, Cambridge.

The Ancestry of the Chordata.

Preface.—In view of the facts relating to the structure of the Enteropneusta which form the subject of the accompanying paper and of those which have preceded it, it seemed necessary to attempt some analysis of their import and bearing upon morphological problems, and especially upon the vexed question of the ancestry of the Chordata.

But at the outset it was impossible to attempt such an analysis without first clearing the way by a discussion of the morphologic meaning of Segmentation. Since the Enteropneusta are essentially "unsegmented" animals and the Vertebrata are "segmented," this preliminary discussion was necessary. Moreover, having shown reason for not accepting the view that the vertebrate segmentation was of such a kind as to necessitate the existence of a series of segmented ancestors to account for it, it became also necessary to treat the whole question of the origin of segmentations of this class upon a wider basis. This must be the apology for the introduction into this paper of some matter and speculation not otherwise immediately relevant to the subject.

The decision that it would be profitable to analyse the bearing of the new fact in the light of modern methods of morphological criticism, does not in any way prejudge the question as to the possible or even probable error in these methods.

Of late the attempt to arrange genealogical trees involving

hypothetical groups has come to be the subject of some ridicule, perhaps deserved. But since this is what modern morphological criticism in great measure aims at doing, it cannot be altogether profitless to follow this method to its logical conclusions.

That the results of such criticism must be highly speculative, and often liable to grave error, is evident.

Part I.—The Segmentation of Amphioxus and the Vertebrata, compared with that of the Annelids.

From the time when the theory of descent in some form or other became generally accepted amongst zoologists, the question of the pedigree of the Vertebrates has been the subject of much speculation and controversy. The amount of attention which has been bestowed on this question has perhaps been greater than is warranted by the actual importance of the problem considered as a contribution to general biology; but when it is borne in mind that the question is that of the history of the human race, the fascination which has been found in it is not surprising.

Beyond, however, this more sentimental side, there is another source of special interest to be found within the terms of the problem itself; namely, that which is afforded by the obscurity of the solution; for when the relation of any one group to the rest of the animal kingdom is sought, in most cases there are some cardinal features of anatomy common to it and to some other group, which appear to point to some affinity between them. For example, the structure of the Tracheata at once suggests Crustacean affinities, while there is a strong apparent resemblance between the whole Arthropoda and the Annelids. Even a group so isolated as the Mollusca has points of obvious harmony with other groups as soon as the characters of the Trochosphere are known, and similarly with most other groups. Each and all of these "obvious" resemblances may be illusory, but still they furnish something

which, temporarily, is satisfying, and at least provides a point of departure for criticism. But in the case of the Chordata there are none of these common features. The three characters which unite them, the notochord, the gill-slits, and the relations of the nervous system, are limiting and exclusive, and without parallel in any forms outside the Chordate group. So strongly has this fact been felt by many of those morphologists who have already dealt with the pedigree of the group, that they have practically abandoned the attempt to find homologies for these features among the Invertebrates; for it is impossible to take seriously such suggestions as, for example, that the notochord may be compared to, generally, the sacs of the Capitellidæ, the "siphons" of any of various Invertebrates the "giant-fibres" of Earthworms, or the crystalline style of Anodon. Each of these structures has been in turn suggested, together with many others, as offering something with which to compare the notochord. In the same way Semper argues that the vetebrate gill-slits have an obvious similarity to certain pores which he has found in the heads of certain Oligochæta (Nais), while other authors see a striking resemblance between them and the Chætopod segmental organ, and so on.

In seeking, then, for the proximate ancestors of Chordata, the Chordate features have been disregarded, and another character of the vertebrate animal has been selected as offering a more probable basis of operations. The character which has in this way been chosen as the point of departure is that of metameric segmentation. By thus setting aside the questions arising out of the notochord, &c., and speculating upon the segmentation of the body, the conclusion is soon reached that some Annelid was the immediate ancestor sought.

This view has found its chief exponents in Dohrn and Semper, and has been generally supported by Haeckel and by most of the popular exponents of evolution.

It would be unprofitable to recapitulate here the numerous morphological difficulties as to the primitive mouth, &c., which arise if this theory be received. Many objections of this kind

have been raised and have been variously replied to, and in this condition the matter rests. By those who support it, it is assumed that the common feature of segmentation is so binding and unique a property as to suffice to link together groups whose morphology is otherwise widely different.

In the following pages it is proposed to examine the propriety of employing the character of metameric segmentation as one of first importance in forming a phylogeny of this kind. And before referring to the evidence derived from the fact that the three characteristic features of Chordata are found in Tunicata and Enteropneusta, which are unsegmented forms, it will be best first to discuss the meaning of the phenomenon—"segmentation"—for if resolved into its elements it will be found to be by no means a peculiar feature of a few groups, but rather the full expression of a tendency which is almost universally present.

The term "metameric segmentation" has been used to describe several anatomical features, which reach their highest development in the Annelids, the Arthropods, and the Vertebrata. If an attempt be made to reduce this expression to its simplest terms it appears to mean, in the first place, that certain organs of the body are serially repeated from before backwards, and in the second place that, in the case of the Vertebrates and Annelids at all events, the body cavity is at some period of life divided into a series of compartments, each of which is closed off from its neighbours. But when a more precise account of this phenomenon is required, and when it becomes necessary to particularise as to which of the various organs of the body is thus repeated, difficulty at once arises from the fact that this repetition is irregular, and even within narrow limits may vary considerably. In the case of many of the errant Polychæts all the mesoblastic organs, together with certain apparently serially homologous parts of the nervous and digestive systems may recur for a seemingly indefinite number of times in one individual, or even the whole animal may be repeated in a chain, thus giving the highest expression to the phenomenon. On the other hand, as in Lumbricus,

&c., one or more of the mesoblastic organs may not be repeated; while in both Oligochæts and Polychæts there is a marked tendency to a division of labour between and specialisation of structure of individual segments or even regions of segments in various parts of the body. It thus appears that even among Annelids alone the fact of segmentation is not a circumscribed idea, but may include several phenomena which clearly differ from each other in degree, and possibly are also unlike in kind. For while in the case of Nais, &c., this repetition is complete, and is thus used as an obvious and simple mode of reproduction, yet in other worms it appears only to be concerned in increasing the length of one individual without adding to the number. Now, if these two conditions are merely various expressions of the same phenomenon the question at once arises as to which is its more primitive manifestation. Was segmentation originally a repetition of all the organs for purposes of reproduction, which process has become subsequently commuted into mere increase in bulk, or is this complete repetition to be regarded as the final term in a series of which the first was increase in bulk? Segmentation, as we know it, may clearly be viewed from either of these two standpoints. With regard to the Annelids, many authors have held that the former is the correct one; the question whether this is so or not cannot be discussed here, but in the case of the Chordata examination will show that their segmentation is of the latter class, and is the result of a summation of repetitions; and, being so, it is by no means a unique condition, which can unite forms otherwise unlike, as Chordata and Annelids, but is rather a result of the common tendency to repeat parts already present, which tendency occurs more or less in almost all animals. But before communicating the features of Chordate anatomy, which point to this as the mode of origin of the segmentation of the class, it will be best to establish the fact that repetitions of this sort are common, and to examine the comparative evidence as to the manner in which they occur. It will then be seen that segmentation on the plan found in the Vertebrates are really

extremely common, and appear to arise suddenly and in forms nearly allied to those in which they are not found.

Firstly, among the ciliated Platyhelminths a striking case is offered by Gunda segmentata, in which, as described by Lang, the diverticula of the gut, the testes, the yolk-glands, the tubules of the excretory organs, the transverse commissures, and the nerve-cord, are all regularly and synchronously repeated. Now, this case stands alone merely in the completeness of the repetition. All through the Turbellaria are to be found many instances of animals with great numbers of gut diverticula, with testes and yolk-glands scattered all over the body, with branched excretory systems, with anastomosing nervous networks, &c. Not only this, but instances are common in which some of these structures are repeated regularly, and others irregularly or not at all, as, for example, Polycelis pallida (Quatrefages), in which the ovaries are scattered and the testes are not, while the reversed condition is more frequent. It becomes probable that the repetitions of these organs did not phylogenetically occur simultaneously, but that repetition occurred at various times in each set of organs.

Again, among Nemertines in some species the saccules of the gut, the generative organs, and the circular blood-vessels are all repeated together and with great regularity, so as to produce a segmented whole. In other species these repetitions are not all formed or are more or less irregular, thus pointing to the fact that these repetitions have been acquired within the limits of the group. The development (*v.* especially Salensky, 'Arch. de Biologie,' 1884) precludes at once the possibility of the ancestral form of Nemertines having been "segmented;" hence they, together with the Planarians, offer a type of a high degree of repetition being acquired within the limits of a group. Nor do these forms alone exhibit this feature as one peculiar to themselves, for there are few groups in which it is not found. Even among Mollusca, which are, perhaps, the most typically unsegmented of all forms, the Chitons may be instanced as examples showing that such com-

plicated organs as shells may be repeated within the limits of a small group. Moreover, in some Chitons bunches of calcareous setæ recur along the sides symmetrically to the scutes, producing an appearance not far removed from that of Arthropoda.

Another case is to be found among the Nudibranchs, in which the liver diverticula, which are peculiar to and characteristic of the group, not only recur in an obviously segmental manner, but may be arranged in several ways among the Æolidæ, being in some (as Æolis papillosus, Æolis pulcher, &c.) arranged in more or less regularly paired oblique rows, while in others (as Dendronotus) the liver cæca stand in paired, arborescent tufts, which are as definitely symmetrical in their repetition as any system of organs of a Vertebrate. In cases of this kind the regularity of these repetitions is obviously secondary, and all the other anatomical features show no trace of segmentation, which constitutes the great interest of cases of this kind from the point of view of the present argument.

The cases which have been so far mentioned have all been selected from bilateral animals, with a definite long axis in the direction of which they move. But the belief that repetitions of this sort are of constant occurrence as a factor in effecting modifications of general form, derives most remarkable support from the facts of the anatomy of radiate animals, especially of the Echinodermata. From embryonic evidence it may be regarded as almost certain that these animals are descended from a bilateral ancestor, and that their present form has been since acquired. Whenever this change took place it came to pass in some entirely unknown manner that the various organs came to be repeated round a central axis. However this may have been brought about, the fact remains that the number of such repetitions did not become a fixed and definite feature common to all the divisions of the group. For while the number five appears to be the limit of the repetition in the Echinoidea, Ophiuridea, and Crinoidea, among the Asteroidea the arms of different genera have not the same number, nor do they necessarily occur in multiples

of any number. For example, while in the divisions Asteridæ and Asterinidæ the prevailing number is again five, among the Solasteride we find that the arms of Solaster may be thirteen or nine (as in S. endeca), in Heliaster from twenty-nine to forty. Not only is this true of living forms, but in the case of the fossil Cystidea the plates were irregularly arranged and the perforations of the feet scattered, and in the Blastoidea the basal plates were three, though bearing five radials and interradials. All these facts point to a history of the occurrence of repetitions among the various parts around a central axis. And perhaps more remarkable still is the extreme variability to be seen among individual members of living species.

For example, though Asterias rubens ordinarily possesses five arms specimens possessing six or seven arms are very common, while individuals with only four are not rare (the latter may possibly, however, arise from mutilation). In like manner specimens of Brisinga coronata are said to have from nine to twelve arms. Thus, in these cases the arms, with all the organs which they contain, may be spasmodically repeated as a mere individual variation.

All these animals move on the oral surface, and though, of course, the body may be regarded as arranged bilaterally round a longitudinal axis, yet in the locomotion of the animal this fact is not conspicuous (?) But in the Holothurians in which a long axis does again assume importance, though repetitions of this magnitude do not occur, yet there is a tendency for certain organs to arrange themselves in a series of longitudinal repetitions closely imitating segmentation. In this connection the Elasipoda (Holma Théel, 'Challenger Monographs'), which crawl about on the "trivial" surface in the direction of the long axis are of great interest. The body of these animals is long and flat, and its margins are produced into long processes, resembling parapodia, which are regularly arranged in pairs down the sides. The regularity of this arrangement is so great that some of the species figured by Théel might easily be thought at first glance to be segmented

worms[1]. Thus, in animals whose long axis has been suppressed, it appears that repetition may arise of most of the organs of the body radially arranged; next, that not only the specific but also the individual number of these variations is liable to great variations, pointing to the fact that the power to repeat in this way is one which may be easily called into action producing great differences of form.

It may also be observed in this connection that similar casual repetitions are frequent in the case of the Gonozooids of Hydromedusæ, in which animals also they are radially arranged. As in the case of the Echinodermata this is shown by the great diversity in the specific and individual number of those organs which are radially repeated. The latter may be seen, for example, in Clavatella prolifera. The Medusa of this animal creeps about on its tentacles, which are long and stiff, and which carry short suctorial processes on their oral faces which support the animal, giving it the appearance of an Ophiurid. The number of these tentacles and of the radial canals varies with age, from six to eight (Hincks). In the specimens which have come under my own observation in the undetached buds the number of these arms was five, while those of the free Medusa was generally six. The number of the organs in Cladonema radiatum, another creeping form, is also very variable, the number of oral lobes being five or seven, and that of the tentacles and canals eight or ten (Hincks).

The facts of Echinoderm and Cœlenterate anatomy above quoted, suffice to illustrate the statement that in animals whose organs are already radially repeated, variations consisting in the repetition of one or more of the peripheral organs is of common occurrence, and may affect large numbers

[1] In relation to this acquisition of the appearance of longitudinal repetition or segmentation by a radiate animal, an example of the inverse phenomenon may be given. Among the Operculate Cirripedes, though in the Balanidæ the arrangement of the six plates composing the "cone" are so placed as plainly to indicate the original long axis, yet in the Coronulidæ this feature becomes obliterated, and the plates are disposed in a radially symmetrical manner.

of organs as in the case of the arms of Asteroidea, and may be of specific occurrence as in Asterias rubens and Brisinga coronata, or even ontogenetic as in Clavatella, &c.

All the instances of repetition of organs which have been so far selected, whether in the case of animals with a marked long axis or in the radiate forms, have been examples of the recurrence of parts or organs in some more or less definite relation to the axis of symmetry of the animals. These have been chosen especially as more markedly illustrating the possibility that the segmentation of some forms at all events may have been derived from the continual recurrence of this phenomenon until it became more or less regular and transmissible to the offspring as the definite course of development. But it must be remembered that repetitions of this kind are of an extreme type. The recurrence of whole sets of organs, as in the case of the arms of Asterias or the gastric pouches and generative organs of the Nemertines, must be regarded as the higher manifestations of this phenomenon, and consequently of more or less occasional occurrence. Since, however, it is in these cases that the nearest approach has been made to metameric segmentation as we now see it, they have necessarily been selected as of the first importance. But if repetitions of this magnitude are of rare occurrence, repetitions of smaller parts or organs are extremely common, if not universal. There is hardly one of the larger or more organised types in which whole tracts of the body are not composed of almost precisely similar and "serially homologous" parts, which are of very variable number. The scales and fin-rays of fishes, the tufts of hair and markings on many caterpillars, the teeth of Vertebrata, the joints of the Arthropod appendages, or of the stems of a Crinoid, the ossifications in the ambulacra of the Echinodermata, and many others, suggest themselves at once.

Especially noticeable are the casual repetition of large complex structures, such as the mammary glands and of exoskeletal organs, as the horns and dermal scutes of Vertebrates. The

number of these is liable to great variations, not even being constant in the species. For example, certain deer and also certain sheep have specifically more horns than two; and in the case of Iceland sheep the horns may be three, four, or five (Youatt, 'The Sheep'). By the nature of the case none of these repetitions can be atavistic; and it is interesting to notice how, just as it was shown that irregular repetitions of parts about the axes of symmetry of the body often take up regular secondary relations to them, recurring either in segmental pairs or in radial symmetry, so these minor repetitions take up regular relations (secondary in some cases, probably primitive in others) to the axes of the limb or part of the body in which they occur. Thus the ossifications in the Crinoid stem or the Starfish arm are so regularly related to the axis of the part that in the latter case they have suggested to Haeckel his extraordinary view of the phylogeny of the group, appearing to him precisely similar to the segmentation of a Chætopod. The case of the scales of fishes and the hairs and markings of caterpillars should perhaps have been more properly quoted in the former connection, as being an instance of irregular repetitions which have become definitely related to the symmetry, as in the case of the Sturgeon, and among caterpillars the Tussocks and the Spherigidæ. One very curious instance may be quoted of a series of repetitions which, though essentially arranged with reference to the axis of a limb, have yet a definite relation to the long axis of the body. This instance is that of the Vertebrate tail, which has often been adduced by opponents of the Annelid theory of Vertebrate descent. Now, the structures which repeat themselves in the Vertebrate tail with great variability of number, namely, the vertebræ with their neural and hæmal arches, the segmental vessels and nerves, &c., are precisely those structures upon whose repetition in the trunk the view of the primitive character of the segmentation of the Vertebrata mainly depends.

In the foregoing pages the attempt has been made to show that greater or less repetition of various structures is one of the chief factors in the composition of animal forms, that these

repetitions may be of greater or less extent, affecting single or many organs, and may be at first irregular, and finally culminate in regularity, and that even this regularity may afterwards vary so as to become a symmetry of a different order. It is further contended that between repetitions in these varying degrees it is impossible to draw any hard and fast distinction, for nothing more can be affirmed as yet about them than that they are repetitions. The reason for their appearance is as yet unknown, and the laws that control and modify them are utterly obscure. But in view of what has been adduced it is surely not too much to say that enough of their mode of working can be seen to enable us to realise that they are at least powerful enough to have produced anatomical features of high importance, and further that the metameric segmentation of the Vertebrata is distinctly of the kind which could be brought about by their operation. That in this case they have attained a degree of completeness far exceeding that which they elsewhere present must be admitted; but there is no evidence to show that this result differs in kind from that which occurs on a smaller and more restricted scale in almost all animals. Whether the repetitions which occur in the Annelids and Arthropoda are also the products of this force in a still higher degree cannot yet be certainly stated.

General Conclusions as to the Mode of Occurrence of Repetitions of Organs.

In the present state of biological knowledge no guess can be hazarded as to the cause of the facts above quoted. The solution of the problem must be sought in a fuller knowledge of the laws of growth and variation, of which we are still ignorant. As yet only one or two features in these repetitions may be mentioned as possibly of importance, though even these can only be selected in the most tentative manner.

In this connection the first noticeable fact is that the structures repeated in the Triploblastica are very generally of mesoblastic origin, and that when other structures have become involved this would appear often to be a secondary

occurrence. To such an extent is this true that in a recent contribution to this subject (Caldwell, 'Quart. Journ. Mic. Sci.,' 1885), a suggestion has been made which proposes to give a simple physical explanation of all the phenomena of segmentation. Caldwell suggests that owing to the early acquisition of the long axis of the body and the consequent elongation of the blastopore, the mesoblast has become, so to speak, left behind in blocks, in consequence of the more rapid growth of the epiblast. That this extremely simple theory will not account for all cases of repetition is shown, firstly, by the fact that though the repeated structures are generally mesoblastic, yet they are not always so; secondly, that the mesoblast does not thus originally segment as a whole, but rather that separate organs repeat themselves separately, as has been already urged, especially in the case of the Turbellaria; and finally, these repetitions are by no means universally embryonic or even larval features, but their whole history rather points to their having very generally originated in the adult condition, and to the view that they have come to be thus earlier in development, the opposite of which is assumed by such a hypothesis as Caldwell's.

This belief that these repetitions have had their origin in variations which occurred in the first instance late in life is founded upon several considerations. Firstly, the cases in which the generative organs are repeated are very numerous; in fact, both organs or the testis, at all events, are repeated in nearly all the cases in which much repetition is found (in most Dendrocœles, Chætopods, Nemertines, Balanoglossus, Amphioxus), even if few other systems are repeated. In the case of these organs it is most likely that the repetition first arose in adult life, and, in fact, in most of them it does still so arise; that is to say, the masses of cells which are to form generative organs are not specially broken up at an early age. And in the second place, the original late origin of repetitions is likely from the fact that most of them still so arise; it is only in exceptional cases as that of the mesoblastic pouches of Vertebrata, Phoronis, Enteropneusta, and the horns of the

water-vessel of Echinodermata, that some of the repetitions are presented early in the development.

Besides the probability that most repetitions occur in the first instance in adults, or, at least, in mature individuals, it may also be noted as a general feature of them that they are at first very similar to, if not identical with, each other. For on their first appearance in an individual they do not generally arise phylogenetically in the condition which may be supposed to have been that in which the original organs of the same series first arose, but rather from the first they are found as fully differentiated copies of the other members of the series, and not as rudiments. For example, the horns and teeth of mammals, whose number varies greatly, are, in those forms which possess additional ones, not repeated as tubercles or as plates, but rather as fully developed horns, teeth, &c. Though this is not universally true it is yet sufficiently well marked a feature to be of great importance in estimating the probability of the recurrence of such a complicated organ as a vertebra with its correlated parts within narrow limits of race. But no less noticeable is the tendency towards a subsequent differentiation and division of function among members of a series of similar parts as soon as the series is formed or any new member is added to it. This is of course to be seen in the case of the tentacles of Hydromedusæ, the division of the ambulacra of Echinoderms into bivium and trivium culminating in the bilateral symmetry of Holothurians, differentiation between vertebræ, &c.

Beyond this little can be predicated of the mode of occurrence of repetition of parts. Nothing is attained by analysis of the known facts which can be felt to be in any way a basis from which to interpret them. This much alone is clear, that the meaning of cases of complex repetition will not be found in the search for an ancestral form, which, itself presenting this same character, may be twisted into a representation of its supposed descendant. Such forms there may be, but in finding them the real problem is not even resolved a single stage; for from whence was their repetition derived? The

answer to this question can only come in a fuller understanding of the laws of growth and of variation which are as yet merely terms.

Preliminary Remarks on the Repetition of Organs of the Chordata.

In the foregoing pages it has been attempted to show (1) that repetition of organs and sets of organs is of common occurrence among animals, and (2) that however far back a segmented ancestor of a segmented descendant may possibly be found, yet ultimately the form has still to be sought for in which these repetitions had their origin. Hence it follows that in no case must it be held à priori impossible that an unsegmented form showing no degeneration should be related to a segmented stock. But when inquiry is made in the special case of the Chordata as to the condition of the repetitions found among them, it will be seen that so far are they from suggesting that their immediate ancestor of the group must have been segmented, that they even preclude this view. As will be shown, there is a history of the actual steps by which several of the organs (the nervous system, the axial skeleton, and the mesoblast) acquired their repetitions within the group, and certain other structures (the notochord, &c.) persist in an unsegmented form. So that instead of regarding a fully segmented form as their possible ancestor it is necessary to search for a form in which these particular sets of structures at least are not repeated.

For in the first place, taken generally, the development of a Vertebrate consists in the gradual appearance of repetitions, first of one organ and then of another, until at last a climax is reached. The mesoblast divides into blocks, paired peripheral nerves grow out, and segmented tubules arise in connection with the excretory ducts, but the mesoblastic plates were at first unbroken, the medullary plate continues without transverse divisions, though its peripheral organs may be repeated, and the excretory ducts are single tubes with single openings. That many of these structures roughly correspond with each other

is no doubt true, but these correspondences are only partial, and, as will be shown in the sections on the nervous system and vertebral column, a history is preserved to us of the steps by which some, at least, of these repetitions have been attained and of stages in which these correspondences were still more irregular.

The attempt to find the ancestor of the Chordata resolves itself first into the question as to whether the Chordate features, viz. notochord, gill-slits, and nervous system of a particular type were first associated in a form which possessed repetitions in a high degree or not. Now, since the notochord is always unsegmented, it is à priori likely that it arose in an unsegmented form; for, having in view the early period of development at which it arises and the situation which it occupies in the body, and the fact that it is found in the dorsal wall of the gut, the sacculation of which is one of the commonest features in segmented forms, it could hardly have thus arisen without participation in such segmention. On the hypothesis of Annelid descent the facts of the morphology of the notochord are inexplicable; for, seeing that no homologue of the notochord exists among Annelids, on the theory that Vertebrates are their descendants, the notochord must have arisen subsequently to that segmentation, to account for which the Annelid ancestor is postulated. If this were so the notochord, by every rule of phylogenetic interpretation, might be expected to arise late in development, and to exhibit marked segmentation, instead of which it is almost the earliest organ formed, and is absolutely unsegmented.

Similarly from the first, the medullary plate is distinctly a single structure, and without suggestion of transverse division. Not until the peripheral nerves arise is any serial repetition to be found in it, and were it not for theoretical considerations it would not have been supposed that the nervous system of a two-day Chick was a segmented structure. Further, in Amphioxus and the Marsipobranchs the serial repetition, even of the peripheral nerves, is not regular and opposite, the further meaning of which facts will be discussed later.

Lastly, the gill-slits are by their nature repeated structures; but, seeing that nothing resembling them occurs outside the group,[1] their origin and, à fortiori, their repetition has been acquired within it.

It becomes then probable, from preliminary examination of the morphology of the three typically Chordate features, that their first origin was not in a segmented form. There is also one other structure which certainly points in the direction of an unsegmented animal as the immediate ancestor of the Vertebrate. This structure is the liver. Now, the liver is essentially a unique structure in the body which is not repeated. On the Annelid theory of Vertebrate descent it would have to be supposed that the liver either arose as an enlargement of one of the segmental saccules of the gut, or by the coalescence of several. The evidence attainable on this point is distinctly against either of these possibilities; for the liver of all the Vertebrates, and especially of Amphioxus, is markedly and obviously a single structure, not formed by the coalescence of several, while its asymmetrical position and general appearance favour the view that it is a structure newly formed within the limits of the group, rather than a relic of a paired sacculation.

Having then disposed of the à priori objections to regarding an unsegmented form as a primitive member of the group, the attempt will be made to show that the Enteropneusta occupy this position. After this we will proceed to consider the light which this admission will give on the history of the steps by which the organs of the other Chordata acquired their present arrangement, and finally to determine the relation which the various forms included under this head bear to one another.

The Enteropneusta as Members of the Chordata.

The general features of the anatomy of the Enteropneusta place them in a very isolated position. They are extremely

[1] For Semper's suggestion that the cœlomic pores on the heads of some Oligochæts are of the same nature cannot be seriously considered.

like one another, but apparently very unlike any other group of animals. Before Tornaria was known to be a stage in their development they were assumed to be worms of some kind, but after Metschnikoff had succeeded in proving Tornaria to be the larva of a Balanoglossus this was felt as an impossible view of its affinities. Up to this time Tornaria had been regarded by Joh. Müller, who first described it ('Berl. Akad.,' 1849, 1850), and by others who examined it as a varied form of Bipinnaria, which, indeed, it very closely resembles, differing only in the presence of eye-spots, and of a peri-anal ring of cilia; both of which structures are liable to great variation. When, then, Metschnikoff discovered its real destiny, it appeared at first sight necessary to suppose the Enteropneusta closely connected with the Echinodermata, and accordingly Metschnikoff ('Zool. Anz.,' 1880) proposed to include them in a division Bilateralia under the Echinodermata, the remainder of the group forming a parallel division, Radiata. But this generalisation with regard to the group was made solely on the characters of the larva, and almost without reference to the structure of the adult, which, indeed, was little known. So certain, however, did the conclusion seem, that Metschnikoff was led to suppose that the gill-slits of Balanoglossus were mere amplifications of the water-vascular system of Echinoderms, which could hardly have been suggested had it not been felt that no other solution was possible. Since this time the anatomy of the adult has become more fully known, and another mode of development has been shown to occur, and from neither of these additional sets of facts can any confirmation of the Echinoderm theory be derived. Hence we must conclude that the characters of Tornaria are not to be looked to solely in attempting a solution of the problem.

In the development of Balanoglossus Kowalevskii the following important features occur: (1) the origin of the central nervous system is by longitudinal delamination from the skin in the dorsal middle line; (2) at the anterior end of the body a portion of hypoblast is constricted off on the dorsal side to form a supporting structure, i. e. a notochord; (3) the

gill-slits are formed as regular fusions and perforations of the body wall and gut from before backwards. Hence the three features which alone distinguish Chordata from other animals are present, and associated from an early period in development. Added to this the minor features of Chordate anatomy are also represented by (1) the origin of the mesoblast; (2) the remarkable asymmetry of the anterior parts; (3) the opercular fold; (4) the excretory funnels opening into the atrial cavity thus formed. From all these facts we may form a preliminary conclusion that the Enteropneusta bear some relation to the Chordata. We will now discuss what relation this is, and before doing so we must determine what relative importance is to be attributed to the two modes of development known to occur, the one largely embryonic the other pelagic.

In our present state of ignorance as to the mode of development of Tornaria and of the details of its later stages, it is difficult to compare these two modes, but the question as to which is to be regarded as primitive is probably a part of the larger question as to the comparative likelihood of the preservation of ancestral features in the free or in the protected developments. This question cannot be fully gone into here. No general answer has as yet been given to it, and since the balance of probability is very nearly divided between these two possibilities we may be right in assuming either of them to be correct. For the purposes of the following argument it will be assumed that, on the whole, development within an egg-shell, as involving a less complicated struggle with environmental forces, is less subject to variation than that in the open sea, and consequently is more likely to preserve ancestral features. Besides this, in the special case before us, the adult structure is practically conclusive against Echinoderm affinities, to which the pelagic development would point if regarded as primitive.

Assuming, then, that the development of B. Kowalevskii is more primitive than that involving a Tornaria stage, the following features are of great importance:

(1) The animal is ciliated and inhabits muddy sand.

(2) The præoral lobe is enormously developed.

(3) The notochord arises at the anterior end of the hypoblast and grows forwards.

(4) The origin of the central nervous system consists in the delamination of a solid cord of epiblast in the dorsal middle line of the middle third; this, by invagination of its two ends, s afterwards extended as a tube in both directions.

Other collections of nerve-fibre are afterwards deposited in various parts of the body, and finally a general network of nerve-fibre occurs at the base of all the skin of the body, especially in the line of the gill-slits.

(5) The mouth originally faces ventralwards, but comes afterwards to open forwards, being not a sucking but a digging mouth.

(6) The gill-slits for a long time are only one pair, but subsequently are repeated in pairs, increasing in number with increase in the size of the body.

(7) The mesoblast arises as one unpaired pouch, followed by two pairs of pouches.

(8) The blood-system is entirely peculiar, consisting of an anterior heart and a dorsal and ventral vessel, and in B. minutus of two lateral vessels in the intestinal region. The two former are united by a plexus of trunks, which are placed under the skin and below the walls of the gut.

(9) The generative organs are repeated through a large part of the body; in the branchial region more or less following the repetition of the gill-slits.

(10) Of the excretory system little can be affirmed. The cells of the mesoblast appear to have a power of forming concretions, probably excretory, in their substance, and then throwing them into the body cavity. Here they form small aggregations. A large gland (containing a plexus of vessels), apparently performing their function, exists in the proboscis cavity attached to the end of the notochord.

From the proboscis cavity opens an asymmetrical ciliated

pore, placed on the left side of the body, which in B. Kupfferi is stated to be paired.

From the middle body cavities open a pair of pores into the atrial cavity, which is partly enclosed by

(11) A rudimentary operculum.

Having these facts in view, and having set aside the preliminary objection that no high degree of segmentation is present in Balanoglossus, we may consider their bearing on theories as to the ancestry of the Chordata.

Previous Suggestions as to the Ancestry of the Chordata.

Setting aside the possibility of Annelids having been genetically connected with the Chordata, the most notable alternative suggestion is that of Balfour, that the Nemertines might be thus regarded. This view has been supported and extended by Hubrecht. It has thus been thought that the Chordate nervous system might have arisen by the longitudinal coalescence of two such cords as are present in Nemertines. But even the facts of other Chordate developments almost preclude the view that their nervous system is a double structure; the medullary plate of Amphioxus is distinctly single, and it is only in the medullary folds of higher and more complex forms that even an appearance of a double structure is produced, while no really double origin occurs. This being so, the mode of origin in Balanoglossus is practically conclusive against the theory of double origin. It is possible, and even likely, that Nemertines bear some distant relation to Chordata, as will be further discussed subsequently, but if this is so it can no longer be supposed that their nervous system is other than a special development within the group.

In most speculations as to the origin of Vertebrata, it is assumed that all the lower forms of Chordata are degenerate. The supporters of the Annelid theory especially are compelled to resort to this view severally in the case of the Ascidians Amphioxus, and the Marsipobranchs. These, with the exception of the Enteropneusta, are the only forms which could have

been used to throw light on the origin of the group, and they had to be expressly excluded because the suggestion as to the origin of the group had been made without regard to them. In the case of Amphioxus and the Marsipobranchs this theory of degeneracy will not bear examination.

It rests solely in the one case on the fact that Amphioxus has no developed sense organs and lives buried in the sand, and in the other on the semi-parasitic habit of life of the group. This degeneration is postulated to explain the lower degree of segmentation presented by these forms; and the fact remains that of all animals the worms which live most underground are the most segmented types which are known. Hence it cannot be assumed without ontogenetic evidence that degeneration in this direction has occurred. This ontogenetic evidence is entirely absent. Degeneration in this sense means a phylogenetic change of plan; and this change of plan should then leave a mark on the ontogeny, as occurs in Echiurus, &c.; but no event in the development of Amphioxus or of Lampreys points to any such change of plan. The development of these forms is a steady progress up to the point which the creatures finally reach, and in a case of this kind it is gratuitous to postulate degeneration in order to support a preconceived view of the morphology of the group. (Even in the Ascidians, though a well-marked change of this kind does occur, yet it is not a deviation from a segmented to a less segmented form; for with the doubtful exception of Appendicularia, Ascidian tadpoles are quite without trace of segmentation.)

Again, no such evidence of a change of phylogenetic plan is found in the case of the Enteropneusta. Highly modified, no doubt, the adult animals are, but not degenerate. For these reasons the presumption of universal degeneracy on the part of all the lower Chordata will be dismissed, and an attempt made to systematize the facts as they are found.

The Habits of Life and Form of the Body of the Primitive Chordata.

Habits of Life.—The presence of gill-slits in all the Chordata may be taken as positive evidence that they arose in an aquatic habitat. Moreover, such a structure as the notochord cannot be conceived as having arisen in a fixed form. Hence they probably led a more or less free existence. This being so, they may either have been pelagic creatures, as the larvæ of Amphioxus, or may have crept in mud as the larvæ of B. Kowalevskii. Between these two possibilities there is little or no determining evidence. The only feature which seems likely to affect the question is the question as to the original point in the body at which the notochord first segregated itself from the gut. Unfortunately the evidence upon this point is divided. For if we suppose that the condition in Balanoglossus is primitive, and that notochord began as a rod in the dorsal wall of the anterior end of the hypoblast, then this origin would more or less point to a burrowing habit, the notochord functioning as a support for the head in this operation; but if the separation of the notochord in the middle of the body, as in Amphioxus, be held to be primitive, then this would point to a pelagic habit, the notochord serving as a fulcrum, from which the movements of the animal in swimming might be maintained. The absence of fins on the young Balanoglossus and on the young Amphioxus, though pelagic, appears to point slightly in favour of a burrowing habit, though no reliance can be placed on such slight negative features.

Primitive Mouth.—There is one more point that does point in favour of a pelagic habit, namely, the fact that the anteriorly-directed digging mouth of both Balanoglossus and of Amphioxus is of secondary origin, being formed by a modification of a more primitive ventrally-directed mouth.

Balfour, having the mouth of Lampreys and Tadpoles in view, held that the original Vertebrate mouth was suctorial. This the ventrally-directed mouth might have been; but this

fact does not interfere with the obvious possibility of a digging mouth having again intervened, from which such a mouth as that of the Lampreys could easily be derived.

Taking into consideration, then, the fact that in the most primitive forms the mouth is anteriorly directed, and that in the Lampreys it is also anteriorly directed, though of different function, we may tentatively suppose that though the mouth of the possibly original pelagic form was directed ventralwards, and was possibly suctorial, yet probably the mouth of the Marsipobranchs is derived from a digging ancestor, in which the mouth of the hypothetical pelagic form had come to be anteriorly directed in correlation with an acquired burrowing habit. In any case the facts of the Enteropneusta entirely confirm Balfour's view, that the Vertebrate jaws have been developed comparatively long afterwards.

The Skin.—That the skin was originally ciliated there can be little doubt; also it is probable that at first plexuses of nerve-fibre were formed at the base of the ectoderm cells, such as may be seen in many if not in all animals with ciliated skins of this type.

The Nervous System.—The next question relates to the position and mode of the first formation of a differentiated nervous system. The evidence of Enteropneusta, Ascidians, and Amphioxus is united in showing that this first occurred in the dorsal middle line, and not by the coalescence of two lateral cords. The structure of the nervous system of Balanoglossus further shows us a stage in the process by which this nervous cord separated from the skin. By many authors it is supposed that this was accomplished in the first Chordata by an invagination, but the evidence of Balanoglossus is decidedly for the view that a process of delamination preceded this; and, indeed, this being the simple process, might naturally have been expected to have occurred first. In Balanoglossus we see in the trunk the cord still in the skin, in the collar the cord delaminated, and at the ends of this cord the process of invagination commencing and leading to the presence of a lumen. More than this, the mode of

origin of the peripheral nerves is also seen; for those portions of nervous tissue which remain in the skin consist of fibres and a few cells. Into the nervous tissue thus composed run the tails of ectoderm cells, and out of them, on their inner sides, run many fibres into the subjacent mesoblastic tissues. Now, the fibres entering this nerve-substance on its outer side are plainly sensory, or at all events afferent, and the fibres passing from it on its inner side are presumably motor, or at least efferent, seeing that they innervate the mesoblast.

It is clear, then, that on the separation from the skin of a cord thus composed the relations of the efferent fibres will not be changed, as they still remain in contact with the mesoblast. But, on the other hand, if this nerve-cord be entirely separated from the skin the supply of outer or afferent fibres is cut off from it, unless cords of epiblast remain to connect it with the skin. Applying this reasoning to the particular case of the separation of the dorsal cord, we see that the afferent fibres are entering it on its dorsal side, and that the efferent fibres are leaving it on its ventral side. If, then, the cord sinks in from the skin, the efferent fibres coming out on the ventral side to supply the muscles can still do so without being gathered into cords, remaining irregular as they do in Balanoglossus, but without dorsal cords connecting the main cord with the skin afferent impulses could only enter at the two ends which remain connected with the skin; hence I submit that it is probable that the three median cords in Balanoglossus minutus, &c., are to be regarded as the homologues of the dorsal roots of other Chordata. It is at once evident, from the physical exigencies of the case, that if the nervous system arose in this way the dorsal roots were from the first sensory, and that they did not arise as differentiations of roots of mixed function, as has often been supposed. If this is true, then, as the cord phylogenetically comes away from the skin from before backwards the number of these dorsal cords will increase, until finally the cord lies connected all along the body with the skin by a series of median dorsal cords placed at intervals.

Now, returning to what is found in Balanoglossus, it is to be noted that, first, the cord separates from the skin as a solid rod connected at the two ends to the skin, and upon this condition invagination supervenes at the two ends, forming a neural tube in these regions. Let us follow the effect which an extension of this system of invagination along the cord will have upon the origin of the dorsal roots; for it is nearly certain that invagination in this case is secondary to delamination; the condition in Amphioxus, in which the medullary plate folds up after being enclosed, offering a stage of transition between the condition found in Balanoglossus and that of an Elasmobranch, for example. Since the invagination of a plate of tissue differs from the separation of a cord in the fact that it is not the central line, but the two edges of the plate, which remain last in connection with the skin, it follows that, as the process of invagination phylogenetically arrives at the point of attachment of any one of these median dorsal roots, it must take up its new attachment at one of these two edges. It is thus not possible, supposing these views correct, that the dorsal roots could in the first instance have been paired, except on the hypothesis that as the process of invagination phylogenetically reached its point of attachment each dorsal root split into two; which is almost impossible, and which the condition of Amphioxus shows not to have occurred. The other alternatives would be (1) that all the dorsal roots should remain attached on one side to the cord; (2) that they should be attached irregularly to one side or the other; and lastly (3) that they should have been attached alternately to either side. From the nature of the case they could not be opposite. Now, the fact of their alternate arrangement in Amphioxus is almost a proof that the latter alternative was the one which occurred. (It may be observed that, as a physiological convenience, they probably supplied the two sides of the body alternately while yet attached in the middle line.) Thus the opposite origin of the dorsal roots is almost certainly secondary to an alternate arrangement. The fact that it is the foremost pairs which are opposite in

Amphioxus seems to indicate that the process by which they became so occurred first anteriorly.

Let us now follow the history of the ventral roots as preserved to us. In Amphioxus the large nerves or dorsal roots supply the skin and certain sense organs placed among the muscular tissue (Rohon); but into each myotome, opposite each dorsal root, runs a bunch of loose nerve-fibres from the cord. This was stated by Rohon, but denied by Balfour. Improved methods of section cutting leave no doubt, however, that Rohon's observation was correct, and, indeed, these fibres may be easily seen. The presence of these bunches of fibres clearly gives us another step in the formation of the "segmented" nervous system. For in the simplest case, that of Balanoglossus, the muscles are not gathered into bunches, and the nerve-fibres likewise are irregular. In Amphioxus the muscles are already gathered into bundles, and the motor nerves follow them in this arrangement, but remain distinct from the dorsal roots. This therefore is a stage towards the gathering of the efferent fibres into a "ventral root;" in Bdellostoma this is already done, and though the dorsal roots are already approximately, though not quite opposite each other, yet the ventral roots are not at the same level with them. Besides this, in Lampreys, the anterior and posterior roots are still not united into a common cord, though in Myxine they are thus arranged (Schneider and others).

In this the nervous systems of Balanoglossus, Amphioxus, Lampreys, and Myxine form a graduated series leading up to the condition found in higher Vertebrates, showing the evolution of the nervous system of Vertebrata from a solid cord in the skin to its condition as a closed tube whose walls give off a series of "segmental" nerves arising by roots of different functions.

[It will be seen that if this view be accepted it becomes very doubtful whether efforts to analyse the segmentation of the head can lead to any result, seeing that it almost follows that the head was differentiated as such before any complex metamerisation was present; and, indeed, were it not for theoretical

considerations, it could hardly have been supposed that the head of a three-day chick, for example, was a highly segmented structure, seeing that the regular segmentation of the body conspicuously stops at its junction with the trunk. No doubt the cranial nerves may, by arbitrary divisions and combinations, be shaped into an arrangement which more or less simulates that which is supposed by some to have been present in the rest of the body, but little is gained by this exercise beyond the production of a false symmetry.]

The Axial Skeleton.—The notochord of the Enteropneusta is so partially developed that it is not difficult to conceive that its presence in the middle third of the body may indicate a stage in its phylogenetic appearance. If while in this condition it was used as a fulcrum in swimming it seems further conceivable that if this organ grew backwards the condition of the Ascidian Tadpole's tail would be produced, though no stress can be laid on this view. As will be shown later on, it is likely for other reasons that the Ascidians separated themselves from the other Chordata before Amphioxus, or even the Enteropneusta.

By extending the separation of the notochord the condition of Amphioxus is reached. And next, the axial column of the Marsipobranchs shows us the notochord enclosed in a mesoblastic sheath as yet unsegmented. This process is foreshadowed by the presence of rings round the neural canal, placed between the nerves whose segmentation they follow. Finally, in the other Vertebrata the column itself is segmented, so that this is another instance of the appearance of a typical segmentation in a system of a Vertebrate whose origin within the limits of the group is unmistakeably traceable.

The Myotomes.—Intermediate conditions between the condition of the muscles of Balanoglossus and of Amphioxus are as yet unknown. I submit, however, that it is not impossible to conceive the formation of myotomes by a simple mechanical process of gathering the muscular fibres into bundles. Their origin as archenteric pouches may then be supposed to have originated from the fact that the ancestral

mesoblast already arose thus, and when new bundles of muscles formed in the adult began to arise in the larva they arose in the same manner as the primitive mesoblast. That provision is made for the production of more mesoblast than that of the original fourteen pairs of pouches is shown by the presence of mesoblastic pole-cells in Amphioxus (Hatschek). In any case the existence of Balanoglossus proves that the notochord, gill-slits, and Chordate nervous system were present together before the myotomes were formed.

The Gill-slits.—It is unfortunate that the facts of the Enteropneusta seem to throw no new light on the original meaning of gill-slits. That they do not do so tends, however, to show that probably gill-slits were from the first developed as such, and not as modifications of any previously-existing organ, as has been sometimes held.

The folded skeletons of the gill-slits of Balanoglossus are remarkable in their resemblance to those of Amphioxus. Until the development of these latter is fully known no further comparison can be instituted. It is clear from their origin in Balanoglossus that no "myotomes" are obliterated between them (as has been suggested by some, with the hope of increasing the symmetry of the body), for plainly their repetition preceded that of the myotomes.

The Excretory System.

Upon the origin of the excretory system of Vertebrata nothing can be affirmed from a study of Balanoglossus. The excretory systems of Vertebrata cannot be easily derivable from anything found in either Balanoglossus, Ascidians, or Amphioxus. The absence of any regular excretory system in these three forms may, perhaps, be correlated with the extraordinary development of their respiratory systems, which may possibly assist in this function. The one fact which is derivable from the morphology of Balanoglossus, Ascidians, and Amphioxus, is that it is nearly certain that the excretory system of other Chordata has been developed within the group.

The Pituitary Body and Proboscis Pore.—Though

no insistance is placed on the following suggestion, the plausibility of it is such that it cannot be omitted. On a previous occasion I have called attention to the fact that the pore which in Amphioxus leads into the left anterior body cavity is obviously homologous with the proboscis pore of Balanoglossus, which leads from the left horn of the anterior body cavity. In some species of Balanoglossus the opening of this pore is placed medianly, though opening into the left horn. Now, supposing the præoral lobe to atrophy, as in an Ascidian, so that the neural pore came to open into the buccal cavity, as occurs in these forms, it is clear that any pore placed dorsally between the neural pore and the mouth will then be directed ventrally, and open into the pharynx below the end of the nervous system. This is precisely the position occupied by the ciliated pit of an Ascidian, which leads into the gland described by Julin ('Arch. de Biol.,' 59). Hence with this pore and gland of an Ascidian the proboscis pore and gland of Balanoglossus may be compared. Next, supposing the end of the nervous system to dilate and form a brain which bends up by a cranial flexure it follows that on the atrophy of the proboscis (or rather before the proboscis was formed, this being peculiar to Enteropneusta) this pore will lie in the dorsal wall of the stomodæum, i. e. in the position of the pituitary body. More than this, any gland attached, as is the proboscis gland, to the end of the notochord, will, when this is flexed by the cranial flexure, be bent backwards with it to the place where its end comes to lie, i. e. above the pituitary involution. In this way the double structure of the pituitary body becomes intelligible. If these views are correct the pituitary body and its pore is to be regarded as the rudiment of a primitive excretory organ, which originally opened dorsally.

I have elsewhere shown the primâ facie resemblance of the anterior body cavity with its pore in Amphioxus to that of Balanoglossus, which in the Tornaria development is formed from the water-vessel (Spengel). This water-vessel is precisely similar to that of Echinoderms, being otherwise without parallel among animals.

The Affinities of the Chordata.

Having thus examined the history of those organs which the morphology of Balanoglossus enables us to trace, let us consider the relations of Chordata (1) to other groups, (2) to each other.

Of the Echinodermata.—Unlikely though it may seem, if any reliance can be placed on the characters of pelagic larvæ, we must assume some affinity between Echinodermata and Chordata, for Tornaria is not very like, but practically identical with, Bipinnaria. The case is like that of Mollusca, which may be supposed to be allied to Annelids, as is indicated by the trochosphere larva.

Of the Nemertines.—So much has been said by previous writers as to the Chordate affinities of Nemertines that the subject cannot be omitted. The suggested homology of the nervous system has already been dismissed. Hubrecht has further suggested (1) that the notochord is homologous with the proboscis sheath of Nemertines, (2) that the cephalic pits are gill-slits, (3) that the proboscis is the pituitary body.

With regard to (1), what can be adduced from a study of Enteropneusta seems rather to be opposed to this view. If this were true, the notochord must have arisen in some such body as that of a Rhabdocœl, into the wall of the endoderm of which a præoral lobe could be invaginated, rather than as a hard thickening which is constricted off to form a lumen. Into the free end of such a structure it is impossible to conceive the invagination of a proboscis, which is what Hubrecht's suggestion seems to require. All that can be said is that the notochord of Balanoglossus suggests that it arose as a supporting structure and not as a modification of something else.

But supposing the larva in Stage G to represent a phylogenetic phase, several points of Nemertine anatomy can be derived from it. At this stage it has one pair of gill-slits, a short nerve-cord, one median anterior mesoblastic pouch, and two pairs of posterior pouches. Now, on the hypothesis of

Hubrecht that the œsophageal pouches of Nemertine were the homologies of gill-slits, and supposing the proboscis invaginated and around its base a quantity of nerve-tissue deposited as in Balanoglossus, the proboscis would then have the same relation to the nerve-ring as that found in Nemertines. Hubrecht's view of the pituitary body falls if the alternative here given is accepted. Though the points of anatomical resemblance are not striking, yet when taken with the ciliated skin, the ventral mouth and position of the generative organs they form a basis for comparison.

If these resemblances were found to be real the nervous system of the Nemertines would have to be supposed to have arisen within the limits of the group. As both animals possess a nerve-plexus in the skin this does not seem impossible. Also the excretory system lately described by Oudemans (' Quart. Jour. Mic. Sci.,' 1885), would have thus arisen as a specialization of parts of the body cavity; since in Balanoglossus this function appears to be generally distributed over the body cavity, this also might be conceived.

Of the Tunicata.—Next, since all the Chordata at some period of their development agree with the larva in Stage H, in possessing a dorsal nerve-cord more or less invaginated, one or more pairs of gill-slits and a notochord, let us pass on to Stage H, in which the notochord is forming at the anterior end of the gut. From such an animal as this the Ascidians may have been descended. For, as has been suggested by van Beneden and Julin (' Archives de Biologie,' 1885) it may be, that all the Ascidians have but a single pair of gill-slits; for that Appendicularia has only one pair is known; while in some genera the atrial cavity arises as an increase in the size of the pair of ciliated chambers by which the gill-slits open; and this increase may take place in the hypoblastic half of the chambers, or in the epiblastic; by the fusion of these two chambers the atrial chamber of these genera is formed. Van Beneden and Julin then suggest that the atrial pore is the actual opening of the two fused gill-slits, and that the rows of slits placing the pharynx in communication with the atrial

chamber are to be regarded as secondary perforations. Whether this ingenious theory be adopted or not, the fact remains that Appendicularia is almost certainly a very primitive Tunicate, and also that the arrangement of the pharyngeal perforations of other Ascidians makes it unlikely that they are homologous with the gill-slits of higher forms.

The increase in size of the tail, which would speedily follow the first use of the backward directed notochord as a swimming organ is not difficult to understand. In connection with the increase of the tail the curvature of the gut would also be intelligible. From atrophy of the præoral lobe in correlation with the future sessile habit, coupled with increase of the lower lip to bear the suckers, the relations of the neural pore to the mouth would result. The gland of the præoral lobe would then, as before described, be placed below the nerve-ganglion and open into the pharynx.

It has been remarked by Seeliger (' Jen. Zeit.,' 1885) that the body of the Ascidian tadpole appears to consist of one head and two trunk segments. It may be observed that though the reasons for this belief are not very obvious, this view, if correct, would coincide with the possibility of its descent from such a larva as Balanoglossus, Stage G, which also possesses one head and two trunk segments.

However the various points that have been raised in the preceding paragraph may be decided, it has seemed necessary to point out what conclusion with regard to the structure of Ascidians may be drawn from the development of Balanoglossus. That these are so meagre is to be regretted; the only tangible point appears to be the confirmatory evidence that it offers to the view that the atrial folds of Tunicata are not homologous with those of Amphioxus.

In this way only can the absence of mesoblastic repetitions in Tunicates be accounted for. Their development gives no support to the view that their ancestors possessed repetitions of this kind.

Of the Enteropneusta.—That the Enteropneusta might possibly have had an ancestor in an animal possessing the

structure of Stage H is of course shown by their ontogeny. They are derived from it chiefly by increase in size of the præoral lobe, change in direction of the mouth, growth of a rudimentary operculum, serial repetition of the gill-slits, and appearance of the generative organs also as a serial repetition. That any animal possessing a large præoral lobe should acquire a thick sheath of nervous tissue (especially when consisting of fibres for the most part) is easily understood. As shown in the foregoing pages, this mass of tissue is probably mainly composed of afferent fibres connecting the proboscis with the dorsal cord. As soon as the ventral nerve-cord arose as a concentration of nerve-tissue, this would naturally be followed by another circular concentration in the nervous sheath connecting the ventral cord with the central, invaginated, nervous system, also as an afferent mechanism.

In all probability the enormous increase in size of the larger species was a comparatively recently acquired feature, as also the peculiar odours which they emit; to this latter power it is possibly not too much to attribute the preservation of such a group.

Of the Cephalochorda.—The relations of the Cephalochorda is the next subject for consideration.

The young Balanoglossus agrees with Amphioxus, especially in the following anatomical features:—

(1) The digging mouth.

(2) The repetition and folding of the gill-slits.

(3) The repetition of the generative organs.

(4) The peculiar fate and remarkable asymmetry of the anterior mesoblastic pouch and proboscis pore.

(5) The presence of atrial folds.

(6) The absence of (*a*) any developed sense organs; (*b*) any excretory glands differentiated as such.

(7) In the presence of excretory tubes opening into the atrial cavity.

On the other hand it differs from it in—

(1) The relative size of the præoral lobe.

(2) The degree of its mesoblastic repetition.

(3) The degree of the invagination of its nervous systen and the extent of the neural tube.

(4) The extent and degree of isolation of its notochord.

(5) The extent of the atrial folds.

(6) The absence in B. Kowalevskii of any definite liver sacculi, and the presence in B. minutus, &c., of liver saccules differing from those of Amphioxus.

The points of resemblance taken together are so considerable as to suggest that they were possessed by a common ancestor of the Hemichordata and Cephalochorda. On the other hand, the points of difference are nearly all differences of degree, and (1), (2), (3), (4), (6) are points in which the Vertebrata agree with Amphioxus. In the case of (5), however, the Vertebrata more nearly agree with Balanoglossus.

Of the Vertebrata.—The common ancestor, then, of the Cephalochorda and the Vertebrata may be presumed to have possessed the features of mesoblastic repetition, invaginated nerve-cord, and consequent extension of the neural tube, raised, so to speak, to the degree in which they are found in both those divisions. Also it may be believed that the præoral lobe had somewhat diminished and that the atrial folds were still small. The origin of such a liver as that of Amphioxus, as a specialisation of part of the wall of the digestive region of a young B. Kowalevskii is easy to imagine, for the histology of these two tissues is still almost identical. [The presence of peculiar liver saccules in B. minutus, &c., presents no difficulties, as their absence in the more primitive B. Kowalevskii shows that they have arisen within the limits of the group.] Animals possessing those features would answer nearly to the Protochordata of Balfour, though the structures now attributed to it are somewhat different.

The Protochordata thus constituted would then differ from the Enteropneusta in the possession of a serially-repeated mesoblast, in addition to serially-repeated gill-slits, and possibly generative organs; also in the complete separation of the nervous system and notochord. The serial repetition of the gill-slits, the small operculum, &c., they must be presumed to

have acquired from the ancestor common to them and the Enteropneusta.

In this way the connection of the Protovertebrata of Balfour with the other division becomes explicable on the new facts derived from the Enteropneusta.

The peculiar fact that so many of the features of the Enteropneusta differ from those of the Cephalochorda in degree of expression only is very remarkable, and suggest that their further evolution towards the Protochordate type proceeded by correlated variations affecting the several systems.

From the Protovertebrata thus constituted, which in all probability possessed an unsegmented mesoblastic sheath for the notochord and a brain, the Cyclostomata may be easily derived without the necessity of any hypothesis of great degeneration, which cannot be well supported.

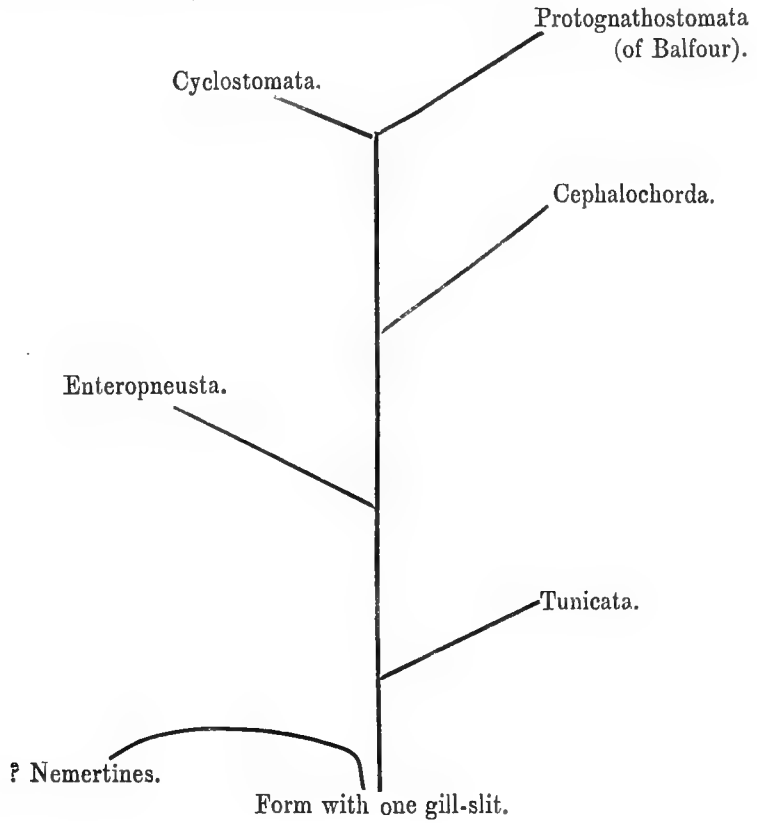
Balfour has fully discussed the question of the origin of his hypothetical group of Protognathostomata, and upon the question of their immediate origin no new light can be thrown.

The above suggestions entail many difficulties. The chief of these is that they involve the hypothesis that the rudiment of the notochord of the Archichordata developed itself as a separate structure, once in the case of the Ascidians, and again in the case of the Protochordata. In the first case, owing to the atrophy of the præoral lobe and use of the tail in swimming, it came to lie in that organ, and in the second case extended through the whole length of the body. Also does this suggestion of the origin of the Tunicates involve the proposition that the rudiment of the dorsal nerve-cord extended itself twice along the body, once in the case of the Ascidians, and again in the case of the Protochordata. If this occurred there is no difficulty in supposing it to have been twice invaginated, this being a more less common feature among nervous systems.

Another difficulty which affects all these suggestions arises from the epiblastic origin of the generative organs of Enteropneusta, in which they resemble the Echinoderms.

Though it is likely that many of the suggestions here made may be shown hereafter to be wrong, still it has seemed well, on the whole, to analyse the facts as they stood, and to endeavour to reconstruct the past stages, whose existence is indicated by the lacunæ in the sequence of these facts, avoiding as far as possible a reliance upon phylogenetic changes of whose occurrence we have no evidence.

The foregoing views are, perhaps, more clearly expressed in the following table, which is not meant so much as a genealogical tree as to serve as an exhibition of the logical relation of the various forms, showing their points of divergence.



The Development of the Mole (Talpa Europea).

STAGES E TO J.

By

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With Plates XIII, XIV, and XV.

DURING the preparation of the following paper I have been conscious that a considerable proportion of the matter included is of little special interest; at the same time it has appeared to me that the course of the development of certain organs in the Mole deserves to be recorded, and in order to do so satisfactorily I have been compelled to mention much which is not different from embryological phenomena already observed in other Vertebrates.

I have further been led to hope that a somewhat complete account of the development of one of the Insectivora will not be without value.

To facilitate reference I have described the development of the embryo in stages, which, in continuance with the stages of growth described in a former paper (No. 8), will be called Stages E, F, G, H, and J. A summary of the various sections of this paper will be found on p. 132.

EXTERNAL FEATURES.

Stage E.—The youngest embryo which I have figured (fig. 1) lies flat upon the surface of the blastodermic vesicle. The embryo is ·76 mm. long, and is narrow in the centre and wider

at each end. A shallow medullary groove runs down the centre of the long axis of the embryo, which in its turn is narrow in the centre and wider at either end. On each side the medullary groove in the central narrow portion of the embryo, three protovertebræ may be seen already formed.

The hinder end of the embryo is thickened owing to the growth of the mesoblast of the primitive streak, while anteriorly it is flattened out to form the cephalic plate. The shaded portion surrounding the embryo (*a. p.*) is the extent of the area pellucida at this age.

Fig. 2 represents a slightly older embryo of the same stage of growth (1·82 mm. long). The medullary folds have here begun to form, they are raised somewhat, and in the centre of the embryo are already approximated. At the anterior end the floor of the medullary groove, on either side, is swollen, and on the outer and anterior edge of the two masses so formed a deep narrow groove indicates the commencement of the formation of the optic organs and will be referred to as the "optic grooves."

This early appearance of the organ of sight is, so far as I am aware, peculiar, and is worthy of notice; even at this age the grooves are directed outwards and downwards, and have their origin from the most anterior portion of the medullary groove. The curved condition of this embryo is due to careless manipulation whilst it was in a fresh and soft state.

Stage F. — Fig. 3 represents an embryo of this stage of growth; it is 1·96 mm. long. The medullary folds have met, although they have not yet coalesced, in the middle of the embryo, and have extended thence forwards.

The anterior end of the medullary canal is, however, still widely open, and the two thickenings of the floor and sides of this portion are shown. The optic grooves are also indicated in the same manner as they were in the previous figure.

It will be observed that the sides of the medullary canal at the anterior end have grown forwards in advance of the floor. At the hind end the medullary canal is widely open, forming

the sinus rhomboidalis. On either side of the embryo, just behind the widely open anterior end of the medullary canal, a ridge extends backwards and onwards over the blastodermic vesicle; these ridges are the first traces of the two tubes which will eventually form the heart (compare fig. 5, *ht.*).

Figs. 4 and 5 are two drawings of an embryo of about the same age as that last described (Stage F). The length of the latter is, however, greater than that of the former embryo, being 2·12 mm., while the medullary groove is not so far advanced in development. My object in drawing fig. 4 is not only to show these points but to represent the amnion, which is as yet developed only at the hind end of the embryo, and has already grown nearly half way over the back of the embryo.

Fig. 5 is a transparent view of the same embryo, and indicates the position of the first five protovertebræ, and of the commencing tubes (*ht.*, *ht.*) which eventually will form the heart. The blind lateral prolongations of the medullary groove at the cephalic end are the optic grooves. In this figure also the floor of the sinus rhomboidalis at its posterior end is seen to contain a much thickened, forwardly projective knob, which, as will be shown in sections, is the anterior end of the primitive streak. The medullary folds may therefore be described as extending posteriorly behind the front end of the primitive streak.

Stage G.—Stage G is represented by the embryo drawn in fig. 6. The hinder portion of the medullary canal is much the same as before; anteriorly, however, development has progressed, and the edges of the medullary folds have come together and partially fused at the anterior end of the embryo. At the extreme end, however, a pore is left, owing to the more rapid growth of the sides than of the floor of the canal as pointed out above. At this stage, therefore, the neural canal is still open to the exterior, both anteriorly and posteriorly.

The optic grooves are now closed, and have given rise to the

optic vesicles; these are shown as two bud-like vesicles projecting outwards and backwards, and slightly downwards from the front end of the neural tube; behind them the swelling of the fore-brain is discernible, while still further backwards and at the edge of the body of the embryo the two tubes of the heart are indicated.

The folding off of the embryo from the yolk-sac has at this stage made some progress, and, indeed, the whole of the head of the embryo as far back as the line *so. pl.* now lies projected freely above the blastodermic vesicle.

Stages H and J.—These stages are depicted in figs. 7 and 9, the embryo represented in the former figure being 2·2 mm. long, that in the latter figure 3·06 mm. long. The more complete closure of the medullary canal and the constriction of its anterior region into fore-, mid-, and hind-brains is to be noticed. The optic vesicles are still seen in fig. 9; in fig. 7 they are barely noticeable, owing to the curved position of the embryo when drawn.

The increase of the protovertebræ and the gradual reduction of the sinus rhomboidalis is also seen, while the thickened anterior end of the primitive streak is now enclosed within the posterior walls of the medullary canal, and projects upwards as a rounded knob at its hinder end.

The direction of the increase of the protovertebræ is a difficult matter to determine, but a careful examination and measurement of figs. 5, 7, and 9 leads me to believe that in all probability the increase is almost altogether posteriorwards during those stages. The embryo (fig. 7) of Stage H has, however, apparently one protovertebræ more anteriorly than the embryo of Stage F (fig. 5), and the embryo (fig. 9) of Stage J one more than that of Stage H (fig. 7). The embryos of Stages E and F are more difficult to compare (figs. 1 and 5), but I think it is highly probable the increased number in the latter is due to a backward growth.

The amnion at Stage H completely covers the embryo (fig. 7), an anterior limb having grown over the head as the

posterior limb grew over the tail at an earlier period (Stage F, fig. 4).

The anterior fold of the amnion (*vide* p. 128) is the so-called pro-amnion of Beneden and Julin (No. 2). It must be noted that up to the close of Stage J no signs of a folding off of the tail end of the embryo can be observed, and, indeed it is not until considerably later that this process takes place.

The first junction of the two tubes to form the heart takes place during Stage H, and is shown in fig. 8; while the side view of the head of the embryo drawn in fig. 10 (Stage J) shows the relation of the heart to the visceral arches, and the arrangement of the latter.

There are at this stage five visceral arches. Faint grooves indicating the partial formation of two and even three visceral arches may be discerned during Stage H, but it is not until Stage J is reached that they can be satisfactorily outlined.

For the comparison of these figures with figures of other mammalian embryos I would refer to papers Nos. 3, 4, 5, 6, 7, 9, and 10.

The Epiblast.

Soon after the epiblast is first definitely produced it is in the form of a plate of columnar cells of uniform thickness over the whole embryonic area, and passing abruptly at the edge into the flattened epiblast cells which cover the remainder of the embryonic vesicle. This stage is figured in a former paper, No. 8, fig. 30.

During the primitive streak stage of growth and the early formation of the medullary groove, the lateral epiblast becomes reduced in thickness and at the edge of the area the cells gradually assume a flattened condition and blend without a break with those of the vesicle (l. c. figs. 32—36, and 43—46).

The appearance of protovertebræ and the deepening of the medullary groove is attended by a further modification of the epiblast of the embryo.

During Stages E to G the median portion becomes thickened

and forms the medullary plate (fig. 15) while the lateral portions become gradually still more reduced in thickness, until in those portions of the embryo where the medullary groove has attained its greatest depth prior to its conversion into a canal the lateral epiblast is formed for the most part of a single row of somewhat cubical cells, continuous, without modification, either over the vesicle or across the embryo as the inner fold of the amnion (fig. 17).

Where the lateral epiblast joins the wall of the medullary groove there is now an abrupt transition from the columnar cells lining the latter to the cubical cells of the former.

Subsequently, Stages H. J., in that portion of the embryo where the neural canal is formed, the closure of the medullary groove causes the approximation of the lateral portions of the epiblast, which fuse, and thus form a continuous layer across the embryo. The cubical epiblast cells at the same time become much flattened on the dorsal surface of the embryo (figs. 26, 29, and 47), while (1) in the trunk, the cells of that portion of epiblast which overlies the somatic mesoblast remain cubical (figs. 26, 27, 29, and 47); and (2) in the anterior region, the cells of certain portions which either give rise to sensory structures (figs. 25 and 46), or which surround externally the visceral arches (figs. 23 and 46) assume again a columnar form.

In that region of the trunk where the medullary canal is still open the lateral epiblast cells remain as before, cubical.

The Medullary Groove.—At the commencement of Stage E a deep medullary groove exists about the middle of the embryo; anteriorly and posteriorly it is shallower however, finally terminating in the latter direction upon reaching the anterior end of the primitive streak, while in the former direction all trace of the groove is lost some considerable distance behind the front end of the embryo.

Beyond the anterior end of the medullary groove the epiblast is thickened to form the "cephalic plate."

Fig. 1 is a transparent view of an embryo with three protovertebræ, and shows the relations above mentioned; I have

also figured three transverse sections, which indicate the structure and form of (1) the cephalic plate (fig. 12); (2) the groove in its anterior portion (fig. 14); and (3) the groove in its posterior portion (fig. 15).

In fig. 12 the thick cephalic plate is shown, becoming folded off from the yolk-sac; fig. 14 is taken from the region in front of the protovertebræ, and depicts the wide and shallow groove, the wall at the bottom of which is considerably thinner than at the edge of the groove; and in fig. 15, taken from the region of the second protovertebra the medullary groove is V-shaped, and the columnar cells of which it is formed pass abruptly into the lateral epiblast cells, thus indicating the extent of the "medullary plate."

At the hind end the wide and shallow medullary groove forms the so-called "sinus rhomboidalis." A section through this region of an embryo during Stage F is shown in fig. 18.

At the close of Stage E the groove has considerably increased in length, and during Stage F it reaches to the anterior end of the embryo (figs. 3 and 16). The latter figure is a transverse section through the anterior end, and shows—

(1) The median medullary groove.

(2) The commencement of the curvature upwards of the lateral portions of the cephalic plate and the formation of the two "optic grooves" (*op. gr.*), seen in surface view in fig. 4, which give rise when the neural canal is closed, to the optic vesicles.

The Medullary Canal.—The medullary plate is now sharply marked off from the lateral epiblast from a considerable distance in front of the first protovertebra backwards to the posterior end of the embryo, and the groove itself commences to close in the region of the protovertebræ.

The closure is effected by the approximation of the peripheral edges of the medullary plate, a sharp angle being thus formed at the junction of the lateral epiblast with the edge of the plate (fig. 17).

The closure commences at a late period of Stage G in the region of the first provertebra, extending thence forwards and

backwards; it proceeds very rapidly, being at the end of this stage, although open at its immediate anterior end (fig. 6), closed from there posteriorly until the fourth protovertebra is reached, after which point it gradually widens out into the sinus rhomboidalis (figs. 28 to 33).

At the close of Stage H a narrow slit-like pore is all that remains open at the anterior end (fig. 20), while posteriorly it is closed as far back as the eighth protovertebra; and at the end of Stage J the whole groove is converted into a canal until the last, the fourteenth, protovertebra is reached.

The sinus rhomboidalis is now narrow and shallow (figs. 48 and 50). The swelling in the floor at the hind end of the sinus rhomboidalis is caused by the mesoblast of the front end of the primitive streak (figs. 33 and 35; 48 and 50).

When the canal is first formed, its lumen—except in the anterior region which is described below—is a narrow slit and its walls are thicker at the sides than they are dorsally and ventrally (fig. 28); soon afterwards, however, during Stage H, the middle portion of the lateral walls thickens still more and projects into the narrow lumen of the canal, thus converting it into an hour-glass form (fig. 29).

The cells of the cord are much elongated, and their nuclei, in general, oval (fig. 43).

I may in this place mention there appears to me to be great likelihood of the migration of mesoblast cells into the walls of the medullary canal during Stages H and J. Sections of an embryo belonging to the former stage present strong evidence of this process (fig. 43). Two masses of mesoblast cells are to be seen in very close connection with the lateral walls of the canal in the region of the neck, and from these masses I feel inclined to believe certain cells grow into the tissue of the nervous system.

As I will show below, these masses of cells are in connection with two blood-vessels, which are in process of formation, and it would appear highly probable that these ingrowing mesoblast cells give rise to the blood-vessels of the spinal cord.

The Brain. — When the medullary groove first closes in

(Stage G) it is wider in front of the first protovertebra than it is in the latter and posterior regions, and faint indications of a division of the brain into portions may be discerned in section, and to some extent also in the surface view of this stage; the hind-brain, with its somewhat thinner roof, is of considerable length and blends into an anterior portion in which the roof is thicker. Stage H shows some little advance upon this; the cranial flexure has begun (fig. 34) and the cavity of the brain has increased in size, the roof of the hind-brain also is thinner and wider than before (fig. 23).

At the close of Stage J three divisions of the brain are indicated (fig. 49). There is a well-marked cranial flexure, and at what is now the anterior end of the animal the mid-brain is situated. The cavity of the mid-brain is partially separated from that of the fore-brain by a constriction of the walls at the junction of the two, but the structure of the wall is very similar in both portions. The hind- and mid-brains pass into one another without any such constriction, but the thin roof of the former distinguishes it from the latter. The lower wall of the hind-brain at the posterior end is now much folded. The lower wall of the fore-brain is curved downwards, forming a short and wide diverticulum which marks the first appearance of the infundibulum. The apex of the infundibulum comes into close connection with the anterior end of the alimentary tract and with the notochord overlying it (fig. 49).

The Optic Vesicles.—The optic grooves seen in the head in surface view in figs. 2 and 5 are the rudiments of the optic vesicles; they are shown in section in fig. 16. Later (Stage H), when the medullary groove forms a closed canal in the head region, these grooves become wide lateral diverticula projecting from the anterior portion of the brain, and constitute the optic vesicles (fig. 20). They are situated dorsally on each side the middle line, and are projected outward and somewhat downwards and backwards.

Such a condition is clearly shown in surface view in fig. 9. Sections of this stage show a very similar condition as regards the development of the vesicles; they merely extend slightly

further outwards, but do not at this stage fuse with the external epiblast (fig. 21).

The wall of the optic vesicles is similar in structure to the wall of the remainder of the fore-brain.

It is interesting to note that for a considerable period after Stage J the optic vesicles show but very slight advancement on the condition then attained; their growth appears now to be retarded in as marked a degree as it was advanced in the early stages. The early appearance of the optic grooves will probably be recognised as a mammalian distinction when the embryology of more species of Mammalia has been worked, but the sudden checking of the development in the Mole we may expect is due to the specialisation of this species. Any modification of an important sensory organ would doubtless rapidly affect the development of the organ, but such an extended modification as is apparent here says much for the primitive nature of the habits of the animal.

The Ear.—The first indication of the ear arises during Stage H as a thickening of the external epithelium on each side the hind-brain (fig. 25). The thickening extends along a great portion of the hinder half of the hind-brain, and during Stage J increases in thickness and becomes grooved along the greater part of its length (fig. 46).

The Cranial and Spinal Nerves.—I do not propose to describe the development of the cranial and spinal nerves in this paper. I hope to make a separate communication upon this portion of the development at some future time.

The Hypoblast.

The hypoblast in the earliest condition of Stage E is similar to what it was in Stage D (described in my former paper, No. 8), and is composed of a single layer of flattened cells extending on all sides over the embryonic area (figs. 13, 14, and 15).

The cells in the median line give rise to the notochord, and the changes they undergo will be described in detail in another section of this paper.

The formation of the deep medullary groove in Stage D and

the thickening of the vertebral portions of the mesoblast causes the hypoblast cells underlying those structures to be stretched out as it were and flattened (No. 8, fig. 45).

In Stages E and F this condition may still be seen where the groove is deepest in front of the protovertebræ (fig. 13); anteriorly the groove becomes shallower and the hypoblast cells more rounded in consequence (fig. 14), while posteriorly the formation of protovertebræ forces the lateral hypoblast downwards, and the axial hypoblast cells are again thickened (figs. 15 and 17).

In the region of the sinus rhomboidalis the medullary groove again projects considerably below the level of the peripheral body wall, and, forcing the hypoblast cells downwards also, flattens them.

This condition in the anterior region and posteriorly below the sinus rhomboidalis is, however, soon modified; the thickening of the peripheral mesoblast and the gradual depression of the body wall brings the lateral portions of the hypoblast on a level with the axial portion throughout the length of the embryo, and at the close of Stage J the cells of the whole layer, wherever it is not converted into the alimentary canal, become rounded.

The Alimentary Canal.—The first trace of the alimentary canal appears during Stage D (*vide* No. 8, fig. 46) at the anterior end of the embryo as a short tubular diverticulum. In the paper referred to I described this tube as the notochord, an error which I have corrected here and in more detail on p. 118 of the present paper in the section devoted to that organ.

This structure is indicated in figs. 11 and 12, Stage E. The diverticulum has but a small lumen, and is situated close against the cephalic plate; the cells of which it is formed are columnar.

Stages G and H witness further changes; the fore-gut is now considerably longer (fig. 34). It is rounded anteriorly (fig. 22), but farther backwards is widened out laterally (fig. 19) and becomes flattened and crescent shaped, the lateral horns

of the crescent being projected upwards and somewhat closely approximated to the lateral epiblast of the embryo (figs. 19, 23, and 24).

The epithelium of the dorsal border of the sac is thinner than that of the ventral border, the difference being more apparent in the hinder portion than in the front portion of the sac. The points of the lateral horns are lined with cylindrical cells.

There is no distinct evidence at this stage (H) of outgrowths of the fore-gut in the position of the future visceral arches, but slight indications of the invagination of the epiblast may be seen corresponding to the grooves mentioned in the description of the surface view of an embryo of Stage H.

On the ventral surface at the anterior end of the fore-gut in Stages G and H (figs. 19 and 22) two slight invaginations of the epiblast may be seen one on either side of the middle line, and a few sections further backwards the epiblast and hypoblast are closely applied in the middle line, and there is a deep median groove in the epiblast (fig. 23).

At the close of Stage J there is a still further change in these relations. The lateral outgrowths of the fore-gut are now directed towards invaginations of the epiblast which correspond to the grooves mentioned in the description of a surface view of an embryo of this stage (Stage J). The outgrowths are directed outwards and downwards from the lateral portions of the lumen of the canal (fig. 46). The hypoblast and epiblast have met and are partially fused in the case of the anterior diverticula, although there is as yet no perforation constituting a definite cleft, but in the more posterior diverticula the hypoblast does not meet the epiblastic involution.

Now also the fore-gut is a little longer, and the fusion of epiblast and hypoblast on the ventral surface near the front end is closer, although the perforation to form the mouth has not yet taken place (fig. 49).

This invagination of the epiblast is clearly seen in an embryo of this stage to be in the form, anteriorly, of two shallow grooves which converge posteriorly, these forming a deep

median invagination (figs. 44, 45). These grooves are formed along the anterior border of the first visceral arch. The epiblast and hypoblast are in close contact along the whole of the V-shaped groove, but become actually fused posteriorly at the apex, where the mouth will eventually be formed (compare figs. 44, 45, and 49).

It will be seen by the foregoing description that the mouth is formed somewhat behind the anterior end of the fore-gut at the apex of a V-shaped groove on the ventral surface of the head, the diverging limbs of which groove are directed forwards. The section of the gut which is placed anteriorly to the mouth is identical with the blind tube first formed by the folding-off of the embryo from the yolk-sac, and this anterior diverticulum exists for some time after the ventral enlargement of the gut towards the external groove.

These facts appear to indicate that a more primitive mouth, the terminal position of which is indicated by the primary anterior diverticulum of the fore-gut, has been replaced by a secondary formation, the paired origin of which is rendered possible by the two converging grooves in the epiblast of the ventral surface.

If these observations are correct, they must be considered to some extent confirmatory of Dr. Dohrn's theory of the paired origin of the existing mouth of the Vertebrata, but I would suggest that such evidence cannot be used as argument for the paired formation of the primitive Vertebrata mouth, the terminal position of such being exceedingly probable.

As in the earlier stage, the cells forming the dorsal wall of the fore-gut are throughout thinner than those lining the remainder of the cavity, and in the posterior section of its length are much flattened; on the other hand the cells of the ventral wall, the lateral horns, and the outgrowths to form the visceral clefts, are cubical or even columnar in form.

The Notochord.—The notochord, as I have before described (No. 8, figs. 37—48), is a hypoblastic structure and is primitively in connection with the hypoblast and the lateral plates of mesoblast of the embryo. During Stage D it becomes first

separated from the lateral mesoblast, then reduced in thickness, and finally converted (1) in the anterior region into an arc formed of a single row of columnar cells; (2) towards the central deepest portion of the medullary groove into a single row of considerably flattened cells; while (3) in the hinder region it remains thickened and forms posteriorly the anterior wall of the neurenteric canal, thus joining the epiblast.

During this stage (Stage D), the notochord is, throughout its whole length, never actually isolated from the hypoblast, but remains a portion of that layer, although an obviously specialised portion; it is in fact the remnant of the primitive hypoblast (l. c.).

In this same paper (l. c. fig. 46) I described as a portion of the notochord a short tube formed of columnar cells lying below the medullary plate at the anterior end of the embryo. I must here correct that error. This tube does not represent the notochord solely, but constitutes the anterior end of the alimentary tract (figs 11 and 12), and, as I shall show below, the cells only of the dorsal portion of this tube give rise eventually to the anterior end of the notochord.

During Stages E and F the relations of the notochord remain very much the same as they were during Stage D (figs. 14, 15, and 17); it is noticeable, however, at the close of Stage F, that in the trunk of the embryo, where the medullary groove is deep, the axial hypoblast has increased in thickness (fig. 17).

The deepening of the medullary groove towards the anterior region which occurs during Stage G causes the notochord cells situated there to be reduced in the same manner as they were reduced in the central region during Stage D. Similarly the axial hypoblast is reduced in bulk in the posterior region of the embryo, while in the central region, where the protovertebræ are forming, there is a further increase in the size of the notochord.

At this stage of growth (Stage G) the notochord exhibits a tendency to become separated from the hypoblast layer in the

same manner, although not with precisely the same result, as when the neurenteric canal was formed in Stage D.

The process in the latter stage involved the ingrowth of the lateral portions of hypoblast and the conversion of the axial portion, containing the neurenteric canal, first into an arc and then into a complete tube. Now the lateral hypoblast grows inwards below the axial portion of primitive hypoblast and unites to form a continuous layer, merely causing the isolation of the axial portion as either a solid rod or band of cells which lies freely between the hypoblast and the medullary canal. It is, however, true that a lumen may appear in some of the portions of the notochord which are rod like, although its conversion thus into a tube is, so far as I can determine, a secondary matter, and is not connected with the method of isolation.

The isolation of the notochord first occurs in the region of the first protovertebra during Stage G, and extends during Stages H and J anteriorly and posteriorly. The separation does not, however, appear to be a continuous process, and the shape of the isolated notochord is very various. To demonstrate these facts I have figured several sections of an embryo with nine protovertebræ (Stage H, figs. 24 and 36 to 42).

In this embryo, in front of the first protovertebra, the notochord is isolated for some distance as a rod or thickened band (figs. 24 and 37), in which a lumen may occasionally be seen (fig. 36: compare also figs. 23 and 25, which are drawings of sections through another embryo of this stage).

In the region of the first protovertebra, it is in the form of a flattened band consisting of a single row of cells (fig. 38), and this condition persists, except here and there, where the notochord is not completely isolated (fig. 39), until the fourth protovertebra is reached; here it increases in size. From this point it is more frequently attached to the hypoblast (fig. 40), and posterior to the seventh protovertebra is not isolated at all. Immediately behind the seventh protovertebra it is in the form of an arc (fig. 41), which further backwards flattens out, and the mass, increasing in size, joins the front end of the primitive streak (fig. 42).

Such is the condition of the notochord during Stage H. At the close of Stage J, however, the whole of the notochord, except at the immediate anterior end, backwards to the ninth protovertebra, is isolated as a rod of varying size and shape (figs. 46 and 47). Behind the ninth protovertebra it becomes band shaped and continues in this form, still distinct from the hypoblast, for some distance behind the last (fourteenth) protovertebra. It then again assumes the form of a rod, although of much larger size than in the anterior region, in the centre of which a lumen may here and there be seen, and joins the anterior end of the primitive streak becoming thus connected there with the epiblast, hypoblast, and lateral mesoblast (fig. 50).

The phenomena I have here described, viz.: (1) the presence of a mass of primitive undifferentiated hypoblast in the median line (Stage D); (2) its reduction to a thin, even single layer of cells (Stages D, E, and F), and (3) the conversion of those cells into the notochord (Stages G, H, and J); these phenomena, in my opinion, indicate without doubt that this organ is of hypoblastic and not of mesoblastic origin.

Further, during the isolation of the notochord, (*a*) the appearance, vague though it be, of an arc of notochordal cells; (*b*) the fact that the isolation of the solid rod or band commences at the two sides and gradually extends across the median line (figs. 39—42); and (*c*) the occasional appearance of a lumen in this rod,—these appearances indicate that it is formed in the same manner as the notochord of Amphioxus, that is to say by the ingrowth of the lateral hypoblast and the constriction of the axial mass of primitive hypoblast cells.

I have already discussed the views of other observers upon this subject (No. 8) and need not again refer to them.

Figs. 39 and 40 are especially interesting in regard to the isolation of the notochord. In both these drawings the process of isolation is shown taking place; in both the notochordal tissue is in the form of a pair of knobs connected by a median more slender portion; and in both cases when the notochord is actually isolated it will be isolated as a band of

greater or less substantiality. It will be noticed the knobs are more or less free from the underlying flattened hypoblast cells, while in the median line there are no flattened cells, thus showing the process of the growth of the lateral hypoblast below the axial primitive hypoblast.

The relation of the notochord at the front end of the embryo requires special notice; it will be best understood by a reference to figures of longitudinal sections through embryos of Stage E (fig. 11), Stage H (fig. 34), and Stage J (fig. 49). In fig. 11 the notochord is not separated from the roof of the fore-gut; in fig. 34 it remains attached to the anterior wall of the fore-gut, although isolated posteriorly; but in fig. 49 the notochord, although joined at its anterior extremity to the hypoblast, is separated from it throughout its extent posteriorly.

The hooked anterior end of the notochord, so characteristic of this organ, is seen to be due, in the Mole, to the fact that it is derived from the anterior wall of the alimentary tract after the cranial flexure has commenced.

At the close of Stage J, therefore, the notochord is continuous with the epiblast at the front end of the embryo, by means of the front wall of the fore-gut, which is fused with the epiblast at the point where the mouth will eventually be formed; and posteriorly, at the anterior end of the primitive streak, where epiblast, hypoblast, and mesoblast are all joined together (compare figs. 49 and 50).

The close relation of the fore-brain to the notochord, a relation brought about not so much by the cranial flexure as by the ventral enlargement of the brain at this point, will be referred to in another communication, which I hope shortly to make, upon the pituitary body of the Mole.

There is one other point of interest in the growth of the notochord in the Mole, and that is its size compared with the nervous system. The relative size of the notochord compared with the nervous system is less in the higher than it is in the lower Vertebrate embryos. In the Mole the notochord is relatively smaller than it is in any other Vertebrate embryo I am acquainted with, and it appears to me the reduction in size is

due to the comparatively early development of the nervous system. During the early part of Stage D there is a considerable mass of primitive hypoblast along the axial line of the embryo, but the rapidly forming medullary groove pressing on to this mass before it has become formed into a rod capable of resisting much pressure, causes it to bulge inwards and thus flattens out its cells, administering an effective check to the development of the organ. Such a check occurs during Stages D and E. Subsequently the thickening of the lateral mesoblast plates and the consequent depression of the lateral hypoblast, removes the strain from the axial cells and admits of the isolation of the slender rod or band which exists for the greater portion of the length of an embryo Mole at the close of Stage J.

The Mesoblast.

At the close of Stage D the mesoblast in front of the primitive streak is in the form of two lateral plates which are connected together across the middle line by means of a mass of undifferentiated hypoblast, except during a short space where they are separated by the deep portion of the medullary groove.

At the periphery these mesoblastic plates are split into two layers, an upper somatic and a lower splanchnic layer, along the whole of their extent posterior to the cephalic plate. The split is entirely peripheral, however, and does not extend into the embryonic area.

The Mesoblastic Somites and the Body Cavity.—During Stage E the splitting of the mesoblast extends further forwards, and also inwards towards the medullary groove. I have never been able completely to satisfy myself that this splitting ever extends to the innermost portion of the mesoblastic plates; but, as I have before explained, the small size of the embryo and the dense compact nature of the middle layer renders it exceedingly difficult accurately to determine such a point.

The nearest approach to a continuous split of the mesoblast from the axial portion to the periphery which I have seen is

represented in fig. 13; and here it will be seen, although there is no positive division into somatic and splanchnic layers, yet such a division is indicated in the section by the arrangement of the nuclei of the cells on each side a line, which is represented by a narrow band of a lighter shade than the surrounding tissue.

In sections of three other embryos which I have examined, about the centre of the medullary groove there is similarly an indication of the splitting of the mesoblast from the periphery to the axial portion, the cells being arranged in two parallel rows along the inner edges of the two layers of mesoblast, although no cavity is actually formed. Thus, although it cannot be said that a split actually occurs through the whole plate of lateral mesoblast in the Mole, yet there is without doubt a tendency to such splitting in embryos of Stage E about the centre of their body.

In the same stage of growth (Stage E) is to be observed:

(1) The separation of the axial and peripheral portions of the mesoblastic plates, these two portions being connected by a narrow neck of cells, the intermediate cell mass; and (2) the formation of protovertebræ by means of clefts in the axial mesoblast at right angles to the long axis of the embryo, which divide this portion into cubical masses. The indication of the splitting of the mesoblast at the same time becomes more definite, and results in a cavity (fig. 15) within both the protovertebræ and the peripheral mesoblast, a cavity which does not, however, extend through the intermediate cell mass (compare also fig. 17, Stage F).

The cells of the protovertebræ are radially arranged round a narrow elongated cavity, and form in a transverse section through the middle of a somite a triangular mass, the apex of which is situated at the base of the medullary groove.

The cells of the peripheral mesoblast in the region of the protovertebræ are columnar on their inner side and border, a narrow slit extending to the periphery. At the edge of the area the cells become flattened, and form a thin somatic and thicker splanchnic layer, extending over the yolk-sac.

An examination of consecutive sections reveals, in front of the protovertebræ, the axial and peripheral mesoblast in the form of a continuous solid plate, with no cavity in the axial portion; while in the peripheral portion the cavity gradually recedes outwards (fig. 14) until it no longer exists within the limits of the embryonic area.

Behind the protovertebræ the cavity in both axial and peripheral mesoblast becomes at once and simultaneously obliterated, and two thick solid lateral plates of mesoblast extend backwards, and join the mesoblast of the primitive streak.

As the medullary groove closes in, the protovertebræ become more cubical and compact (compare sections of Stage H, showing these points (figs. 31, 30, and 28)), and the narrow slit reduced to a small central pore, which about this time becomes very generally partially filled up by a core of cells derived from the lower and inner portion of the protovertebra.

The protovertebræ then (Stage H) commence, first in the anterior region, and gradually assuming in subsequent stages the same relation posteriorly, to divide into two portions, an outer and dorsal arched portion composed of columnar cells, and a lower and inner portion formed of irregularly rounded cells (fig. 29; compare also fig. 52 of Stage J), the former giving rise mainly to the muscle-plates, the latter to the bodies of the vertebræ and the connective tissue surrounding them. It will be shown subsequently, however, that the inner portion also participates in the formation of the muscle-plate.

A very marked cavity exists between the two portions on the outer side of the somite (fig. 29), and the vertebral portion of the mesoblast is continued ventrally below the neural canal towards the notochord.

The cavity is derived from the small cavity present in the earlier stage (Stage E); and it is worthy of notice it is not first obliterated and then again formed, as has been stated by some observers to be the case in the Chick, nor does it entirely disappear, as has been supposed to be its fate in Mammalia (*vide* No. 1, p. 553).

Anterior to the protovertebræ scattered mesoblast cells

exist below the neural canal, closely approximated to the slight rod-like notochord (figs. 24 and 26), while in the region of the protovertebræ the mesoblast is more compact, and does not extend so far beneath the medullary canal (fig. 28).

In Stage J the anterior protovertebræ exhibit still further changes:—(1) The vertebral portion of the somite has increased very considerably in depth; (2) the cavity has almost entirely disappeared, remaining only as a mere slit (fig. 47) within; (3) the muscle-plate, which is now formed of two rows of columnar cells. The second row lies inside the first, close beside and parallel to it. It is formed from the cells of the vertebral portion of the somite, which have hitherto occupied this position. The two rows are continuous with each other at their dorsal and ventral ends, and the cavity before spoken of lies between them, reduced to a narrow slit.

Posterior to the three anterior protovertebræ the muscle-plate consists of only a single layer of columnar cells, as was the case in the earlier stage (H).

The muscle-plates are therefore first formed anteriorly.

When examining this stage my attention was drawn to the histological characters of the cells of the outer layer of the muscle-plate in the anterior protovertebræ.

These cells were observed with an ordinary Zeiss D lens to be continued outwards into more or less fine processes, and upon examining sections with a high power (Powell and Lealand's $\frac{1}{12}$th oil immersion and Reichert's $\frac{1}{15}$th oil immersion) it was found that these fine processes were branched or simple prolongations of the mesoderm cells, which on the one hand joined with the ectoderm cells, and on the other formed a fine network immediately below the ectoderm.

These processes are voluntary muscular fibres, which are thus early developed from the outer portion of the muscle-plate. This structure is fairly satisfactorily represented in fig. 51.

The fact being observed that these mesoderm cells actually joined the ectoderm cells led me to make a renewed examination of my sections of earlier stages, and I found that from the time the hypoblastic mesoblast was formed in Stage c

(No. 8) it was always possible to trace processes from mesoderm cells into the overlying epiblast cells.

The elongated mesoblast cells shown in fig. 51 are more extended in Stage J than they hitherto have been.

In Stage H (figs. 27—29) a tendency to elongate may be observed in these cells and so also in Stages G and F (fig. 17), but it is not until Stage J is reached they can actually be described as muscular processes. Further, this condition in Stage J only exists in a marked degree in the first few anterior protovertebræ; further backwards these processes gradually decrease in length.

With regard to the inner layer of the muscle-plate, certain of the cells already show a differentiation into elongated muscular fibres, but they are not all of them as yet so metamorphosed.

The protovertebræ remain at the close of Stage J still separated from one another throughout their depth, and between each, short blood-vessels run, which are dorso-lateral branches from the dorsal aorta (fig. 52).

The mesoblast at the front end of the embryo now extends between the notochord and the floor of the neural canal (figs. 44, 45, and 49), the embryo having increased dorso-ventrally.

I find no trace of the body cavity in the head. As was stated above, the splitting of the mesoblast never extends to the axial portion of this part of the mesoblast, and no cavity, as far as I have been able to see, makes its appearance secondarily.

Pericardial Cavity.—The separation of the pericardial cavity from the remainder of the body cavity has only commenced during Stage J, and at the close of that stage the mesenteries in which the ductus Cuvieri run from the body wall to the sinus venosus, divide the body cavity into two dorsal sections, one on each side, the pleuro-peritoneal cavities, and one median ventral section, the pericardial cavity.

These three sections are all continuous at the anterior end into a single cavity surrounding the heart, which is prolonged a considerable distance further forwards.

Posteriorly the pleuro-peritoneal cavities are each continuous with the body cavity contained between the diverging folds of the somatopleure and splanchnopleure.

The Primitive Streak.—During Stages E and F the relations of the primitive streak are almost exactly similar to those described for Stage D (No. 8), the only difference being the extension of the medullary folds backwards round the front end of the primitive streak (fig. 18). The lumen of the neurenteric canal disappears, but the point where it originally existed is shown by the fusion of the epiblast, hypoblast, notochord, and primitive streak mesoblast at the front end of the latter (Stage J, fig. 50).

In my paper, No. 8, I endeavoured to prove the mesoblast of the primitive streak did not extend beyond the point where the neurenteric canal was situated, and I showed that over the whole of that part of the embryo situated anterior to the primitive streak, mesoblast was formed from the hypoblast ("hypoblastic mesoblast").

Now if this be true, it follows that the mesoblast of the primitive streak takes no part in the formation of the body of the embryo anterior to the neurenteric canal, and that the growth of the embryo is caused by a multiplication of cells anterior to the primitive streak.

The mesoblast of the primitive streak is, however, a considerable and hitherto a constantly increasing mass, and it extends backwards and outwards beyond the embryonic area. It thus occupies the position where eventually the allantois is formed, and it is, in fact, the primitive streak mesoblast which forms the walls of that organ.

During the stages now under discussion (E—J) the primitive streak becomes partially—almost entirely—divided into two portions, an anterior and a posterior portion. The division is caused by the formation of two pits—(1) a dorsal pit which eventually gives rise to the anus, and (2) a ventral pit which projects upwards and backwards into the primitive streak, and forms the cavity of the allantois (figs. 35 and 50).

These two pits constrict the blastoderm and partially divide

the primitive streak into a short anterior portion which projects upwards along the floor of the medullary groove at its hind end, and into a larger posterior portion which forms the wall of the allantois (figs. 33 and 35, Stage H, and figs. 48 and 50, Stage J).

The dorsal pit I have mentioned gives rise to the anus; this structure is therefore formed in the middle of the primitive streak in the Mole, in the same position as Weldon (No. 11) pointed out the anus of Lacertilia is formed.

The Amnion.—The amnion is first formed, as I have before described (p. 109), at the hind end of the embryo; extending thence forwards, and being met by the lateral folds of the amnion which also grow, in the first place, from behind forwards (figs. 26, 28, 35, and 50). This portion of the amnion is formed as in the Chick of a double fold of somatopleure. Immediately upon the junction of the two lateral folds and the formation of true and false amnion, the epiblast of the false amnion, which is shown in fig. 28, unites eventually with the neighbouring uterine tissue, and the thin sheet of somatic mesoblast alone remains between the uterine wall externally and the splanchnic mesoblast within.

At the front end of the embryo a different structure is found to exist. The lateral folds in this region are similar to the posterior portion of these folds, but the median anterior fold of the amnion is different inasmuch as it is formed solely of epiblast and hypoblast (fig. 34). Although the amnion at the anterior end is not formed until some considerable time after the mesoblast of the embryo has extended to the front end of the embryonic area, and although this mesoblast has extended laterally over the vesicle throughout the whole length of the embryonic area, it only extends forwards for a very short distance, and does not grow between that portion of the epiblast and hypoblast which gives rise to the anterior fold of the amnion. Consequently, when the head of the embryo becomes projected anteriorly over the yolk-sac, as it does first in Stage G (fig. 6), and then bends downwards, forming for itself a pit on the surface of the yolk-sac, the walls of this

pit constitute the anterior fold of the amnion, and are formed solely of epiblast and hypoblast. This portion of the amnion does not come in contact with the wall of the uterus.

The relations of these parts have recently been very fully described by Beneden and Julin in Rabbit and Bat embryos (No. 2). These authors have named this anterior fold of the amnion the "pro-amnion," and have most ingeniously, and as it appears to me correctly, compared it with the internal stalk of the "träger" of inverted types of mammalian embryos.

I should mention that the mesoblast present in the median line in the longitudinal section of an embryo of Stage E (fig. 11) is concerned in the production of the heart, the anterior fold of the amnion having its origin in front of this mesoblast (compare figs. 11 and 34).

The Allantois.—The allantois is, in an embryo of Stage F, a short wide diverticulum of the hypoblast projecting into the posterior portion of the primitive streak mesoblast behind the point where the epiblast and mesoblast curve over to form the amnion, and therefore also behind the point where the anal pit is forming.

This diverticulum increases in size during Stages G, H, and J, and forms at the latter stage a very considerable vesicular cavity opening by a narrow neck into the (future hind-gut) yolk-sac beneath. The hypoblast diverticulum is formed of rounded cells, and is surrounded by a mass of mesoblast through which blood-vessels already ramify. The relation of these parts is shown in figs. 35 and 50.

The Vascular System.—In the earliest embryo I have examined of Stage E, viz. one with only a single protovertebra, the position of the heart is already indicated, and vessels are already formed in the splanchnic mesoblast of the blastoderm outside the embryonic area. Blood-corpuscles are, moreover, to be seen within these vessels even at this early age.

At the close of Stage F, the rudiments of the dorsal aorta are present, lying some distance on each side the notochord and extending from a point on a level with the front end of the heart backwards to the last protovertebra (fig. 17). They

do not, however, as yet form continuous tubes. From the front end of the aorta on each side a short vessel is given off which lies dorsal to the aorta and immediately below the nervous system; it does not, however, extend far. There is no communication between the aortæ and the heart tubes at this stage.

At the commencement of Stage H the two tubes of the heart have met at their anterior end, and form a single wide tube for a short distance (figs. 24 and 25), a single pair of aortic arches are formed and the dorsal aortæ extend backwards as two separate tubes some distance beyond the last protovertebra; just before they terminate they give off two vitelline arteries.

A series of short diverticula project from the aorta dorso-laterally between the somites, and ventrally, below them at this stage and during Stage J (compare figs. 27—31 and 52).

From near the front end of the aortæ, a little posterior to the point where the aortic arch runs into it, two internal carotid arteries are projected forwards and extend to the under surface of the optic lobes (fig. 21, *i. c. a.*) ; while from about the same point two vessels run backwards joined at intervals with the aortæ (fig. 24, *v. a.*) on each side of, and closely applied to, the now closed neural canal. These vessels run back to a point just in front of the first protovertebra and are doubtless the vertebral arteries.

Stage J shows little alteration ; the heart is still in the form of a straight tube somewhat longer than in Stage H, but without curvature or any sign of a division into chambers; there is still also only one pair of aortic arches, and two separate aortæ are still present throughout the extent of their course.

A number of small vessels are now given off from the internal carotid arteries, and the aortæ in their anterior portion also send short branches into the surrounding tissue. The vessels which I have before described, running backwards on each side the nervous system, are frequently in communication with the aortæ, and it is these vessels which appear at this stage to project diverticula into the substance of the walls of the spinal canal (*vide* above) (fig. 43).

The vitelline arteries are given off about on a level with the

ninth and tenth protovertebræ as a series of branches, after which the aortæ immediately become reduced to very minute proportions.

The venous system, which is barely distinguishable in Stage H, is very slightly developed in Stage J. The vitelline veins run in the converging folds of the splanchnopleure to the posterior end of the heart on a level with the second and third protovertebræ.

The only veins in the trunk of the embryo are two slightly developed anterior cardinal veins which are situated on the outer edge of the anterior protovertebræ (fig. 47, *a. c. v.*). They communicate with the ductus Cuvieri where the vitelline veins run into the heart between the second and third protovertebræ, and run forwards as far as the first protovertebra.

Traces of a posterior cardinal vein may be seen for some little distance behind the ductus Cuvieri; but as a vessel it exists only for a few sections, and is situated at the point where the somatopleure commences to turn upwards to form the amnion

Thus it may be observed the arterial system is in a far more advanced condition than is the venous system in the body of the embryo.

The Structure of the Heart.—In Stage E the heart merely consists of a small tube in the thickened splanchnic mesoblast on either side, in front of the protovertebræ (fig. 14). Then (Stage F) the thickened portion is bulged outwards into the body cavity and splits up into two layers. The outer layer bounding the body cavity forms the wall of the heart itself, the inner the flattened epithelial lining of the cavity of the heart. The space between these two layers increases and in Stage H (fig. 25) is considerable. In this figure the epithelial layer is connected with the outer layer of the heart by long protoplasmic processes stretching from cell to cell across the space. In Stage J the wall of the heart has increased in size more in proportion than has the inner epithelial layer. The latter is now an elongated bag within the space contained by the outer wall and connected with the latter by marvellously delicate

simple or branched cell processes (fig. 47). At the points where the cavity of the heart is continuous with the vessels entering into and emanating from the heart, the epithelial layer is continuous with the wall of these vessels. As I have stated above, the heart shows no indication of curvature or of division into chambers.

The Blood-Corpuscles are formed from stellate mesoderm cells. The nuclei of these cells become darker, the stellate processes are then withdrawn and a meagre coating of protoplasm surrounds the now rounded nucleus. Such conditions and changes are shown in many of the figures I have drawn; notably in fig. 25 in the heart, and in fig. 28 in the vitelline vessels.

Summary.

External Features. — The early appearance of the optic grooves (Stage E) which give rise to the optic vesicles; the existence of five visceral arches in Stage J; the formation of the amnion first at the hind end of the embryo; and the folding off of the head end of the embryo only, are the chief points to be noted. The enclosure of the front end of the primitive streak within the medullary fold; the formation of protovertebræ, chiefly from before backwards; the closure of the medullary groove; the appearance of three divisions of the brain, and the formation of the heart are also detailed.

The Epiblast.—The epiblast of the embryo (Stages E—G) becomes formed into a median thickened portion, the medullary plate, and into lateral portions which are formed of cubical cells and are continuous with the flattened epiblast cells which cover the vesicle. The closure of the medullary groove (Stages H and J) causes the union of the lateral epiblast which thus forms a continuous layer across the embryo. The medullary groove commences about the centre of the embryo, widening out into the sinus rhomboidalis behind and into the cephalic plate anteriorly. The optic grooves are formed one on each side of the middle line in the cephalic plate (figs. 4 and 16).

The Medullary Canal.—The closure of the medullary groove commences in the region of the first protovertebra during Stage G and proceeds anteriorly and posteriorly, and at the close of Stage J a complete canal is formed as far back as the last (fourteenth) protovertebra. The lateral walls of the canal thicken, and are converted into an hour-glass form in places. The migration of mesoblast (nutritive) cells into the walls of the canal is noted in Stages H and J.

The Brain.—The three divisions of the brain are indicated in Stage J, and a well-marked cranial flexure is then present. The infundibulum is just apparent at this stage in close connection with the front end of the alimentary canal and notochord (fig. 49).

The Optic Vesicles are formed from the optic grooves by the closure of the medullary canal. These organs first appear extremely early, but their development is soon checked, doubtless in consequence of the habits of the adult animal.

The Ear in Stage J is merely indicated as a deep groove in a thickened mass of mesoblast on either side of the hind-brain.

The Cranial and Spinal Nerves are not described.

The Hypoblast may be divided into axial and peripheral portions. The peripheral hypoblast, a single layer of flattened cells, extends on all sides over the embryonic area during Stage E. The deepening of the medullary groove stretches these cells and flattens them still more, but the thickening of the lateral mesoblast forces the lateral hypoblast down, removes the strain, and its cells become rounded.

The Notochord is formed of axial hypoblast cells. In Stage C a mass of axial hypoblast cells are continuous with two lateral masses of mesoblast—derived from lateral hypoblast—and with the lateral hypoblast layer also. In Stages D and E the axial mass becomes isolated from the lateral mesoblast plates, and gradually decreases in size below the deepening medullary groove until in that portion where the groove is deepest, i. e. near the centre, a single layer of flattened cells is all that exist.

It does not, however, become reduced to this extent through-

out its length ; at the posterior end it remains thickened, and by the ingrowth of the lateral portions the axial cells first form an arch and then a complete tube, which is the neurenteric canal and which communicates dorsally with the exterior and ventrally with the yolk-sac.

This tube is the homologue of the median dorsal diverticulum of the alimentary tract in Amphioxus, i. e. the structure which gives rise to the notochord of that animal, and it is noteworthy that in the Mole it disappears almost entirely before the notochord is formed.

The single layer of cells to which the greater part of the axial hypoblast is reduced at the close of Stage D (No. 8) again increases in bulk during Stages E to J, and gives rise to the notochord.

As was the case with the lateral hypoblast, the flattening of these cells and their increase in bulk appears to be due, first to the stretching effect of the rapidly deepening medullary groove, and secondly to the release from that strain caused by the depression of the lateral portions of the embryo.

The isolation of the notochord first occurs in the region of the first protovertebra during Stage G, and extends anteriorly and posteriorly during Stages H and J.

The isolation is caused by the ingrowth of the lateral hypoblast below the axial cells, and the latter are isolated either as a solid band or rod, although a lumen may here and there appear in it afterwards.

At the close of Stage J the notochord is completely separated from the hypoblast, except at two points, viz. at the anterior end, where it is connected with the hypoblast and epiblast, where these two layers fuse to form the mouth, and posteriorly where it is joined to both epiblast, hypoblast, and mesoblast, at the front end of the primitive streak (figs. 49 and 50).

The origin of the notochord and the manner of its isolation appear to be sufficient reason to regard it as entirely homologous with the notochord of Amphioxus.

For a review of other opinions on this point I would refer to a discussion in my former paper (No. 8).

The hooked anterior end of the notochord is due to its origin from the front wall of the fore-gut. Its close approximation to the fore-brain is noted.

The relatively small size of the notochord to the nervous system in the Mole is pointed out, and it is suggested the early development of the latter is the cause of the check administered to the growth of the former, a check from which it appears never entirely to recover.

The Alimentary Canal first appears in Stage D as a short tubular diverticulum, projecting below the cephalic plate nearly to the anterior end of the embryo.

The tube enlarges and extends backwards during the progress of the folding off of the embryo during Stages E to J, and the cranial flexure causes a ventral enlargement, which is somewhat posterior to the original anterior diverticulum.

The mouth and the visceral clefts are not formed at the close of Stage J, but the epiblast and hypoblast have fused at the point where the mouth will eventually be formed, and several lateral outgrowths from the now widened fore-gut exist; in the case of one of these, the anterior one, the hypoblast has reached the epiblast, and the two layers are partially fused at that point.

The mouth is formed at the apex of a Y-shaped groove, the diverging limbs of which are directed forwards; these grooves are the anterior border of the first visceral arch.

The primary anterior diverticulum would indicate the existence primitively of a terminal mouth, while the two grooves, at the junction of which the mouth is formed, would suggest a paired origin for the existing mouth of the animal.

The Mesoblastic Somites and Body Cavity.—The lateral plates of mesoblast are split horizontally into somatic and splanchnic layers, but the split is not actually carried through both peripheral and axial portions of the plates, being merely indicated in Stage E in the axial portion. The mesoblast of the head also is not split, and no cavity is formed there.

Protovertebræ are formed and the axial and peripheral portions of the mesoblast plates are separated from one another by the intermediate cell mass.

A cavity appears in the protovertebræ, Stage E, which still exists at the close of Stage J.

The formation of the muscle-plate commences at Stage H from the outer layer of cells of the protovertebra, but during Stage J, in the three anterior protovertebræ, a second row of cells derived from the inner (vertebral) portion of the somite, takes part in its formation, the two rows being continuous with one another at their dorsal and ventral ends.

The muscle-plates are first formed anteriorly. The outer cells of the muscle-plate in Stage J are prolonged into fine processes, which are connected with the overlying epiblast cells, and constitute voluntary muscular fibres (fig. 51). Certain of the cells of the inner layer are also differentiated into elongated muscular fibres.

I would further remark the mesoblast and epiblast cells in front of the primitive streak appear always to be connected together by processes.

The Pericardial Cavity only commences to form during Stage J, and is not at the close of that stage entirely separated from the remainder of the body cavity.

The Primitive Streak has the same relations in Stages E and F as in Stage D, except that the medullary folds grow backwards round its front end. The neurenteric canal disappears, but its original position is indicated by the fusion of the germinal layers at the front end of the primitive streak.

The mesoblast of the primitive streak does not give rise to the mesoblast of the body of the embryo in front of the primitive streak, in my opinion, but extends backwards and outwards and forms the wall of the allantois. The anus is formed in the middle of the primitive streak.

The Amnion is first formed at the hind end and from thence extends forwards. This portion of the amnion is formed of a double fold of somatopleure; the epiblast of the outer fold unites with the epithelium of the uterus. The anterior fold

of the amnion, however, is formed only of epiblast and hypoblast, and has been called by van Beneden and Ch. Julin, who first described this structure, the "pro-amnion."

The Allantois commences in Stage F as a short wide diverticulum projecting upwards and backwards into the primitive streak. This diverticulum enlarges during Stages G to J; it is lined with hypoblast cells (figs. 35 and 50).

The Arterial System.—The dorsal aortæ commence in Stage F, and remain double until after Stage J; they are connected with the heart by a single pair of aortic arches during Stages H and J, and give off vitelline arteries at their posterior end. Internal carotid arteries and vertebral arteries are formed, and it is from the latter of these vessels the mesoblast cells are derived which migrate into the walls of the neural canal.

The Venous System is very slightly developed. Vessels are to be seen in the splanchnopleure over the yolk-sac at an early date, but vitelline veins connected with the heart are not seen until Stage H. Two short anterior cardinal veins are present in Stage J, and traces of two posterior cardinals, but nothing more.

The Heart, which is formed of two tubes widely asunder in Stage E, is composed of a single tube for a short distance in Stage H, and is somewhat longer, but still straight and without sign of division into chambers at the close of Stage J. The thickened splanchnic mesoblast which gives rise to the heart, splits into two layers at an early age. The outer of these layers forms the outer wall of the heart, the inner the flattened epithelium of the cavity of the heart.

When the heart enlarges, as it does rapidly, a wide space exists between these two layers, but they are connected together by exceedingly fine processes of their cells which stretch across the space.

The Blood-Corpuscles appear to be formed from stellate mesoblast cells directly.

In conclusion, I may mention that I propose eventually to follow the further development of the organs of the Mole, one by one, and in doing so, to pay more attention to the researches

of other investigators than has appeared to me advisable in the present paper.

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DESCRIPTION OF PLATES XIII, XIV, & XV,

Illustrating Mr. Walter Heape's Paper on "The Development of the Mole (Talpa Europea)," Stages E to J.

List of Reference Letters.

a. arc. Aortic arch. *a. c. v.* Anterior cardinal vein. *al. c.* Alimentary canal. *all.* Allantois. *all. v.* Allantoic vessels. *am.* Amnion. *am. fl.* False amnion. *an. p.* Anal pit. *a. p.* Area pellucida. *aud. ep.* Auditory epithelium. *aud. inv.* Auditory involution. *b. c.* Body cavity. *c. pl.* Cephalic plate. *d. a.* Dorsal aorta. *ep.* Epiblast. *f. br.* Fore-brain. *h. br.* Hind-brain. *ht.* Heart. *hy.* Hypoblast. *i. c. a.* Internal carotid artery. *i. c. m.* Intermediate cell mass. *m.* Mesoblast. *m. br.* Mid-brain. *m. gr.* Medullary groove. *m. pl.* Medullary plate. *msc. pl.* Muscle-plate. *n. c.* Neural canal. *nch.* Notochord. *op. gr.* Optic grooves. *op. v.* Optic vesicles. *p. c.* Pericardial cavity. *pro. am.* pro-amnion. *p. st.* Primitive streak. *p. v.* Protovertebra. *Si. rh.* Sinus rhomboidalis. *so. m.* Somatic mesoblast. *so. pl.* Somatopleure. *sp. m.* Splanchnic mesoblast. *sp. pl.* Splanchnopleure. *v. a.* Vertebral artery. *vs. ach.* Visceral arch. *vt. a.* Vitelline artery. *vt. v.* Vitelline vessels. *vt. vn.* Vitelline vein.

Figs. 1—10 were sketched with Zeiss's A* lens and eye-piece 2 by myself, and were most carefully shaded by Mr. H. A. Chapman under my supervision.

Figs. 11—35, 44—50, and 52 were sketched with Zeiss's B lens and eye-piece 2.

Figs. 36—43 and 51 were sketched with Zeiss's D lens and eye-piece 2.

Fig. 1, Stage E.—Transparent view of embryo ·76 mm. long. It has three protovertebræ. The medullary groove is narrow in the middle of the body, widening out at either end. The anterior end of the primitive streak projects as a dark knob into the wide sinus rhomboidalis. The flattened cephalic plate (*c. pl.*) and the area pellucida (*a. p.*) are to be observed.

Fig. 2, Stage E.—Surface view of embryo 1·82 mm. long. The cephalic plate has now two deep lateral grooves, the optic grooves (*op. gr.*). The curved condition of the embryo is due to careless manipulation.

Fig. 3, Stage F.—Surface view of embryo 1·96 mm. long. The medullary groove has commenced to close in the region of the protovertebræ (which are not shown in this drawing), but the edges of the groove have not yet coalesced. The optic grooves are seen at either side of the cephalic plate.

Fig. 4, Stage F.—Surface view of embryo 2·12 mm. long. Although some-

what bigger than the embryo drawn in Fig. 3, the medullary folds have not advanced so far along the body of the embryo as they have in the latter embryo. At the anterior end, however, they are slightly more advanced, and where the folds meet in front a narrow slit is to be seen. The amnion is shown covering the posterior half of the embryo, and the wide sinus rhomboidalis is indicated below it.

Fig. 5, Stage F.—Transparent view of the same embryo, seen from below. Four fully formed protovertebræ are present, and a fifth is indicated behind the posterior one. The primitive streak projects into the sinus rhomboidalis. The optic grooves appear as narrow lateral prolongations of the medullary groove at its anterior end. The heart (*ht.*) commences to form at this stage, and is indicated by a thickening of the blastoderm on either side the embryo just behind and outside the optic grooves.

Fig. 6, Stage G.—Surface view of embryo 2·33 mm. long. The medullary groove is closed up to the anterior end, where a small pore remains connecting the medullary canal with the exterior. The sinus rhomboidalis is still widely open behind. The head has now been folded off from the yolk-sac as far as the line *so. pl.*, which shows the point of divergence of the folds of somatopleure. Faint indications of the divisions of the brain are shown, and the laterally projecting optic vesicles are very distinct (*op. v.*).

Fig. 7, Stage H.—Surface view of embryo 2·2 mm. long. Ten protovertebræ are present. The closure of the medullary groove has advanced. The sinus rhomboidalis is narrowed, and the primitive streak forced upwards as a rounded knob at the posterior end of the latter. The head is more rounded, and shows partial division into fore-, mid-, and hind-brains. The amnion has been torn away, and the jagged edge of the somatopleure surrounds the body of the embryo.

Fig. 8, Stage H.—View of the under surface of the head of the same embryo, showing the heart and the diverging folds of somatopleure and splanchnopleure.

Fig. 9, Stage J.—Surface view of an embryo 3·06 mm. long. The sinus rhomboidalis is much narrowed, and the medullary groove closed for the greater portion of its length. The optic vesicles and fore-, mid-, and hind-brains are well shown. Thirteen protovertebræ are present. The primitive streak is in the same condition as described for Fig. 7, also the amnion has been torn away as it was in that figure.

Fig. 10.—Lateral view of the head of the same embryo, showing the heart and five visceral arches.

Fig. 11, Stage E.—Median longitudinal section of the anterior end of an embryo with three protovertebræ. The cephalic plate projects slightly over the blastoderm in front, the folding-off process having already begun in this embryo. The commencement of the fore-gut is indicated at *al. c.* A small portion of mesoblast exists between the epiblast and hypoblast of the blasto-

derm at the front end of the embryo; beyond that point no mesoblast is present in the middle line.

Fig. 12, Stage E.—Transverse section through the cephalic plate of an embryo with three protovertebræ. At the point where this section is taken the flat cephalic plate is completely folded off from the yolk-sac. The narrow fore-gut is shown as a tube (*al. c.*) immediately below the cephalic plate. A few scattered mesoblast cells extend between the two layers of epiblast.

Fig. 13, Stage E.—Transverse section through an embryo 1·97 mm. long, with only a single protovertebra. The section is taken in front of the protovertebra, and shows the indication of a split of the mesoblast into somatic and splanchnic layers throughout its whole depth. The medullary groove is wide and deep. The notochord is formed of flattened cells.

Figs. 14 and 15, Stage E.—Tranverse sections through the same embryo which is drawn in Fig. 1.

Fig. 14 is taken in front of the protovertebra, where the mesoblast is split into somatic and splanchnic layers only at the periphery.

Fig. 15 passes through a protovertebra. The body cavity extends inwards as far as the intermediate cell mass (*i. c. m.*) in the peripheral mesoblast, and a small cavity is also present within the protovertebra.

Figs. 16, 17, and 18, Stage F.—Transverse sections through embryos with five protovertebræ.

Fig. 16 is a section through the head. The cephalic plate is grooved in the middle line and at either side where the wide optic grooves are situated. When the external edges meet in the middle line these optic grooves will be converted into vesicles communicating by a wide aperture with the central canal. The notochord is not yet separated from the hypoblast.

Fig. 17 is through the trunk; the medullary groove is narrower, and the notochord more defined than in Fig. 15, which is a section through a similar region of an embryo of Stage E.

Fig. 18 is a section through the sinus rhomboidalis, and shows the anterior end of the primitive streak and the amnion.

Fig. 19, Stage G.—Transverse section through the head of an embryo with eight protovertebræ, 2·49 mm. long. The head at this point is completely folded off, and the medullary groove (still open) will at this point give rise to the mid-brain. A few sections further forward the optic vesicles are cut, projecting outwards from the central canal, and it is on account of the proximity of these structures that the wide space between the external epiblast and the walls of the medullary canal is present here. This space is here filled with stellate mesoblast cells. The two grooves in the epiblast on the under surface on either side the middle line converge posteriorly, and where they meet the mouth will eventually be formed.

Figs. 20—33, Stage H.—Transverse sections through three embryos of this stage.

Fig. 20 is a section through the front of the head; it passes through the point of origin of the optic vesicles, and shows at the same time the pore through which the neural canal is open to the exterior at this stage.

Fig. 21 passes through both the mid- and fore-brains and through the centre of the optic vesicles, which are here seen to be directed outwards, downwards, and backwards.

Fig. 22 passes through the hind-brain and the front end of the fore-gut (*al. c.*). The notochord is not yet separated from the axial hypoblast here. The front edge of the first aortic arch is shown. This vessel is very wide, and may be seen for many sections.

Fig. 23 is also a section through the hind-brain, but at its posterior end. The notochord is here isolated from the hypoblast. The two grooves in the ventral epiblast on either side the middle line, which were seen in Fig. 22, have met in this figure and form a single deep groove closely in contact with the ventral wall of the fore-gut, and here the mouth will be formed. These grooves define the anterior border of the first visceral arch (*vs. ach.*). The alimentary canal in this figure is very considerably wider than in Fig. 22. In Figs. 20 to 23, the head of the embryo is folded off from the yolk-sac.

Fig. 24 is taken from a different embryo from what Figs. 20—23, and 25 are taken. The front end of the heart is shown. The section is not quite transverse, and the first aortic arch is shown on the right side and not on the left side. The extremely wide fore-gut and the separation of the heart into two portions, shown here, is also due to this fact.

Fig. 25. The alimentary canal is here open ventrally. In Fig. 24 the splanchnopleure had formed a complete layer, but the somatopleure had not met below the gut. In this figure the splanchnopleure as well as the somatopleure are still divergent. The heart is here in the form of two tubes, and the two layers of which it is formed may here be seen. The formation of blood-corpuscles from stellate mesoblast cells also may be observed. The thickened epiblast on either side the neural canal is the commencement of the auditory organ.

Fig. 26. A section immediately in front of the first protovertebra. The vitelline vein is seen in the splanchnopleure, branching out over the yolk-sac. The fold of the somatopleure to form the amnion is also indicated (*am.*).

Fig. 27. A section through the anterior protovertebra.

Fig. 28. A section through the middle of the embryo. The large vitelline vessels are shown in the splanchnic layer of mesoblast over the yolk-sac. The true (*am.*) and false (*am. fls.*) amnion are both shown

here. The false amnion is formed of flattened somatic mesoblast and columnar epiblast cells, the latter will eventually fuse with the uterine epithelium.

Fig. 29 is also a section through the middle of the embryo. The cells of the protovertebræ are here seen to be somewhat elongated on the right side of the section, while on the left the cavity of the protovertebra is shown partially filled by a cove of mesoblast cells. This was also shown in Fig. 28. The hour-glass shape of the neural canal at this point is also to be observed.

Figs. 30 and 31 are from the hinder portion of the trunk of the embryo. The neural canal is not closed, the protovertebræ are not so completely isolated from the neighbouring mesoblast, and the notochord, which is larger than in former sections of this stage, is not at all isolated from the hypoblast in Fig. 31. In both these sections the two dorsal aortæ, which were present in all the sections from Fig. 23 to Fig. 29, are here giving off branches to the yolk-sac. The vitelline arteries (*vt. a.*), and posterior to this point, the aortæ themselves, no longer exist.

Fig. 32 is a section behind the former sections, and just in front of the primitive streak. The widely open medullary groove is here called the sinus rhomboidalis.

Fig. 33 is a section through the primitive streak; the medullary folds are growing round it and will shortly completely enclose its front end.

Fig. 34, Stage H.—A median longitudinal section through the head of an embryo, in which the following points are shown:—The cranial flexure; the fore-, mid-, and hind-brains; the notochord separated from the hypoblast, except along the front wall of the alimentary canal; the ventral prolongation from the primitively straight fore-gut (*al. c.*), and the pro-amnion formed of epiblast and hypoblast only (*pro. am.*).

Fig. 35, Stage H.—Median longitudinal section through the hind end of an embryo. The dorsal pit (anal pit) and the ventral pit (allantoic pit) separate the anterior from the posterior portions of the primitive streak.

Figs. 36—42, Stage H.—Transverse sections through various regions of an embryo, to show the formation of the notochord.

Fig. 36 in the anterior region shows a lumen within the notochord.

Fig. 37. In the anterior region: notochord is rod like, and is separated from the hypoblast.

Fig. 38. In the anterior region: notochord is a flattened band-like structure; it is separated from the hypoblast.

Fig. 39. In the middle region: the notochord is not yet separated from the hypoblast in the middle line, although it is so separated at either edge. The lateral ingrowth of the hypoblast is shown.

Fig. 40, from the posterior region, shows relations similar to those seen

in Fig. 39, only the notochordal mass is itself considerably larger the lateral ingrowth of the hypoblast is here also indicated.

Fig. 41. From still further posteriorwards: the notochord is not yet isolated from the hypoblast, but formed into an arc.

Fig. 42. From the hind end of the embryo, immediately in front of the primitive streak: the notochord is a large thickened axial mass, with no indication of the growth of the hypoblast below it.

Fig. 43, Stage H.—A transverse section through the medullary cord of an embryo with eleven protovertebræ, from the region in front of the first protovertebra and behind the hind-brain. Between the lateral mesoblast plate and the cord is a small space, in which several nuclei are seen. The space is continuous with blood-vessels in process of formation, and the nuclei show a tendency to pass into the medullary cord. One such nucleus is shown in the drawing.

Figs. 44 and 45, Stage J.—Transverse sections through the hind-brain of an embryo with fourteen protovertebræ. The alimentary canal is narrow in front (Fig. 44), and wider posteriorly (Fig. 45). The two grooves in the epiblast on the under surface in the anterior section converge in a single deeper groove in the posterior section, where the fusion of the epiblast and hypoblast takes place, and where the mouth will eventually be formed. The dorso-ventral elongation of the fore-gut and the notochord is due to the plane in which the section was cut, caused by the cranial flexure. The presence of mesoblast cells between the notochord and the floor of the brain is to be noticed.

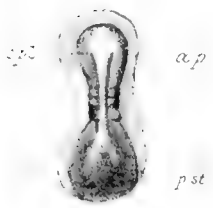
Fig. 46, Stage J, is a section, not completely transverse, through an embryo with fourteen protovertebræ, passing through the hind-brain and the auditory involution. The first aortic arch is shown on one side, and a lateral prolongation of the fore-gut to form the first visceral cleft on the other side.

Fig. 47, Stage J.—Transverse section through an embryo with thirteen protovertebræ in the region of the second protovertebra. Fore-gut crescent shaped. Anterior cardinal veins and dorsal aortæ present. Embryo is completely folded off from the yolk-sac. The heart is enclosed in the pericardium. The thick outer wall and flattened epithelial layer of the heart are here seen to be connected by fine processes of the cells forming one or other of these layers.

Fig. 48, Stage J.—A transverse section through the primitive streak of an embryo with fourteen protovertebræ. The thickened lateral mesoblast will be seen, by comparing this section with that drawn in Fig. 50, to be concerned in the formation of the allantois. Allantoic vessels (*all. v.*) are to be seen in this section.

Fig. 49, Stage J.—A median longitudinal section through the head of an embryo with fourteen protovertebræ. The division between the fore- and mid-brains and the folded floor and thin roof of the hind-brain is shown. The

Fig. 1



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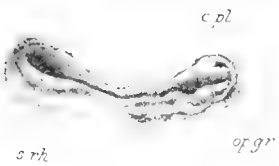

Fig. 3

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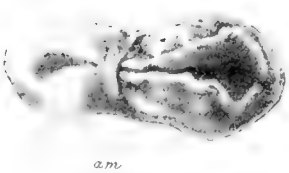

Fig 5

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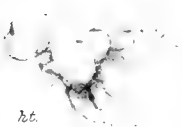
Fig. 8



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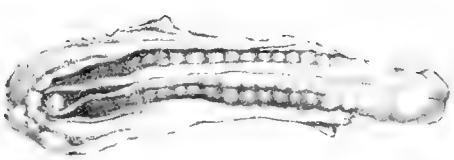

Fig. 10.

Fig. 11.

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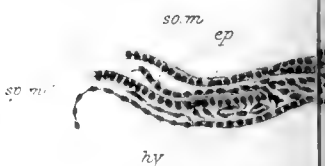

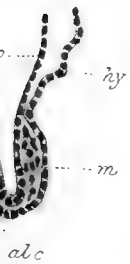

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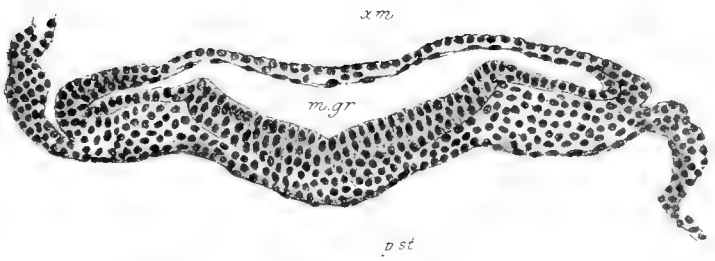

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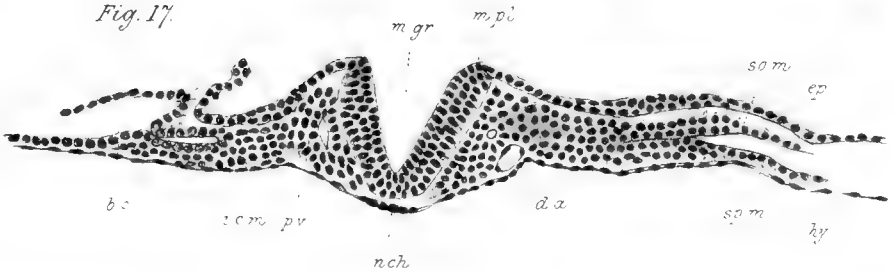

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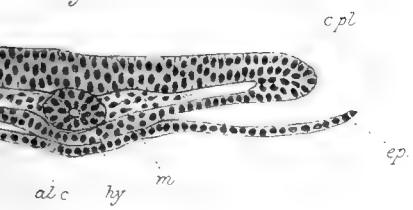

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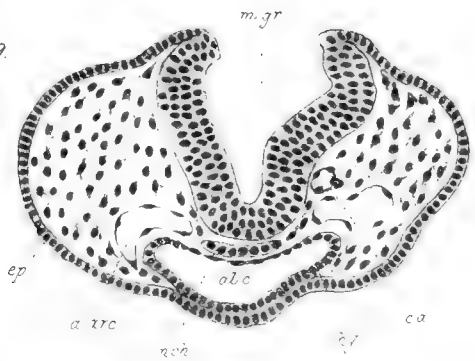

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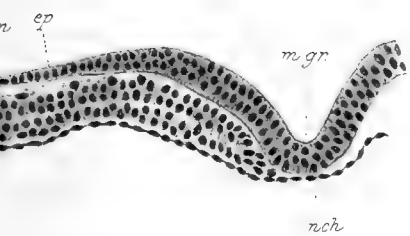

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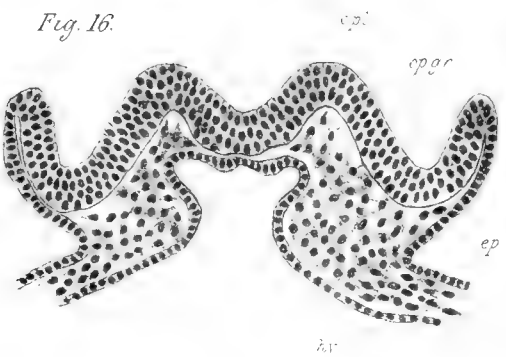

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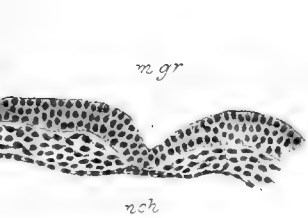

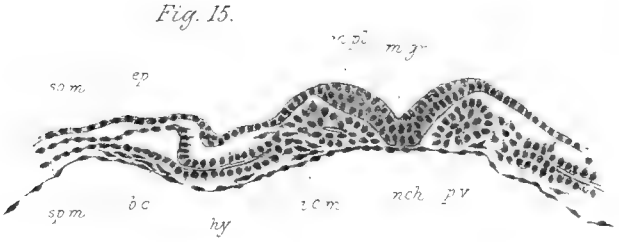

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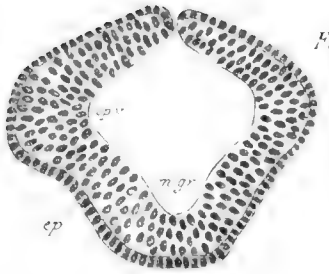
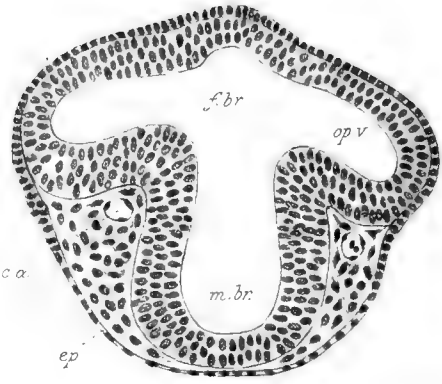


Fig 20



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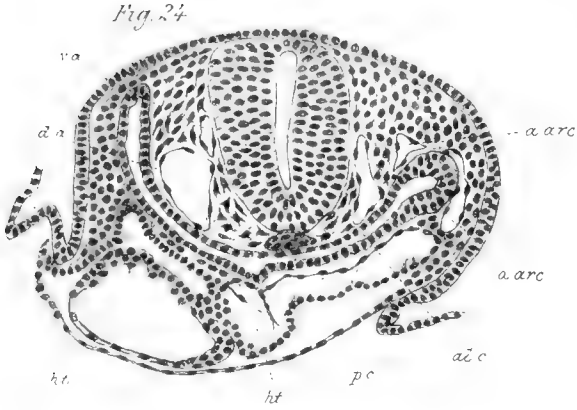

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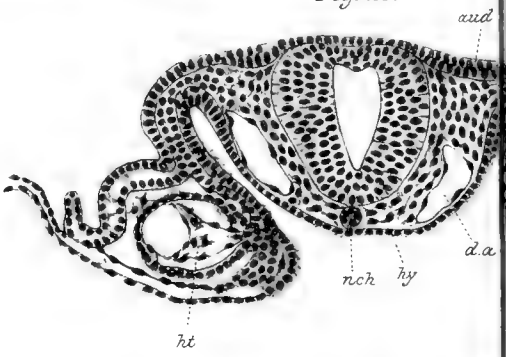

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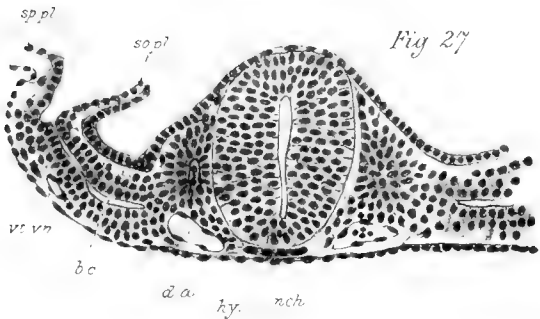


Fig 27

Fig. 28.

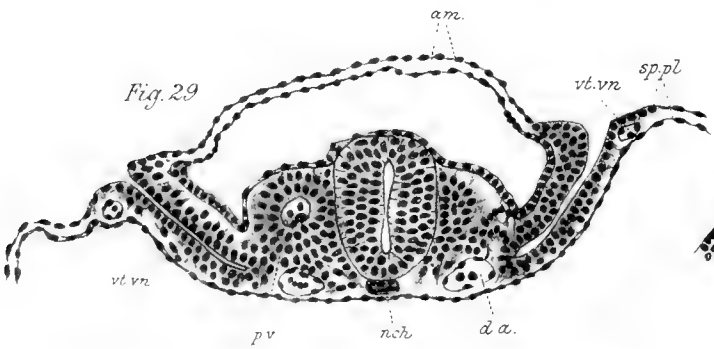
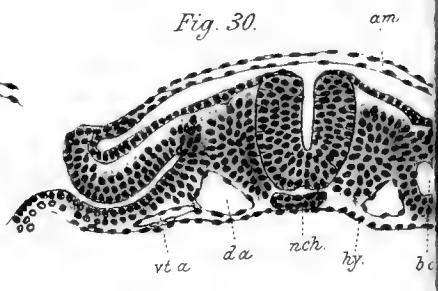


Fig. 29



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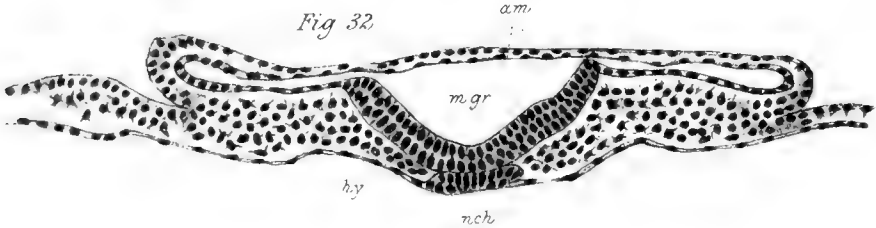

Fig 32

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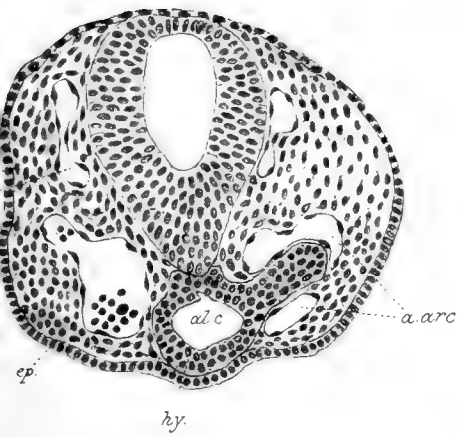

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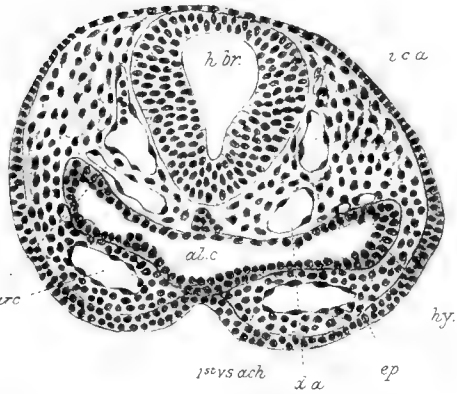

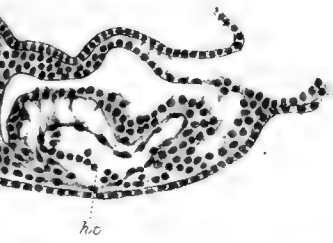

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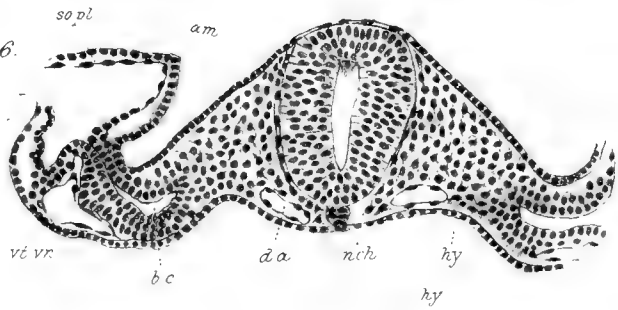


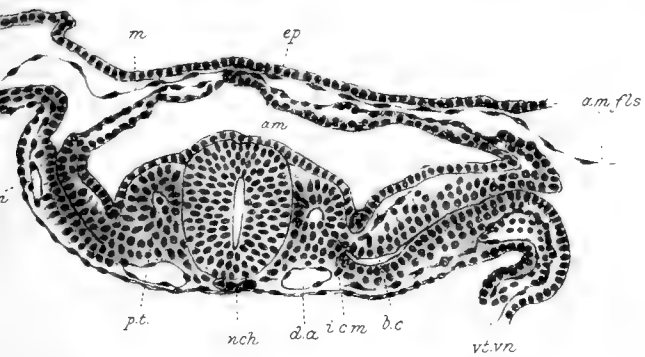
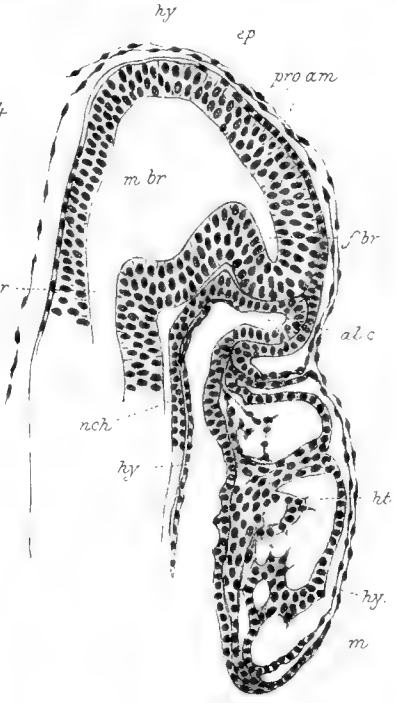


Fig. 34



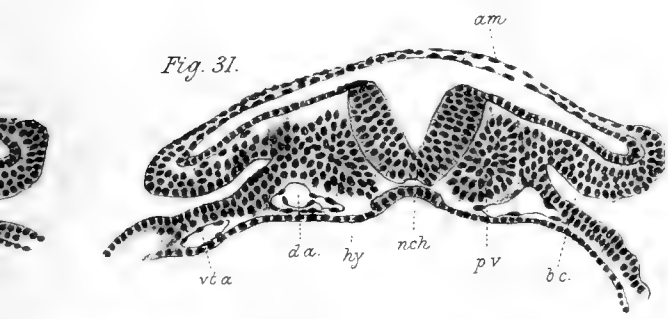
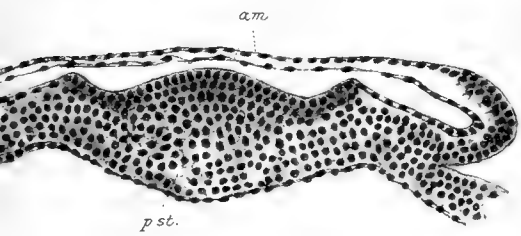


Fig. 31.



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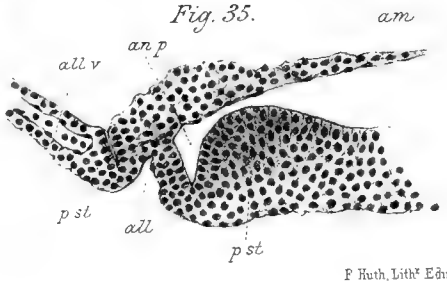

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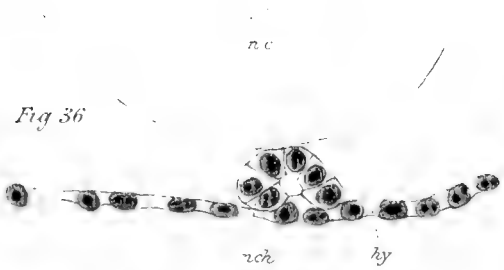


Fig 36

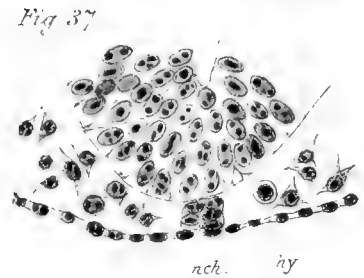


Fig 37

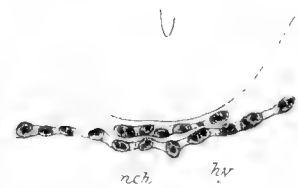


Fig 38

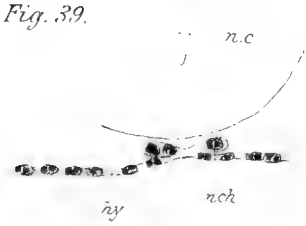


Fig. 39.

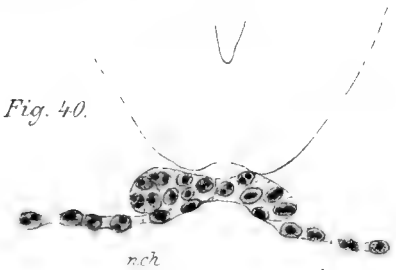


Fig. 40.

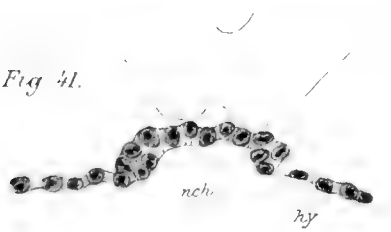


Fig 41.

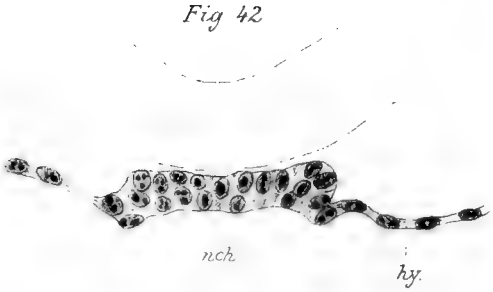


Fig 42

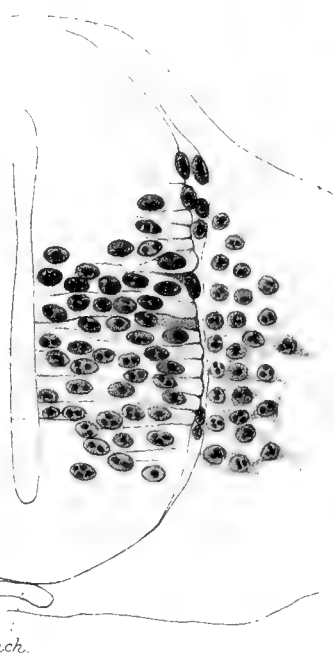


Fig. 43.

Fig. 46.

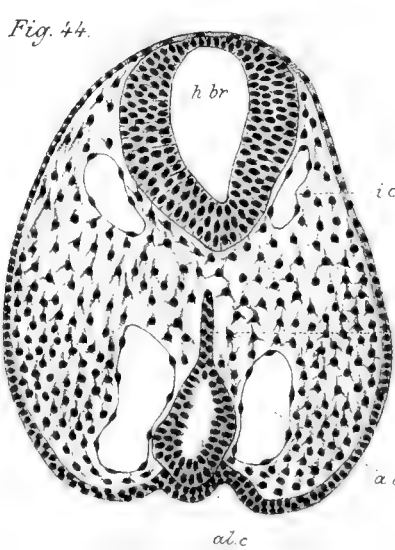
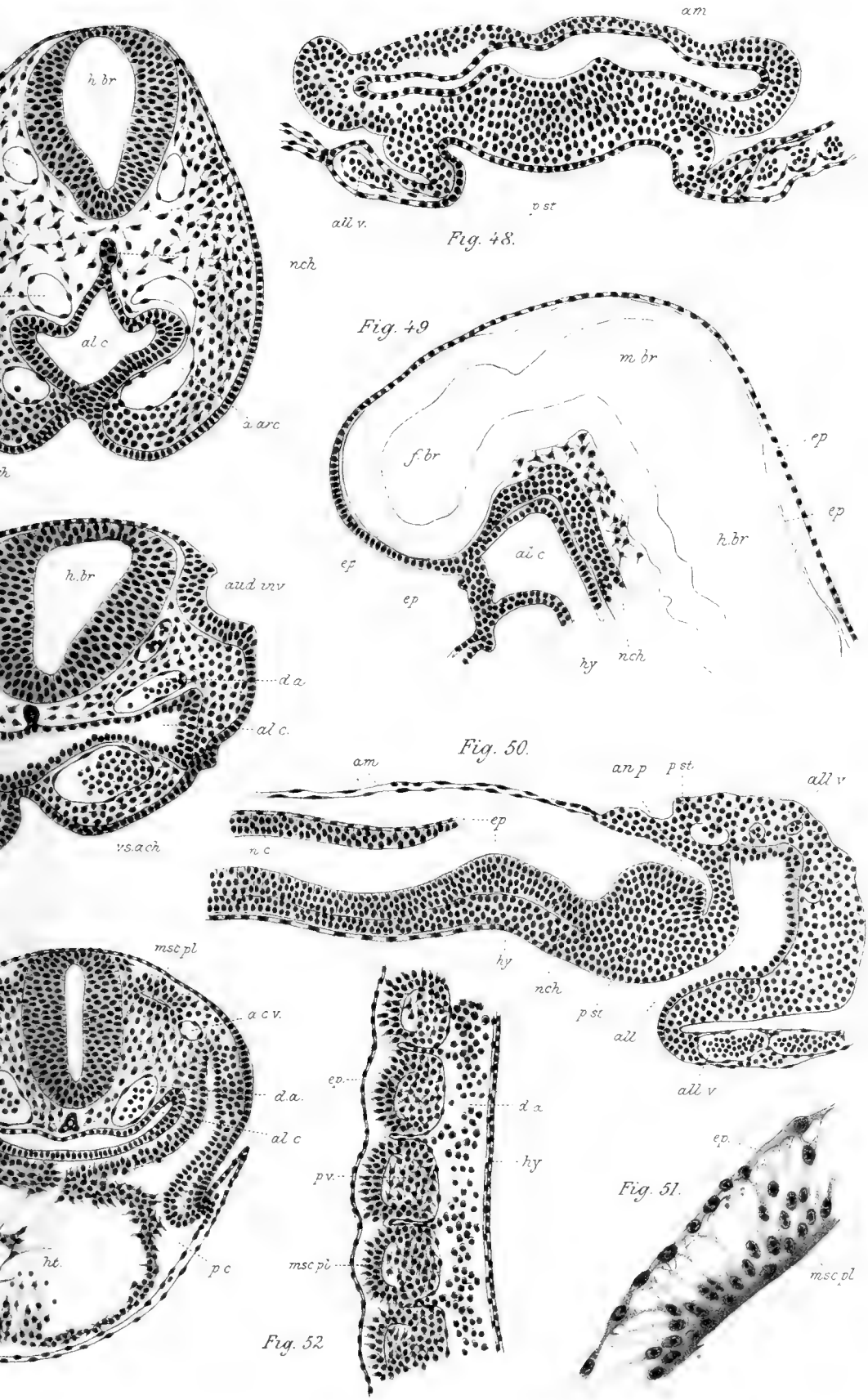


Fig. 44.



F. Huth, Lith. Lith.

cranial flexure, ventral prolongation of the anterior end of the fore-gut, and the hooked anterior end of the notochord is also indicated. The notochord is seen to be continuous at its anterior end with the hypoblast and epiblast at the point where the mouth will eventually be formed. The existence of mesoblast cells between the notochord and the floor of the brain is to be noticed.

Fig. 50, Stage J.—A median longitudinal section through the hind end of an embryo with fourteen protovertebræ. The allantoic cavity has increased in size, and numerous blood-vessels are seen in its walls. The mesoblast surrounding the allantoic cavity is derived from the primitive streak mesoblast. The relation of the epiblast, hypoblast, notochord, and mesoblast at the front end of the primitive streak is seen to be precisely the same as I indicated in a diagram (Fig. 50) of my former paper (No. 8). The neurenteric canal is obliterated.

Fig. 51, Stage J.—A transverse section of a portion of the muscle-plate of an embryo with fourteen protovertebræ. The cells of the muscle-plate (*msc. pl.*) are extended into long processes which are continuous with the epiblast cells (*ep.*) lying above them. These processes are commencing muscular fibres.

Fig. 52, Stage J.—Longitudinal section through an embryo with fourteen protovertebræ. The section is taken through a line about half way between a perpendicular and horizontal longitudinal line, and bisects the muscle-plates and aorta of one side of the body. The hypoblast lining the alimentary canal is cut through slightly to one side of the middle line, and the notochord therefore is not shown. The arched muscle-plate and muscular processes of its cells, the aorta and its dorso-lateral prolongations between the protovertebræ are shown.

On the Life-History of Pedicellina.

By

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With Plates XVI and XVII.

During the summer of 1885, spent in Rocquaine Bay, Guernsey, I succeeded in obtaining material for the study of the metamorphosis of Pedicellina echinata, a form which occurs in great abundance (in Rocquaine Bay) on Coralline growing under the shade of other seaweeds in tide-pools.

The larvæ of Pedicellina invariably refused to fix themselves when kept in a small quantity of water, and I therefore ultimately adopted the following method for procuring the various stages necessary for the investigation.

Adult colonies were placed, after the removal of all superfluous parts of the Coralline on which they were growing, in a small vessel, the mouth of which was closed by a piece of linen. The vessel was then left for a day or more in a tide-pool, after which a careful search (with the aid of a low power) over the Coralline was generally rewarded by the discovery of several young Pedicellina, which had resulted from larvæ hatched in the tide-pool, and which, owing to their inability to escape from the vessel in which they were confined, had been obliged to fix on the Coralline. After preservation with corrosive sublimate and decalcification of the Alga, sections were easily prepared. In this manner, I succeeded in obtaining numerous individuals of various ages, fixed under perfectly normal conditions.

My study of the metamorphosis of Pedicellina has led me in the main to a complete confirmation of the account already given by Barrois (No. 3), and summarized on pp. 312 and 313 of my previous paper on Loxosoma (No. 4), where I have ventured on a criticism of Barrois' conclusions which I do not find to be justified by my own investigation of the subject. In opposition to my previous opinion, I must now conclude that the post-larval changes consist in a remarkable metamorphosis, and that the first bud is formed after the primary individual has acquired its adult characters. Barrois has published no figures illustrative of his statements, the actual details of the process being difficult to understand from his very short description, whilst the morphological nature of the changes remains entirely obscure. The subject appears to me, therefore, to deserve further consideration.

The structure of the larva is well known from the researches of Hatschek,[1] and it will be unnecessary to describe it in more than a few of its details.

In the swimming attitude of the larva, the ciliated ring is everted to the exterior, whilst from the oral face project two prominent structures;—the epistome, with its tuft of long cilia, and the anal cone, on which opens the anus. During the retracted condition, however, the ciliated ring is reflected to the interior of the large vestibular cavity, whose outer walls are formed by the fold of skin which bears the ciliated ring itself (cf. Pl. XVI, fig. 1). The floor of the vestibule is constituted by the ventral or oral surface of the larva, being specially depressed between the base of the epistome and the anal cone, and at the sides of the latter.

As Barrois has correctly stated, fixation takes place by the oral surface, the larva being meanwhile in its "retracted" condition. Pl. XVI, fig. 1, a median longitudinal section, will serve to illustrate the method of fixation. It will be noticed that the long axis of the stomach is approximately parallel to the surface of attachment.

[1] *Vide* the summary of Hatschek's results in Balfour, 'Comp. Emb.,' vol pp. 242—246.

Fig. 3 represents a horizontal section of a larva not long after its fixation: the occurrence of brain (= "dorsal organ," *v.* No. 4), œsophagus, and rectum in the figure sufficiently defines the level of the section. The epistome is cut in the region of its greatest thickness, whilst at the summit of the anal cone is seen the depression into which opens the anus. By comparing this with fig. 1, it will be observed that the anus has already altered its position, since it is now directed somewhat forwards, the rectum being more nearly parallel to the stomach than before. The cells of the vestibular epithelium are very high at the sides of the anal cone, and are characterized by the special readiness with which they take up colouring matters.

Fig. 5 represents a horizontal section through the apices of the epistome and anal cone of another individual of the same age. The epistome is here seen to be continuous, at each side, with a fold of vestibular epithelium; epistome and folds together forming (as seen in this section) a horseshoe-shaped ridge partially embracing the sides of the anal cone, in which region the two lateral folds become evanescent. The result of this arrangement is the formation of a somewhat deep ciliated groove (*o. g.*) running round the greater part of the vestibule, and passing in front into the transversely elongated, funnel-shaped mouth. Posteriorly, however, owing to the disappearance of the lateral folds, the oral grooves fade away at the sides of the anus, where vestibule and oral grooves consequently appear continuous in such a section as that represented in fig. 5. The relations of these structures will become more clear on reference to fig. 4, a larva somewhat older than those previously described, the section passing transversely through the region of the anal cone, in the plane A B in fig. 1. At the sides of the anal cone are the two lateral portions of the vestibule (*l. v.*), these structures being separated from the oral grooves by the folds already mentioned. In the more anterior sections of the series, the lateral folds become continuous with the epistome, and the oral grooves with the mouth. Further back, on the contrary, the folds become lower, and finally dis-

appear, so that the oral grooves are not distinguishable in the post-anal region of the vestibule. The above description, together with a reference to fig. 5, will thus show that the deep post-anal groove (*m. v.*) of fig. 1 is continuous equally with the oral grooves and with the general vestibular cavity. For further clearness, dotted lines in the same figure indicate the position and relations of the right lateral fold as it would appear by looking at the wall of the vestibule from the inside of the latter. The relations of half of the ciliated ring and of the right oral groove are also shown in the figure.

Fig. 2 represents a longitudinal section of a recently-fixed larva, passing in the direction of the line C D in figs. 3 and 4. One of the lateral folds, owing to its projection inwards into the vestibule, separates the latter into two portions, containing respectively the mouth (and oral groove) and part of the epistome. The latter portion obviously corresponds to one of the lateral regions of the vestibule (*l. v.*) in fig. 3. Fig. 2 further explains the continuity of the tip of the epistome with the lateral folds (cf. figs. 1 and 5). In more median sections of the same series the latter are not seen, the epistome being perfectly free at its apex, whilst the separation of the vestibular cavity into two parts is not apparent.

A considerable portion of the base of the epistome and of the sides of the anal cone is formed of a remarkable tissue, composed of large cells, with transparent contents, hardly staining with colouring matters (fig. 2, *x*). The nature of this tissue (which atrophies during the metamorphosis) is unknown to me.

The revolution (about to be described) of the alimentary canal was obviously well understood by Barrois, although I did not formerly succeed in making out his exceedingly concise statements on this head.

Figs. 8 and 9 represent two sections of an obliquely longitudinal series through a more advanced stage. Fig. 9 involves the rectum, whilst fig. 8 shows the mouth and œsophagus. In the latter figure is seen one of the deep portions of the vestibule lying at the sides of the rectum, which is itself of course

not visible. The dorsal organ and the sucker have both degenerated, and are represented merely by the "globules" described by Barrois in various parts of the larva after its metamorphosis. These "globules" are rounded nucleated cells, which do not stain readily with reagents, their general form being shown in fig. 8, &c.

It is obvious, from an inspection of the two sections figured, that the stomach has now taken up a position inclined to the surface of attachment, the concavity of the alimentary canal being directed somewhat backwards.

Remarkable changes, already described in part by Barrois, have by this time occurred.[1]

Fig. 9 shows that the aperture of the vestibule has closed, so that this cavity has no longer any communication with the exterior. The vestibule is partially divided into three portions, which do not, however, quite correspond with those described by Barrois. The most ventral portion (*v. v.* in fig. 9) corresponds to the region near the previous vestibular aperture, and is destined to atrophy completely. The next portion (*v. or.*) is in connection with the mouth (fig. 8), whilst the most dorsal portion (*v. an.*) contains the anal cone, and is at this stage and later the largest and most important part of the vestibule. The second or oral division still communicates with the ventral portion, whilst it is almost separated from the dorsal or anal division by the growth of the epistome and of the lateral folds.

In another section of the series it is seen that the oral and anal divisions of the vestibule still communicate by a small aperture, as in the diagram, fig. 16 (*a. v. v.*).

The anal portion of the vestibule is very large, and is growing, at the previously posterior end of the larva, away from the surface of attachment. The cells lining this part of the vestibule are obviously engaged in active growth and multi-

[1] The following statements will be more readily understood with the assistance of Pl. XVII, fig. 16, representing in a diagrammatic form a median longitudinal section through an individual of the same age as figs. 8 and 9.

plication, their protoplasm being finely granular and staining readily with colouring matters. The backward growth of the vestibule occurs first in the regions at the two sides of the anal cone (cf. fig. 3), but soon extends to the median portion behind the cone (fig. 9), so that this part of the vestibule grows towards the free end of the fixed larva, during the rotation of the alimentary canal, as a single actively extending diverticulum, in which the primary differentiation of median and lateral regions is no longer marked.

Fig. 6 will serve to explain more clearly the relations of the oral grooves and neighbouring structures at a stage very slightly earlier than that of figs. 8 and 9. The section passes in a direction corresponding to the line K L in fig. 16, and consequently involves the apex of the epistome, the lateral folds, and the oral grooves. The anal cone, visible in fig. 5, is, of course, not involved by the section, which in other respects differs from the former figure mainly in the facts that the diameter of this portion of the vestibule has become lessened, and that by the partial rotation of the alimentary canal the apex of the epistome has come nearly into contact with the posterior wall of the vestibule (the manner in which this happens being understood by comparing fig. 1 with fig. 16), whilst the form of the lateral folds is at the same time altered (cf. fig. 6 with fig. 5). By this change of position of epistome and lateral folds, the oral and anal sections of the vestibule communicate merely by a comparatively small round aperture. The oral grooves are no longer continuous posteriorly with the anal portion of the vestibule, although on the left side of the section at least, a trace of the former continuity is distinguishable. During later stages the growth of epistome and lateral folds completely separates the oral from the anal division of the vestibule, the aperture *a. v. v.* in fig. 6 being gradually constricted until it finally disappears.

At the stage of figs. 8 and 9 a considerable amount of histolysis is taking place. This process affects specially the stomach, the epistome, the anal cone, and the ventral portion of the vestibule. In the case of the stomach, portions of the

epithelial cells and some of their nuclei pass bodily into the lumen of the organ (cf. figs. 8 and 9), where they are found quite free at later stages. The more projecting parts of the epistome and of the anal cone lose most of their component cells. The cilia of the former become indistinct, the cell-substance itself obviously degenerating (fig. 9). Ultimately ciliated portions of the cells are thrown off into the vestibule (figs. 9 and 12), in which they can be discovered until a very late stage in the metamorphosis. They no doubt leave the vestibule either by the mouth or by the (adult) vestibular aperture, when the latter is formed.

The histolysis of the ventral portion of the vestibule (fig. 9, *v. v.*) similarly results in the passage of fragments of cells into its own cavity.

This process is again illustrated by fig. 12, a section passing in the plane of the line E F in fig. 9. The permanent vestibule is in this section (cf. fig. 16) completely separated from the degenerating portion, its lumen, like that of the latter, containing fragments of degenerating cells.

The ventral division of the vestibule (*v. v.*) in fig. 9 occupies the position of the future stalk, and in later stages its cavity becomes more and more reduced until it finally atrophies. During this process, the cells previously found in its lumen disappear. In sections parallel to the plane of attachment the cavity (just before its atrophy) appears as a fine tube surrounded by a series of elongated cells radiating from it towards the body wall. It is very tempting to assume that these cells are phagocytes, engaged in the destruction of the vestibule. After the atrophy of the latter, its place is occupied by numerous "globules" (fig. 10), which will themselves be replaced by ordinary connective-tissue corpuscles (fig. 13).

The same assertion may be made of other parts of the "primary body cavity," which is at the stage of fig. 9 almost completely filled with "globules," resulting from the histolysis of the brain, the sucker, the tissue at the base of the epistome and anal cone, and other larval structures. When the primary individual is mature the "globules" have disappeared, and are

replaced by a gelatinous matrix, in which lie connective-tissue corpuscles. Are we not justified in assuming that the "globules" are the active agents in the histolysis, and that they are in fact typical phagocytes?

During the histolysis of portions of the anal cone, the latter structure itself becomes much depressed. This feature of the metamorphosis, although already obvious in fig. 9, may be further illustrated by means of fig. 7, a section passing in a plane corresponding to the line I J in fig. 16.

Owing to the further depression (occurring at a slightly later stage) of the anal cone, the marked bilateral arrangement of this part of the vestibule is, in part at least, lost. At the stage of figs. 8 and 9, as can be easily seen from these figures themselves, the posterior portion of the vestibule is no longer reduced in the median plane to a small slit between anal cone and vestibular wall (as in fig. 1), but is, in this position also, a spacious cavity lined by a columnar epithelium (fig. 9).

After the anal cone has reached the condition of the latter figure the vestibule, in sections parallel to the long axis of the stomach, will usually appear bounded posteriorly by a simple uniformly curved wall, whilst its œsophageal side is floored by the degenerating tissue of the epistome (fig. 7). In later stages, however, the well-developed epithelium of the sides of the vestibule extends inwards, so that the cavity is then entirely bounded by its permanent, partially regenerated epithelium.

In the next stage represented very considerable changes have occurred, whereby the alimentary canal has taken up a position not unlike that which it will ultimately retain. Fig. 10 represents an actual section which passes in the median longitudinal plane of a larva at this stage. Whereas in fig. 16 the axis of the stomach is but slightly inclined to the surface of attachment, in the present instance it has assumed a position almost at right angles to this plane, and the concavity of the gut is now directed towards the primitively posterior end of the fixed larva. In the course of this rotation of the alimentary canal the vestibule, owing to atrophy of one

at least of the portions described in the last stage, has become somewhat simplified. All the more ventral regions (situated in the neighbourhood of the surface of attachment) have completely disappeared, and in their place is found a mass of cells filling a cylindrical stalk, which obviously corresponds to that of the adult Pedicellina. The anal division of the vestibule has continued its backward growth and now lies almost at the free end of the young animal. At about this stage it acquires a secondary opening to the exterior on the side corresponding to the posterior surface of the larva. This opening is formed by a simple concrescence between the vestibular epithelium and the external ectoderm of the body, accompanied by a linear perforation formed at the point of junction of these two distinct portions of ectoderm. My sections have given me no indication of the occurrence of a "labial invagination" (Barrois, q. v.) placing the above portion of the vestibule in connection with the exterior.

The character of the vestibular aperture, immediately after its formation, may be seen from fig. 11, a section passing in a plane corresponding to G H in fig. 10. The vestibular aperture, at the sides of which tentacles (*t.*) are already developing, is shown, by an examination of the remaining sections of the series, to have the form of a slit elongated in the direction of the median plane of the animal. Immediately before the formation of the aperture the vestibular epithelium would appear, in a section of this kind, quite unconnected with the external ectoderm, but already extending towards it in the form of a median groove, similar in appearance to the portion *g. v.* in fig. 11.

The mouth in fig. 10 has, at first sight, the appearance of being closed. By a comparison, however, of fig. 10 with fig. 16, it would seem that the apex of the epistome is really represented (in the former) by the ectoderm closing the (permanent) mouth, and it is thus probable that the commencement of the digestive tube in fig. 10 (*v. or.*) is a part of the oral division of the vestibule. This impression is strongly confirmed by a section (not figured) similar to, but later than,

fig. 9. In the individual referred to, the stalk portion of the vestibule is still present, but is small, and is connected with the œsophagus very much as in the diagram fig. 16; i. e. at some distance from the point where the apex of the epistome ultimately meets the vestibular wall.

In somewhat later stages the permanent mouth is formed by the perforation of the septum between the two portions of the vestibule in fig. 10, and probably in the position of the aperture *a. v. v.* in fig. 16.

In living individuals of the same age could usually be discovered a small projection of the surface of the body in the region marked ?*s.* in fig. 10. This represents the larval "sucker," which, as Barrois has correctly stated, disappears during the metamorphosis. The region of the "dorsal organ" or brain of the larva is doubtless indicated by the marked angle on the left side of the stalk of the individual just referred to. None of the previous histological peculiarities of the organ remain at this stage, and it is in fact already almost impossible to distinguish with certainty its position.

It appears to me that Barrois has suggested the real explanation of the metamorphosis of Pedicellina, although he has confined himself to one or two short statements, which are given without any indication of the manner in which they are to be interpreted. I quote below one or two passages from Barrois' note so many times referred to (3), the given quotations reproducing, so far as I am aware, the whole of Barrois' explanation of this complicated subject.

(i) "La première position" [corresponding, from the description, with my own fig. 10] "représente un état tout à fait analogue au Loxosoma, avec anus en haut et œsophage en bas."

(ii) "L'inférieure" [portion du vestibule] "qui porte la couronne, et dont les éléments viennent former la glande du pied."

(iii) "Les deux organes énigmatiques de l'exoderme" [i. e. sucker and dorsal organ] . . . . "ne sont, suivant moi, que des organes provisoires; tous deux sont rejetés sur la face

dorsale, où ils finissent par disparaître, peu à peu. Sans doute il faut voir, dans les deux soies décrites par Salensky sur la face dorsale du Loxosoma crassicauda, le reste de l'organe des sens antérieur" [i. e. the dorsal organ] "qui, d'après mes recherches, vient occuper cette place."

I have already (4) explained my reasons for the belief that the dorsal organ at any rate, and perhaps the sucker, are important organs, which throw considerable light upon the morphology of the Polyzoa, so that I cannot accept Barrois' conclusion that these structures have no particular significance.

It is obvious that, however accurate Barrois' conclusions (quoted above) may be, they need further explanation. The similarity between larva and adult in the Entoprocta, even in the position of the buds in Loxosoma, is so striking that some means of comparing the two stages is necessary. I therefore suggest the following explanation of the relation between larva and adult.

It does not seem to me that Caldwell's theory of the surfaces of the Polyzoa receives any support from the metamorphosis of Pedicellina. The short line between mouth and anus remains unchanged throughout the metamorphosis, and in order to prove that it is not ventral, it still remains necessary to show that the dorsal organ of the larva is not a brain, and that the larval surfaces do not correspond with those of a Trochosphere.

Figs. 17—19 (Pl. XVII) are diagrams representing a possible explanation of the metamorphosis of the Entoprocta, but although founded on the history of Pedicellina, Loxosoma is the form which is actually (hypothetically) represented.

Fig. 17 explains a possible conception of one of the earlier stages in the acquirement of the sessile habit by the free-swimming Polyzoon ancestors. The form is, however, to all intents and purposes, a Loxosoma larva, with brain, sub-œsophageal ganglion (not discovered in Pedicellina until a stage later than fig. 10), and a pair of buds, one of which is shown. I believe there are no authentic instances of the fixa-

tion of a Polyzoon larva by any other than its oral surface, and it may therefore be assumed that this method of fixation was acquired at a very early stage in the phylogeny of the group. Let us suppose, however, that this "Archi-Loxosoma," on fixing itself by the edge of its vestibule, left an aperture (for the entrance of food), surrounded by the ciliated ring (*vide* fig. 17), leading from the exterior into the otherwise closed vestibule, and situated behind the anus.

Subsequent development may be imagined to give rise to a form like fig. 18, in which the vestibular opening is an elongated slit, extending along the whole of the region formerly occupied by the posterior side, and still surrounded by the ciliated ring. The mouth, in order to obtain its food as conveniently as possible, now faces the posterior side (of the former stage), and this has entailed a rotation of the entire alimentary canal, in the manner shown in fig. 18.

By the growth of the proximal end of the Polyzoon, the mouth would be thrust away from the point of support, and the animal might thereby obtain an advantage in procuring food by means of its ciliary currents. But during this process, the proximal portions of the ciliated ring would become far less efficient for obtaining food than the distal portions, and would tend to atrophy. The final result would be the acquirement of a form like fig. 19, representing in a very slightly diagrammatic form, an adult Loxosoma. The ciliated ring is here represented as consisting of two disconnected portions, corresponding (1) to the ring of tentacles; (2) to the foot-gland (cf. the second of Barrois' conclusions quoted on p. 156). The foot-gland has remained practically as an open groove, a series of ciliated tentacles having been developed round the margin of the permanent vestibule.

The position of the buds in the larval Loxosoma appears at first sight fatal to the above hypothesis. That this larva does actually develop buds normally can hardly be doubted, since I have shown not only that these structures are developed twenty-four hours after hatching (which might, however, be an abnormal circumstance, due to the want of proper conditions

for fixation), but also that ectodermic thickenings, the commencements of the buds, are to be detected some time before the embryo is ready to leave the maternal vestibule, the possibility of the development having been influenced by abnormal conditions being here out of the question.

In figs. 17, 18, and 19, the position of the dorsal organ is represented as not having been much altered during the rotation of the alimentary canal, which has, so to speak, been pulled through the loop formed by the dorsal organ and the somewhat hypothetical subœsophageal ganglion. Assuming for the moment this position for the dorsal organ, we find that throughout the metamorphosis the buds retain their original situation (in Loxosoma) between the dorsal organ and the ciliated ring, and that their position with regard to the œsophagus is practically the same as that which characterised them at their first appearance.

Is there, however, any reason for believing that the position of the dorsal organ is correctly indicated in the diagrams? It seems to me that this question must be answered in the affirmative. In the first place, the degenerating dorsal organ of Pedicellina does in reality occupy this position, and in the second place (*vide* No. 3 of Barrois' conclusions on p. 156), the circumœsophageal commissures may be represented by the strong ganglionated nerves passing from the ganglion to the "posterior sense-organs" in L. crassicauda, as originally described by Salensky (see also No. 4, Pl. xix, fig. 1). Should the metamorphosis of Loxosoma be proved to bear out this suggestion of Barrois', we must assume either that the whole brain has atrophied, or that the adult possesses at most a small portion of the brain at the ends of the two widely separated œsophageal commissures.

With regard to the actual metamorphosis of Pedicellina, I have to point out that I have not succeeded in demonstrating the presence either of œsophageal commissures or of a subœsophageal ganglion. The latter structure becomes distinct only at a stage later than fig. 10, and it then has the position which characterises the adult ganglion.

No. 1 of Barrois' conclusions quoted on p. 156, appears to me perfectly just. It is impossible in fact not to be struck with the great resemblance between the solitary Pedicellina shown in fig. 10 and an adult Loxosoma, and this similarity is quite conspicuous even at much later stages. The obliquity of the lophophore in Loxosoma is hence, on the view already explained, another of the archaic features of this genus, the lophophore having still a marked inclination to the "anterior" side of the animal (fig. 19).

It is unfortunate that the metamorphosis of Loxosoma, which possesses a foot-gland, should be unknown, but we are able to make certain inferences from the phenomena of budding. Both vestibule and foot-gland originate as longitudinal groove-like invaginations of the ectoderm of the "anterior" face of the bud. Fig. 15 is a reproduction of a drawing from Oscar Schmidt, in which the foot-gland is represented as originating from the two proximal cells of the ectoderm of the "anterior" side of the bud, and in which it is further seen that these cells are not in the least marked off from those which are taking part in the formation of the vestibule. The relations of lophophore and foot-gland in this figure are indeed exactly those of the ciliated ring in the diagram (fig. 18).

The Metamorphosis of Pedicellina viewed in its relation to the above Hypothesis.

I have no reason to believe that the position of the ciliated ring shown in fig 1 is in any way altered during the subsequent metamorphosis. This structure in all probability degenerates in situ.

The ciliary apparatus of an ordinary Trochosphere is not, however, constituted entirely by the præoral circlet. In the neighbourhood of the latter there occurs in Polygordius, e. g., (cf. Hatschek, No. 2) a series of smaller cilia forming a postoral circlet, whilst a third part of the apparatus is constituted by "a ciliated groove running between the two ciliated rings, and prolonging itself into the ciliated mouth." This

last portion is obviously represented in Pedicellina by the ciliated oral grooves, continuous, as in Polygordius, with the mouth. The relations of these grooves during the metamorphosis appear to me to deserve further consideration.

We have found that the median postanal portion of the vestibule is continuous with the oral grooves, of which it may, indeed, be said to form a part. Acccording to Hatschek (1) it is, like other portions of the vestibule, lined by ciliated cells.

If we are justified in assuming that the oral groove—a part of the typical Trochospheral ciliary apparatus—extends, potentially at least, from the mouth completely round the vestibule to the postanal region, it seems to me that considerable light is thrown on the metamorphosis. The morphological position of the oral groove will be in no way altered during the rotation of the alimentary canal, and in fig. 16 it will continue to pass from the mouth round the ab-anal side of the altered lateral folds to the median post-anal portion of the vestibule, even though it is no longer distinguishable in the persisting division of the latter structure. In figs. 16 and 6 we observe, however, the commencement of a separation of the oral groove into two parts—one continuous with, and becoming indistinguishable from, the "oral" section of the vestibule (*v. or.* in fig. 16), and the other potentially passing from the free apex of the epistome in fig. 16 to the end of the reference line *m. v.* in the same figure. The position of this latter portion will be the median line passing from *a. v. v.* to *m. v.* Owing to the fact that it is situated behind the anal cone it is, of course, unpaired (cf. fig. 5), and it appears to me that its situation may be very fairly considered to be represented by the linear groove which in fig. 11 has formed the permanent vestibular aperture. From the margins of this groove are developed the tentacles, which, if the above reasoning is legitimate, are formed from the region of the oral groove.

The fact that the tentacles of the adult lophophore of the oral side are on the ab-anal side of the mouth appears to me

to prove that the lophophore itself is developed from a morphologically præoral portion of the oral groove.

The relation between the velum proper and the oral cilia has become, in the Entoprocta, considerably complicated by the formation of a fold of integument (vestibular wall), carrying the former to some distance from the latter. When the Pedicellina larva attaches itself, the distance between the two structures becomes increased. The velar portion maintains its position at fixation, and soon atrophies; the oral groove, on the contrary, growing away from the degenerated velum. Even during the phylogenetic history of the process we may suppose that the velum atrophied at fixation. This is par excellence a locomotive structure, and would be useless in an attached condition. The oral cilia would, however, continue (in the hypothetical stage of fig. 18) to convey food to the mouth, and the cells bearing them would, after a time, become prolonged into tentacles, by which their range of activity would be extended.

During the abbreviated metamorphosis of Pedicellina it has hence resulted (if the above be true) that the velum takes no part in the change of position involved in the passage to the adult condition.

Summarizing the above, I may express my conviction (1) that the metamorphosis of Pedicellina is a simple modification of a more archaic process, due to abbreviation of development, (2) that the oral groove persists in part as the adult lophophore, (3) that the vestibule closes at fixation, and undergoes the whole of its alterations in the interior of the larva, opening secondarily only when the adult condition is practically attained.

The adult form is reached by the elongation of the stalk of fig. 10, and by the replacement of its contained "globules" by characteristic connective-tissue and muscle-cells; by the formation of a stolon and a diaphragm, and by various alterations in the calyx. The more important of these consist in the complete (or almost complete) loss of the obliquity of the lophophore, in the development of the permanent ganglion

and generative organs (if these are formed in the primary individual, as is probably the case) and in the complete formation of the vestibular aperture and tentacles. I have made no special observations on most of the above points, although on the important question of the origin of the colony from the primary individual, I am able to throw some light.

In the first place, it may be stated that adult colonies are by no means restricted to one growing point, as stated by Hatschek (1). Of very common occurrence is the development of two growing points, one at each end of the unbranched stolon: I have noticed this even before the formation of a single secondary calyx. A third growing point may be developed as a lateral branch of the main stolon; the amount of branching is, however, always slight in P. echinata, and apparently in all cases the œsophagus of each calyx is on the side directed to the growing point to which this calyx properly belongs, as already indicated by Hatschek.

The formation of the stolon is shown in fig. 13, a longitudinal section of the stalk of a completely developed but still solitary individual. The young stolon, which is cut medianly, is developed on the œsophageal side of the Pedicellina. The base of the stalk (which is alone represented) consists of a thick cuticle, underneath which occurs a layer of ectoderm, surrounding a gelatinous matrix in which lie connective-tissue and muscle-cells. The section, however,—an extremely good preparation—is contradictory to the theory of Hatschek, according to which the apex of the stolon is provided with a hypoblastic vesicle derived from the dorsal organ, and engaged in the formation of the mid-gut of the secondary calyces. I may at once state that I have entirely failed to convince myself of the occurrence of any such vesicle, at any period, in the stolon, and I am forced to believe that Hatschek has been mistaken in assuming its existence. Neither in sections nor in entire specimens (whether living or treated with reagents) could I discover the slightest evidence of the presence of Hatschek's vesicle, although I have investigated both adult and young stolons in this connection.

It appears to me probable that the growing point of the stolon of Pedicellina (*vide* fig. 13) consists solely of an ectodermic layer secreting a cuticle and of a mass of indifferent mesodermic connective-tissue cells, embedded in a structureless jelly. If this is the case, the only organ derived from the hypoblast of the embryo would appear to be the mesenteron of the primary individual, all other parts of the colony being devoid of any derivatives of hypoblast cells.

This conclusion can hardly be avoided unless we assume that some of the stellate cells of fig. 16 are really hypoblastic in nature, although indistinguishable from the mesoderm cells in their appearance. Owing to the nature of the process by which the dorsal organ degenerates, it is impossible to assert that some of its cells do not become amœboid wandering cells which migrate into the growing point. It can, however, be safely stated that no hypoblastic vesicle is formed from the degenerating dorsal organ. It may further be pointed out that the conclusion arrived at on a previous occasion as to the nervous (epiblastic) nature of the dorsal organ, in Pedicellina as in Loxosoma, is in opposition to the view that this structure plays any part in the budding.

The well-known fact that calyces of Pedicellina may fall from their stalks, which thereupon develop new calyces, appears to me in direct contradiction to Hatschek's view of the budding. The loss of the calyces is probably a normal, periodically occurring process, which is perhaps to be regarded as a means of rejuvenescence, and which is at least analogous to the formation of the "brown bodies" in the Ectoprocta. It is exceedingly easy to discover individuals in healthy colonies in which the calyx has been lost, and a new "bud" (easily recognised by its small size and immature condition) is being developed just below the scar. Specimens kept in captivity seem invariably to lose their calyces if the quantity of water is not very large, the calyx falling off at the "diaphragm." This structure, which is merely a constriction at the base of the calyx, filled by a row of flat cells, is perhaps a special arrangement by which the calyx can break away from the stalk, without

injury to the latter. I have been unable to show that calyces which have thus left their stalks are able to become the starting-points of fresh colonies. The specimens under observation have invariably died after a day or two, even if kept in a tide-pool.

Calyces formed at the scars produced in the manner above indicated, seem to me (from superficial examination of entire specimens) to develop in exactly the same manner as those produced at the true growing point. The occurrence of this phenomenon is undoubtedly adverse to Hatschek's theory of budding; the whole of the stomach falls away with the calyx, whilst the existence of a plug of cells filling up the diaphragm appears to preclude the possibility of the migration of any cells derived from the stomach to the proximal side of the diaphragm. Unless, indeed, it is assumed that some of the "connective-tissue" cells of the stalks as well as of the stolon are endodermic in nature, it must be concluded that none of the cells of the bud are descendants of any of the cells belonging to the embryonic hypoblast.

With regard to the further history of the budding (whether at the growing point or at the apex of an old stalk) I have very little to say. The free end of the stolon (or stalk) before long develops an ectodermic invagination (fig. 14) destined to give rise to the lophophore and, according to my view, to the whole of the alimentary canal of the bud. The latter is from the first continuous with the lophophoral rudiment, and in other sections of the series to which fig. 14 belongs, the stomach and vestibular cavity are separated from one another by means of a septum. The latter does not, however, cut off the whole of the deepest part of the invagination, but, since it is not developed in the position of the œsophagus the vestibule and stomach remain continuous with one another (as in fig. 14). By the formation of a diaphragm and by other processes already described by Hatschek, the bud attains its adult condition. The continuation of the stolon is formed by a lateral outgrowth from that region in the young bud which afterwards becomes the base of its stalk, precisely as in fig. 13 with the exception of the fact that the new growing point is formed long before the

bud is itself mature. It is worthy of remark that the young vestibular invagination does not occur accurately at the apex of the stolon, but on the side of the apex turned towards the growing point. In this respect it exactly agrees with the position of the vestibular invagination formed near the apex of a stalk which has lost its calyx, and again with that of the incompletely rotated vestibule in intermediate stages of the metamorphosis. It may indeed be said that the young vestibule of all the buds is inclined towards the growing point, and that in all cases it subsequently undergoes a rotation in the same direction (but to a less marked degree) as that occurring at the metamorphosis.

The history of the Pedicellina-larva appears to me to point to the existence of a fixation-period in Loxosoma also. In this case, the buds observed by me in the larva of L. Leptoclini would probably have to undergo a change of position, during the metamorphosis, similar to that represented in figs. 17—19. I am inclined to believe that the degeneration of the larval stomach observed in the same species, after a free life of one or two days, was abnormal, and was due to the absence of the conditions necessary for fixation.

On the Nature of the "Brown Bodies" of the Ectoprocta.

The above statements with regard to the life-history of the Entoprocta may, perhaps, give some indication of the manner in which the "brown bodies" of the Ectoprocta have originated. There can probably be no longer any doubt whatever that these structures are degenerated polypides, which are subsequently replaced by new ones budded off from the walls of the zœcia.

In the metamorphosis of Pedicellina the purely larval organs degenerate and form a mass of cells, which subsequently become connective-tissue cells. The degeneration is here slight, and has not yet acquired sufficient importance to give rise to a characteristic "brown body."

Whilst in the adult Loxosoma nothing comparable to the formation of "brown bodies" is known, the adult Pedicellina has developed a special arrangement—the constriction at the base of the calyx—by which the latter may be lost without material injury to the remainder of the colony.

In the adult Ectoprocta there seems to be the same necessity for the rejuvenescence of some of the organs, but here the occurrence of a thick ectocyst, usually intimately connected with that of neighbouring individuals, in general prevents the loss of any part of the body wall, as in Pedicellina. In some of the stoloniferous Ctenostomata, however, the entire zoœcium is deciduous.

But even in Pedicellina one may almost speak of a "zoœcium" in the same sense as in the Ectoprocta. It is a well-known fact that septa occur at intervals across the stolon of Pedicellina, and in most cases are developed in such a manner that a piece of the stolon, connected with the base of each stalk, is cut off from the remainder of the stolon by a pair of symmetrically-placed septa. There are thus typically two septa between the bases of each two stalks, and stalk-bearing and stalkless sections of the stolon alternate regularly with one another.

It is thus possible to consider stalk plus portion of stolon connected with it, the representative of a zoœcium. The distal end of the zoœcium is from time to time segmented off, carrying with it the whole of the alimentary apparatus, whilst a new polypide is developed within the remaining portion by a process of budding. By the formation of a new constriction the distal part of the zoœcium—the calyx—becomes again differentiated from the proximal part—the stalk.

In the Ectoprocta the occurrence of the same process is usually obviously impossible, and the polypide alone degenerates, forming a "brown body" which subsequently passes into the new stomach, and is ejected by the anus. The occurrence of this circumstance is already foreshadowed in two particulars in Pedicellina. We find, in the first place, that a new polypide is actually budded off by the ectoderm of the zoœcium at or

before the loss of the calyx; and, in the second place, that the tissues have already acquired, at the metamorphosis, the power of disposing of degenerated structures.

In the Ectoprocta one may hence suppose that, owing to the inconvenience of losing a portion of the zoœcium at each rejuvenescence, the new polypide is budded off near the preceding one, instead of from an entirely different part of the zoœcium, as in Pedicellina (below the diaphragm). The degenerating alimentary canal and other structures are then worked up by the "Parenchymgewebe" (Vigelius), which has inherited this kind of power from the larval tissues, into the condition of a "brown body," which passes into the new stomach, and reaches the exterior by means of the anus.

In the development of the Ectoprocta an archenteron is formed, in a large number of cases at least. The embryo is, however, richly supplied with yolk; it develops within the interior of the parent, and its alimentary canal is hence, in many cases, functionless.

At its metamorphosis this larva possesses no functional alimentary canal, and must hence form a new one. But since in its previous phylogenetic history our Polyzoon has acquired the power of developing new "polypides" from various parts of its ectoderm, a fresh gut could without difficulty be formed within the body wall of the metamorphosed larva; since the latter is now in the same condition as an adult zoœcium whose polypide has just become a "brown body."

This, indeed, is what actually happens. The larva passes at once into the condition of a zoœcium containing a "brown body," the remains of its larval organs. The complicated metamorphosis of Pedicellina has been given up, the larval structures now degenerating by the method employed during the atrophy of the polypides in adult individuals, and finally leaving the zoœcium by passing as the first "brown body" into the alimentary tract of the primary polypide, and thence to the exterior.

The metamorphosing Ectoproctan larva is probably in the same condition (irrespective of the difference pointed out

in the methods by which the alimentary canal is lost in the two cases) as the primary individual of a Pedicellina colony would be immediately after the loss of its calyx, supposing that it had not meanwhile developed a stolon and secondary calyces.

Unless I am mistaken in my views with regard to the metamorphosis of Pedicellina, it appears to me necessary to conclude that in the Entoprocta the ventral line of the body extends from *a. v.*^{2} in figs. 10 and 19, down the right sides of the figures, as far as *a. v.*^{1} The median dorsal line will in consequence be represented by the entire left sides from *a. v.*^{1} to *a. v.*^{2} These surfaces are most clearly expressed in the young Loxosoma bud, in which the whole of the surface turned away from the parent (characterised by the possession of vestibule and foot-gland) is ventral, whilst the opposite surface of the bud is, conversely, dorsal.

I hope to be able before long to publish some account of the development and metamorphosis of the Ectoprocta. Till that time I prefer to withhold any further expression of opinion with regard to the surfaces and relations of the larvæ of this group of the Polyzoa.

List of Papers Referred to.

1. B. Hatschek.—"Embryonalentwicklung und Knospung der Pedicellina echinata," 'Zeits. f. wiss. Zool.,' Bd. xxix, 1877, S. 502.
2. B. Hatschek.—"Studien zur Entwicklungsgeschichte der Anneliden," 'Arb. a. d. Zool. Inst. zu Wien,' Bd. i, 1878, S. 277.
3. J. Barrois.—"Métamorphose de la Pédicelline." 'Comptes rendus de l'Acad. des Sci.,' T. xcii, 1881, p. 1527.
4. S. F. Harmer.—"On the Structure and Development of Loxosoma," 'Quart. Journ. Mic. Sci.,' vol. xxv, 1885, p. 261.

EXPLANATION OF PLATES XVI & XVII,

Illustrating Mr. S. F. Harmer's Paper on "The Life-history of Pedicellina."

Reference Letters.

an. Anus. *an.c.* Anal cone. *a. v.*[1] and *a. v.*[2] Hypothetical morphologically anterior and posterior ends, respectively, of the vestibular aperture. *a. v. v.* Aperture between oral and anal divisions of vestibule (in position of permanent mouth). *b.* Bud. *br.* Brain (= "dorsal organ"). *c. c.* Fragments of ciliated cells. *c. p.* Ciliated pit of brain. *c. r.* Ciliated ring. *d. s.* Dorsal sense-organ (of Loxosoma). *epst.* Epistome. *f. br.* Fibrous part of brain. *f. g.* Foot-gland. *ga.* Ganglion of adult. *g. p.* Growing point of stolon. *g. v.* Median groove of permanent vestibule, ultimately becoming the vestibular aperture (in position of part of oral groove of larva?). *int.* Intestine. *l. f.* Lateral fold of vestibular wall. *l. v.* Lateral portions of anal division of vestibule. *m.* Mouth. *mes.* Mesoderm. *m. v.* Median postanal portion of the anal division of the vestibule. *œ.* Œsophagus. *o. g.* Oral groove. *rec.* Rectum. *s.* Sucker. *st.* Stomach. *t.* Tentacle. *v.* Vestibule. *v. a.* Its aperture. *v. an.* "Anal" division of vestibule. *v. or.* "Oral" division. *v. v.* Ventral division. *x.* Large-celled tissue at base of epistome and anal cone.

PLATE XVI.

Pedicellina echinata.

Fig. 1.—Median longitudinal section of a larva quite recently fixed (on Coralline).

Fig. 2.—Obliquely longitudinal section (in the plane C D in figs 3 and 4[1]) of a similar larva.

Fig. 3.—Horizontal section of a slightly older larva, passing through brain (= dorsal organ), œsophagus, epistome, and anal cone.

Fig. 4.—Obliquely transverse section (in the plane A B in fig. 1), at a stage very soon after fixation.

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[1] In describing one section as passing in a plane indicated in the figure of another, it is to be understood that the details in the two individuals do not always exactly correspond. This is due, partly to a difference in age between the two larvæ figured, and partly to variations in the position of the internal structures, owing to varying conditions of muscular contraction.

Fig. 5.—Horizontal section, at an early stage in the metamorphosis, passing through the tip of the epistome, the lateral folds and oral grooves, and the apex of the anal cone.

Figs. 6 and 7.—Two sections of a considerably older individual, passing respectively in the planes K L and I J in Fig. 16.

Figs. 8 and 9.—Two sections of an individual of the age of Fig. 16, passing in an obliquely longitudinal direction. Fig. 8 cuts the mouth and one of the lateral portions of the permanent vestibule, Fig. 9 passing through the rectum and the degenerating vestibule of the stalk. In another section of the same series the two parts of the vestibule are continuous, exactly as in the diagram, Fig. 16.

Fig. 10.—Median longitudinal section of an advanced, but still solitary, individual.

Fig. 11.—Horizontal section (in the plane G H in Fig. 10) through a similar specimen.

Fig. 12.—Section of an individual of the age of Figs. 8 and 9, passing in the plane E F in the latter figure.

Fig. 13.—Median longitudinal section through the stalk of a solitary individual with commencing primary stolon. The arrow indicates the position of the oral side of the calyx.

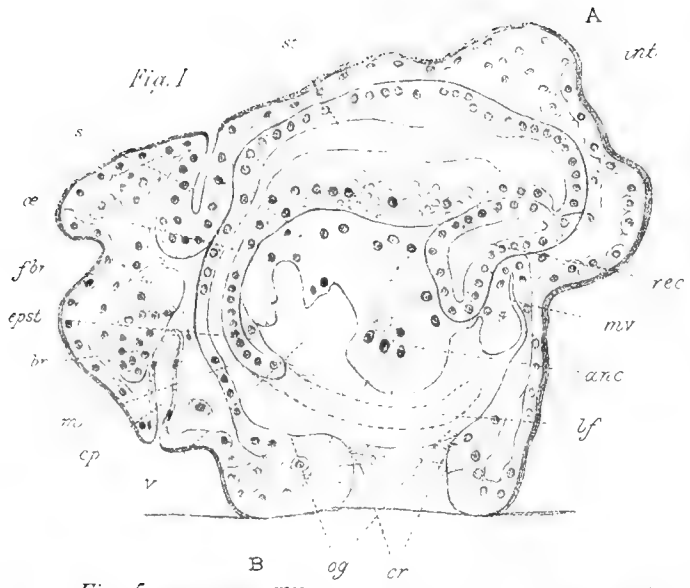
Fig. 14.—Obliquely transverse section of a young bud, developed at the growing point.

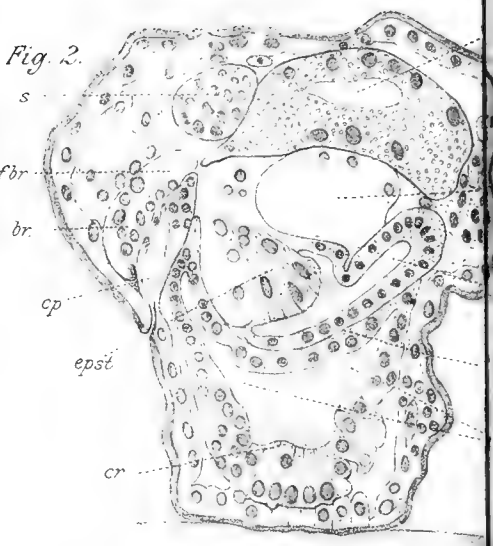
PLATE XVII.

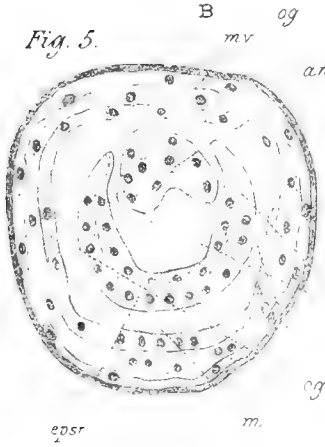
Fig. 15.—Young bud of Loxosoma, from the ventral side. Copied from O. Schmidt, 'Arch. f. mik. Anat.,' Bd. xii, 1876, Pl. III, fig. 17.

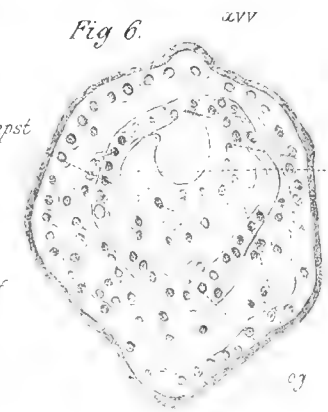
Fig. 16.—Diagrammatic longitudinal section of a metamorphosing Pedicellina at the stage of Figs. 8, 9, &c.

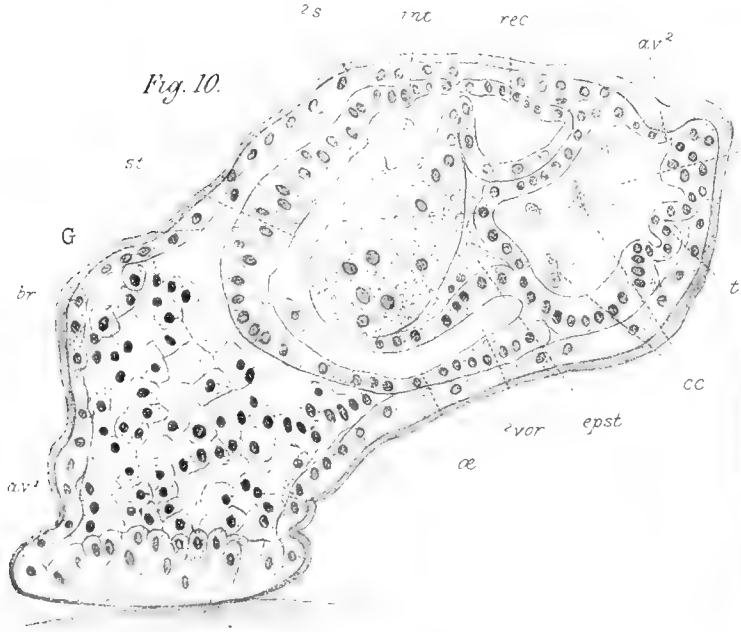
Figs. 17—19.—Diagrams illustrating the supposed morphological nature of the metamorphosis of the Entoprocta. A full explanation is given in the text.

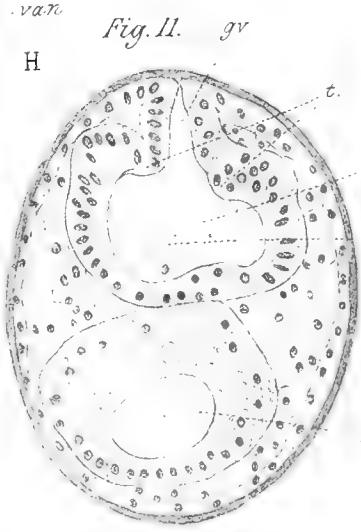












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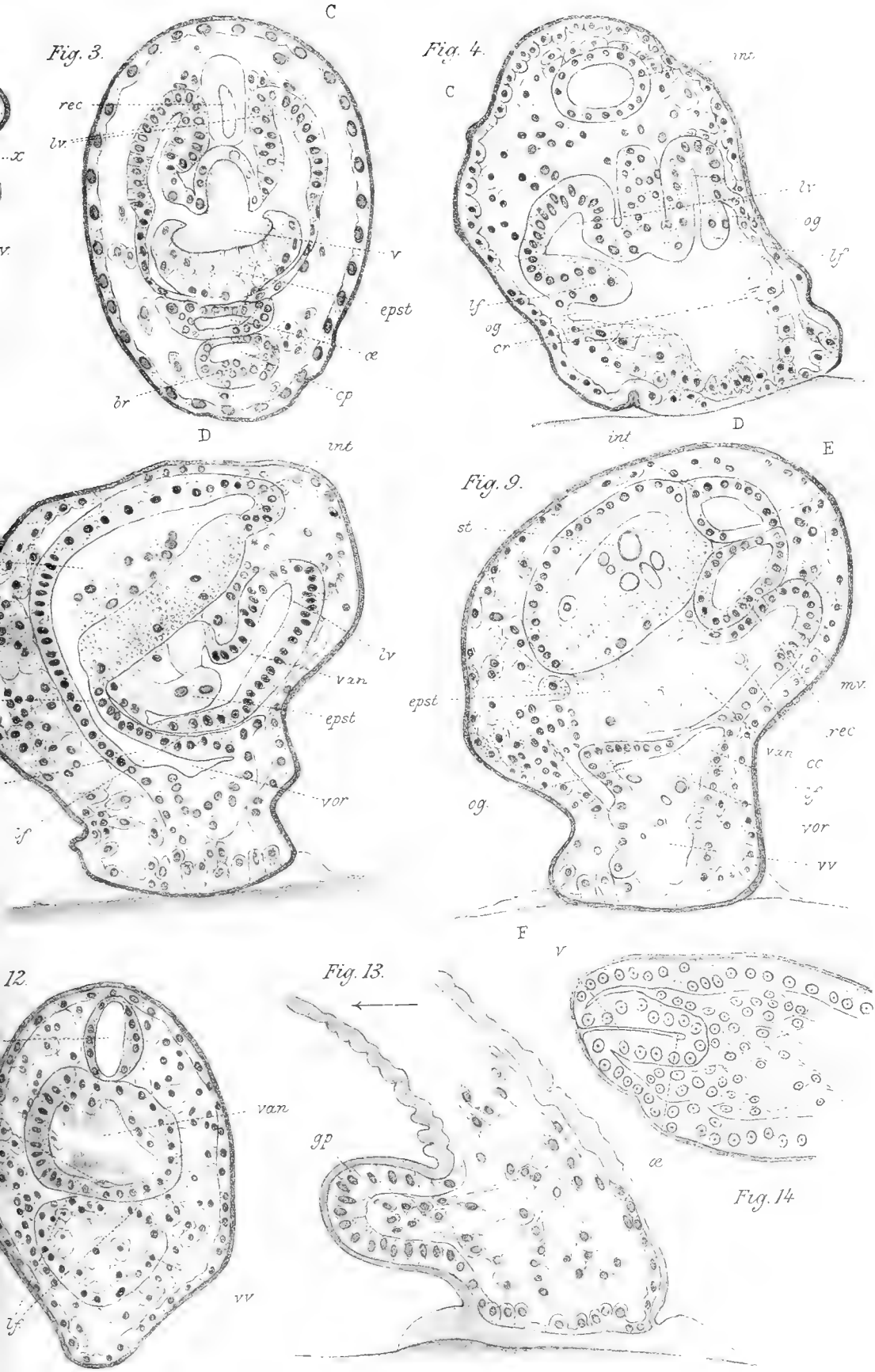


F. Huth Lith. Bath.

Fig. 15

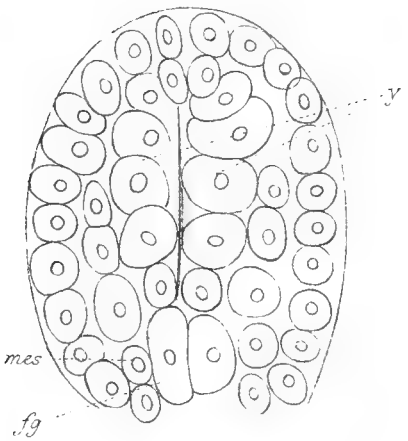


Fig. 16

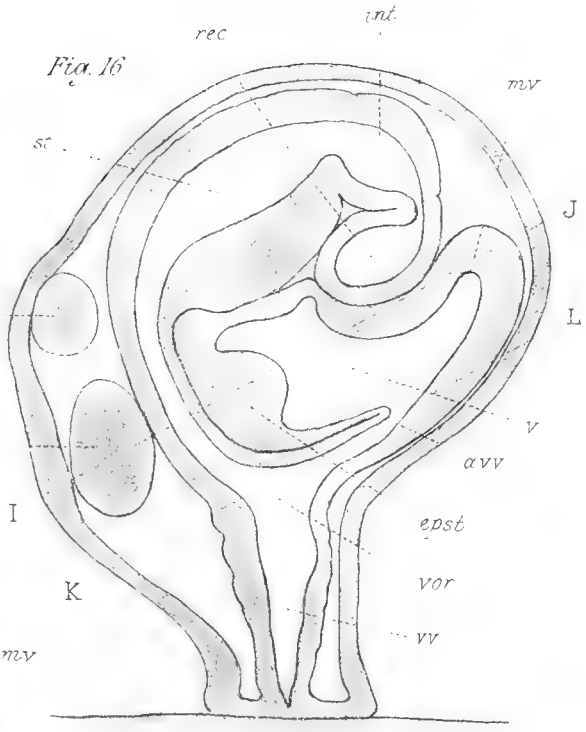


Fig. 17

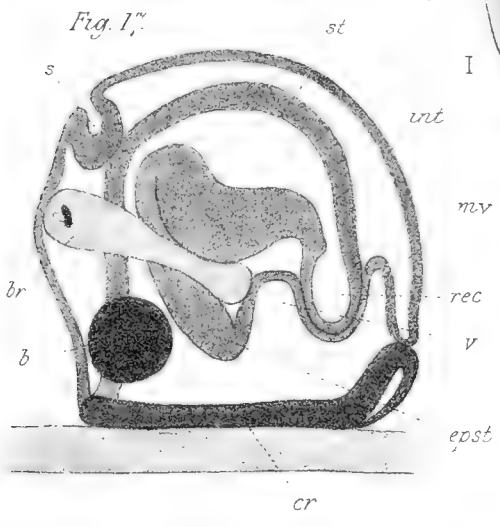


Fig. 18.

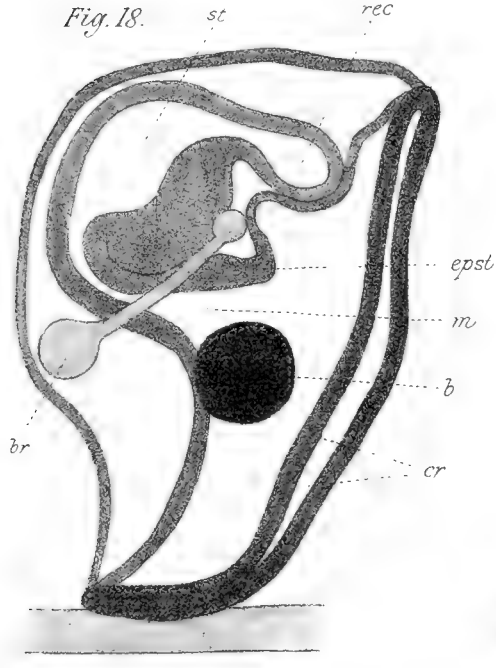
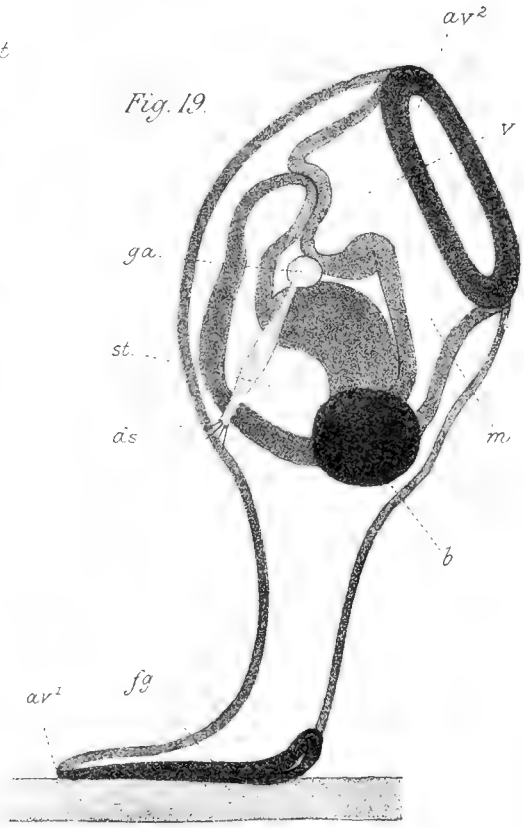


Fig. 19.



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On Some Points in the Development of Petromyzon fluviatilis.[1]

By

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With Plates XVIII, XIX, XX, and XXI.

The development of the Lamprey has occupied the attention of many embryologists during the last fifty years. Of these we owe the most complete accounts of the changes through which the egg passes to Max Schultze, Owsjannikow, Calberla, Scott, Balfour, and Dohrn. I have recently worked through the development of Petromyzon again, and worked out the origin of several organs which have hitherto been incompletely known. In many of the most important points my researches confirm those of the earlier observers, and to these I have only referred at such length as would make the account intelligible; in others, such as the persistence of the blastopore, the origin of the ventral mesoblast, &c., I differ from previous descriptions; and some points, such as the development of the heart, of the parts of the brain and cranial nerves, are worked out for the first time.

The material for this article was obtained by artificially

[1] The differences between Petromyzon planeri and fluviatilis are so slight, and the intermediate forms so common, that I am disposed to follow Anton Schneider, and to consider them as varieties of the same species. This species may conveniently retain the name fluviatilis, as opposed to the larger form Petromyzon marinus.

fertilising the eggs of the ripe female Lampern, hatching the larvæ out, and rearing them in confinement. The breeding time is during the latter half of April and the beginning of May.

The generative products of both male and female were squeezed into glass vessels containing fresh water, and the contents slightly stirred. The eggs at once adhered to the bottom and sides of the vessel, and were left undisturbed for three or four hours. The water was then poured off and a fresh supply added. This was kept thoroughly aerated by means of Semper's aerating apparatus. The number of eggs fertilised were about 70 per cent. of the total, though some hatches were much more successful than others. The rate of segmentation and development also varied greatly, being influenced by the temperature and manner of aeration. The unfertilised eggs very soon could be distinguished from the fertilized; they developed great cavities or craters and were soon attacked by fungi. The fungus, however, rarely affected the developing eggs.

The spermatozoa have elongated heads, pointed at their free end, but thicker at the end from which the tail arises (fig. 1). Their length is from 35 to 40 micro. mm., of which the head forms 3 micro. mm. They move actively about in the water until they come into contact with an ovum. They enter the egg through a micropyle, and Calberla states that the head only enters the protoplasm of the ovum, the tail remaining fixed in the micropyle, thus hindering the entrance of other spermatozoa.

The eggs are almost spherical, with a diameter of about a millimetre. On contact with water the outer cell-membrane swells up and forms a gelatinous coating, by means of which the eggs adhere to the bottom and sides of the vessel. This gelatinous envelope is of considerable thickness; it ultimately disappears shortly before the embryo is hatched. Sections through unfertilised eggs show the protoplasm crowded with oval yolk granules, which stain deeply. These yolk granules vary in size, and this is very evident in the segmenting eggs,

where the yolk granules in the more quickly dividing upper pole are much smaller than those in the more inert lower pole. An attempt has been made to show that those parts of the unsegmented egg containing the smaller granules is destined to form the epiblastic parts of the embryo (16).[1] This view seems to me to need confirmation. The small size of the yolk granules in the epiblast might be due to the more rapid division of these cells, causing a more rapid consumption of the food-yolk.

The unusually deep staining which the yolk granules assume very materially increases the difficulty of observation. Especially in the earlier stages of development the cell limits and nuclei were rendered obscure by the masses of deeply stained yolk granules.

As previous observers have stated, there are two polar bodies extruded one after the other. After fertilization the egg contracts, leaving a cavity between it and the egg membrane.

The first furrow appears about the fourth hour; it appears first in the upper pole and spreads round the egg on each side. Calberla states that the micropyle becomes at first oval, then slit like, and finally passes over into the primary furrow. I have not been able to observe this process in my eggs. He further states that the first furrow divides the egg into two unequal parts, a large epiblastic and a small hypoblastic; the smaller of these divides subsequently more rapidly than the latter. Thus, according to him, the first furrow would correspond with the first equatorial one in the Frog's ovum. Scott, although he had no fresh material to work with, was able to correct this, and, as the latter suggests, Calberla was probably misled by cases of abnormal segmentation. Many of the eggs which apparently had not been fertilized divided by one, two, and sometimes three furrows, and when this took place the furrows were nearly always abnormal in position.

The second furrow is vertical and at right angles to the first, and also appears first in the upper pole. The third is

[1] The figures in brackets refer to the list of papers at the end.

equatorial, but nearer the upper than the lower pole. After its appearance the epiblastic half is separated from the hypoblastic or yolk-bearing half (fig. 2).

The external phenomena of segmentation have been accurately described by Max Schultze, with the exception of the next stage. After the first equatorial furrow he describes two more in the same plane, but in my eggs the equatorial furrow was followed by two vertical lines, which appear at first in the upper pole exactly as they do in the Frog's ovum (fig. 3). These are followed by two more equatorial furrows which divide the egg into thirty-two segments. After this the segments of the epiblastic pole divide more rapidly than those of the lower.

Fig. 5 represents a transverse section through an egg thirty-six hours after fertilisation. In this stage it is a blastosphere, with a segmentation cavity enclosed by a single layer of cells except along the line where the epiblastic and hypoblastic cells join. Here the layer is two cells thick. The nuclei of the large cells appear small, but it must be recollected that the amount of protoplasm is very small compared to the yolk. The latter has been omitted for the sake of clearness. Fig. 6 is taken from an egg twelve hours later. Here both the roof and floor of the segmentation cavity are many cells thick. A similar stage is found in the Frog's ovum, but there is this difference between the two. In the Frog's egg the whole of the roof of the segmentation cavity forms epiblast; in the Lamprey it is only the outermost layer. The following stages are accompanied by a thinning out of the roof of the segmentation cavity, and are represented in figs. 7 and 8. On this point my observations tend to confirm those of Calberla, and are opposed to those of Schultze, who found a many-layered roof to the segmentation cavity just before invagination. The thinning out appears to be brought about by the inner cells of the roof passing round to the sides and floor of the segmentation cavity. Just before the invagination which forms the gastrula the roof of the segmentation cavity consists of a single layer of cells; the segmentation cavity is

large and occupies the whole of the upper hemisphere, whilst the lower hemisphere is solid and consists of larger cells, which we may speak of as yolk-cells. The most external layer of these consists of rather columnar cells. These latter cells soon become smaller than the inner yolk-cells, and about the time of invagination the whole egg is enclosed by a layer of small columnar cells, the epiblast. This is brought about by the conversion of the outermost row of yolk-cells into small columnar cells. As Balfour has shown, this takes place latest in the region of the blastopore.

The invagination which forms the mesenteron commences about 130 hours after fertilisation; it commences at one side of the equator of the egg, in the region where the single layer of epiblast cells passes into the yolk-cells (fig. 9). The invagination at first has a wide-arched slit-like opening, but this soon narrows into a small circular pore (fig. 4). The segmentation cavity is gradually obliterated by the invaginated cells. These from the first enclose a cavity, the mesenteron. In this respect the formation of the gastrula is like that of Amphioxus, and differs from that of the Amphibia, where the mesenteron appears later as a splitting underneath the invaginated cells. The presence of a large amount of food-yolk causes the invaginated cells to be pushed dorsalwards. The mesenteron extends as a tubular cavity about two thirds round the embryo. Its dorsal wall is composed of columnar cells resembling those of the general epiblast; the cells forming the floor have the same characters as the yolk-cells (fig. 12). The dorsal side of the mesenteron lies in immediate contact with the under surface of the epiblast throughout its entire length. In this respect again the Lamprey differs from the Frog, where the invaginated hypoblast cuts off a mass of cells on its dorsal side, which subsequently forms the mesoblast.

The mesoblast now appears by the differentiation of two bands of these yolk-cells, which lie in the angles formed by the mesenteron and the epiblast (fig. 12). This differentiation commences in front and is continued backward. The two bands of mesoblast are separated dorsally by the juxtaposition

of the dorsal wall of the mesenteron and the epiblast, and ventrally by the hypoblastic yolk-cells which are in contact with the epiblast over two thirds of the embryo. Subsequently, but at a much later date, the mesoblast is completed ventrally by the downgrowth on each side of these mesoblastic plates. This takes place at a comparatively early stage in the head and that part of the trunk lying in front of the liver. In the posterior part, which remains swollen with yolk, the ventral completion of the mesoblast is delayed.

The first formation of the mesoblastic plates appears to take place by a differentiation of the hypoblastic yolk-cells in *situ*, and not from invaginated cells (figs. 12 and 13). The subsequent downward growth is brought about by the cells proliferating along the free ventral edge of the mesoblast, these cells then growing ventralwards, pushing their way between the yolk-cells and epiblast (fig. 11).

This account of the origin of the mesoblast differs from that given by Scott. He describes the mesoblast as arising from two sources—(1) cells which are derived from the invagination of the blastoderm, (2) the outermost layer of the hypoblastic yolk-cells, which, according to Scott, split off from the remainder, and form a ventral sheet which completes the mesoblast in that side of the body. The mesoblast in the head is derived only from the first source, as by the time it is completed ventrally the head is raised above the yolk-containing parts.

Shortly before the development of the head fold raises the head from the yolk-bearing part of the embryo, the neural plate becomes evident in the exterior. It extends as a low ridge from the anterior lip of the blastopore to just in front of the blind anterior end of the mesenteron, over two thirds of the circumference of the embryo.

The blastopore is always visible at the posterior end of the neural plate. Schultze has given a very complete set of figures of the exterior of the embryo. As his figures show, with the elongation of the embryo the anterior end curves round and overlaps the posterior, thus obscuring the blastopore. Fig. 10

is a section taken through the blastopore and the head soon after the head is raised above the general level of the egg.

From his observations of the embryo as a whole, Schultze came to the conclusion that the blastopore persisted and gave rise to the anus, and he was supported in this view by Calberla. Later observers, however, who have studied the development of the Lamprey by means of sections, have maintained Benecke's view that the blastopore disappears. Scott describes the neural canal enclosing the blastopore and figures the neurenteric canal thus formed. He describes the formation of the anus, from a protuberance of the alimentary canal which approaches the epidermis and breaks through about the twentieth day. Balfour also states that the blastopore closes and does not form the permanent anus.

My observations of the embryo as an opaque object lead me to the belief that the blastopore remained open. In this I have been confirmed by sections taken through a series of embryos preserved at intervals of a few hours. Primarily the blastopore lies at the posterior dorsal end of the embryo (fig. 4), but by the growth of the dorsal surface and the formation of the tail it comes to occupy a position in the ventral surface. What was the anterior lip in the first position comes to be the posterior in the latter.

Fig. 4 is a view of the embryo twelve days old, as an opaque object, showing the blastopore at the posterior end of the neural ridge. Fig. 16 is an oblique section through an embryo about two days older, showing the nervous cord just separated from the skin, and the notochord both continuing behind the blastopore.

Scott was of opinion that the lumen of the invaginated mesenteron persisted only in the fore-gut. Soon after the invagination is completed this part of the alimentary canal lying in the head and neck becomes raised from the rest of the embryo. It is thus separated off from the yolk-cells, and the hypoblastic cells in this region soon assume a definite columnar appearance, though they continue to contain yolk granules for some days. This region extends to where the liver appears

in older embryos. A similar change in the cells lining the mesenteron takes place at its posterior end. The cells lining the blastopore and extending for some distance into the alimentary canal assume very early a columnar appearance and appear perfectly continuous with the columnar epiblast (figs. 10, 14, and 16.) The cells lining the hind-gut retain the character of the yolk-cells for a long time, but the lumen of the mesenteron in this region never disappears, as Scott and Calberla thought. The lumen of the alimentary canal, with the exception of the mouth, is derived directly from the invagination which forms the gastrula, and no part of it is ever obliterated in the course of development.

A similar persistence of the blastopore to form the anus appears to be common in the Amphibia. It has been shown to occur in the Newt by Miss Johnson, in the Frog by Spencer, and in Alytes by Gasser. Its occurrence in the Cyclostomata seems to point to the fact that it is a primitive feature retained in those eggs whose development is not greatly modified by the presence of a large mass of yolk. Renewed observations in the development of Amphioxus would probably throw some light on this point.

The Central Nervous System.

The early development of the central nervous system has been so fully described by Calberla, Balfour, and Scott, that little is left to be added to their account. But the origin of the neural canal, the relationship of the posterior end of the neural cord to the blastopore, and the later development of the parts of the brain and the cranial nerves present points of interest.

Calberla was the first to show that the central nervous system of the Lamprey arises by a delamination and not by an involution of the epiblast. He described a similar origin for the nervous system of the Teleostei, and Balfour and Parker found the same to be the case in Lepidosteus.

The first trace of the neural plate appears about the eighth day after fertilization, just after the invagination is completed. A

shallow groove is seen running forward from the blastopore, round about two thirds of the embryo and passing a little in front of the blind end of the mesenteron. The groove is a very temporary structure and is soon replaced by a ridge. This arises by the epiblastic cells lining the groove, which are of a columnar shape, budding off cells from their under surface. The result of this is that a keel of cells is formed which forms the neural ridge externally (fig. 12), and internally presses in between the mesoblastic plates. The keel arises solely by the epiblast cells budding off cells in their under surface only. It is much deeper in the anterior third of its course, which region ultimately forms the brain.

The keel in the course of two or three days loses its connection with the epidermis; this occurs at first anteriorly and extends backward, and as Scott has pointed out, it does this of itself and not by an ingrowth of the mesoderm in each side as Calberla described.

Figs. 13, 15, and 16 show the solid neural cord lying above the notochord, which by this time is separated off from the hypoblast. It is important to notice that the neural canal does not arise until after the connection between the neural cord and epidermis is severed. It is about the origin of this neural canal that my observations and those of Calberla and Scott are at variance. They described the epidermic layer of epiblast passing down into the nervous, in such a way that the canal, when it does appear, is lined by this layer. I have not been able to see any trace of this. The cells forming the nervous system appear to me to be all split off from the under surface of the epidermis in the dorsal middle line, and the continuity of the epidermis in this region never seems to be broken by any such invagination as they suggest. Balfour was also doubtful on this point; but in his and Parker's work on the development of Lepidosteus, they state that there is no evidence of the epidermic layer being concerned in the formation of the canal.

The canal seems to arise as a split between the cells in the axis of the solid cord, and not by the absorption of the central

cells, as has been suggested in the case of the Teleostei. It appears at first anteriorly and extends backward, and for some little time the walls of the lumen are by no means sharply defined. Processes from the cells lining the canal project into its cavity and suggest the idea that they have been torn out from between the cells of the other side.

The neural cord remains solid at its posterior end for some time, and here it becomes fused with the surrounding structures in a somewhat remarkable way. It does not fuse round the blastopore as Scott describes, indeed it is not easy, considering its mode of origin, to see how it could; and there is no hollow neurenteric canal. Figs. 14 and 15 represent two sections taken through a larva just after hatching. Fig. 14 is through the region of the blastopore. It shows the neural cord with its canal already formed; beneath this lies the notochord, and beneath this again a solid rod of cells which is continuous with the subnotochordal rod and the dorsal hypoblast. This latter structure is the solid postanal gut. The mesoblastic plates are seen separating off from the hypoblast yolk-cells which occupy the remaining space with the epidermis. Dorsally this is produced to form the dorsal fin. Fig. 15 represents a section through the tail a little posterior to the blastopore. Here the neural cord, notochord, and postanal gut have fused into a rod-like mass of tissue which is ventrally continuous with the hypoblast cells; a few sections posterior to this none of the three embryonic layers are distinguishable except the epidermal portion of the epiblast. A longitudinal median section through the tail is represented in fig. 20. This shows the mass of indifferent tissue which lies in the tail and which by internal differentiation gives rise, as the tail grows, to mesoblastic somites, neural cord and postanal gut. This mass of tissue, which in many respects reminds one of the growing point in a plant, may be called the primitive streak. It is perhaps worth while to point out that it lies at what was originally the anterior lip of the blastopore.

A similar mass of tissue formed by the fusion of the primary layers has been described by Balfour and Parker in

Lepidosteus, Spencer in the Frog, and Miss Johnson in the Newt.

The further development of the central nervous system will be described later after some of the details connected with the mesoblast and hypoblast have been considered.

The Mesoblast.

The origin of this layer from the yolk-cells situated in the angle between the epiblast and the invaginated endoderm has been described above. For some little time the mesoblast remains in the condition of two triangular masses of cells, separated from one another dorsally by the notochord and nervous system, ventrally by the yolk-cells which lie in contact with the ventral epiblast. In the anterior end of the embryo the mesoblast soon unites ventrally by lateral downgrowths; in the trunk, however, which remains crowded with yolk-cells for a week or ten days after hatching, this takes place much later.

Scott has described the formation of the muscle-plates very accurately, and it will therefore be unnecessary to give more than a short résumé in order to make the following account intelligible. About the twelfth or thirteenth day the mesoblastic somites appear by the segmentation of the dorsal part of the lateral mesoblastic plates. These appear at first anteriorly, and the segmentation extends backwards. The most anterior one lies close behind the auditory sac. The ventral unsegmented mesoblast has split into the splanchopleure and the somatopleure on each side, and in the region just behind the posterior gill-cleft these have met ventrally, forming a ventral mesentery, connecting the alimentary canal with the ventral body wall.

The mesoblast somites are shown in fig. 17, which represents a horizontal section through an embryo fourteen days old. They are cubical masses of cells enclosing a small cavity, often entirely obliterated, which represents part of the body cavity. The cells surrounding this are at first uniform in size, and each side is only one cell thick. Like the other cells of

the embryo they contain yolk granules, which are gradually absorbed. In the tail region these mesoblastic somites continue to be segmented off from the primitive streak till five or six days after the larva is hatched.

In transverse sections the mesoblastic somites appear triangular, having a median side against the nervous system and notochord, an external one against the epididymis and a ventral one. Besides these there are the anterior and posterior sides. The cells composing all these, except those of the external layer, develope into longitudinal muscles. Whilst this is taking place the dorsal surface of the embryo has become raised above the general level, so that the embryo in section is no longer round but pear-shaped.

As Stannius, Grenacher, and Langerhans have shown, the muscles of the Lamprey fall into two groups, which differ in structure as well as in their disposition. The first of these form the myomeres, and are derived directly from the mesoblastic somites; the second comprise the muscles of the eye, those belonging to the respiratory system, and those connected with the upper and lower lip and mouth generally. These seem to arise exclusively from the ventral unsegmented parts of the mesoblast, and perhaps, in some cases, from wandering mesoblast cells. The muscles of the heart resemble the latter in many points.

Each myomere in the Lamprey or Ammocœte consists of a number of plates of muscle-substance, lying one on the top of another. Each plate is flat, and more less square in outline. It is bounded anteriorly and posteriorly by the myotomes externally by a connective-tissue layer closely connected with the skin, and internally by a similar layer. Above and below, or dorsally and ventrally, it is in contact with a similar muscle-plate. In some myomeres which have become modified, such as the anterior one which extends far forward over the ear, the shape of the muscle-plate has lost its square outline and become oblong, but in one of the myomeres of the trunk they are almost square in longitudinal section.

From the above description it will be seen that each muscle-

plate or "Kästchen" of Stannius occupies the horizontal space between two myotomes, and that they lie one on another, so that in a horizontal section we see only one, in a transverse or vertical section we see one lying on another like sheets of paper. Each "muscle-plate" contains several nuclei, which stain more deeply than the muscle-substance. It is transversely striated, and faint longitudinal striæ can also be detected; these correspond with fibrillæ, into which the muscle-substance easily breaks up. These latter are especially large, and can be easily recognised in transverse sections near the most external part of the "muscle-plate."

The development of these muscle-plates is as follows:— The outermost layer of cells forming the mesoblastic somite does not appear to be converted into muscles. For a long time it persists as a definite layer of cubical cells with large nuclei lying between the skin and the myomere; this is the case till long after the other cells of the mesoblastic somite have developed into muscles. Finally, this layer seems to disappear, but remains of it can still be distinguished lying just within the skin, even when the myomere has assumed the appearance characteristic of the full-grown Ammocœte. This view that the somatic layer does not take part in the formation of the myomeres, is not in agreement with what Balfour has described in the Elasmobranchs, where both the inner and outer layer become muscular; but, on the other hand, the muscles of the myomeres in Amphioxus appear to be derived from the splanchnic layer only, and the same view is supported by Götte and the Hertwigs.

The remaining cells of the mesoblastic somite begin to grow in between one another, and between each neighbouring somite an intermuscular septum is deposited. The process of growing in between one another is carried on until each cell occupies the whole length from one myotome to the next, and at the same time, each cell becomes somewhat flattened, so that their transverse section, which was at first round, become oval (fig. 24). At the same time longitudinal thickenings occur in the cortical part of the cell, the medullary portion

remaining clear and staining very slightly. The nucleus lies in this medullary portion. The longitudinal thickenings occur at intervals, so that in transverse section the cortex of the cell appears beaded; these fine fibrillæ stain fairly well so that they can easily be distinguished from the medulla. The flattening of the cell goes on until the cell occupies the whole space between two myotomes, not only longitudinally but also transversely (fig. 25). The original nucleus of each cell divides into two or three, so that in each of these plates of muscle-substance two or three nuclei can be seen and an occasional yolk granule, which is, however, soon absorbed. In addition to the longitudinal striation caused by the thread-like thickenings in the cortex, a transverse striation appears. Each plate of muscle-substance remains in this condition, with a clear unstained medulla containing two or three deeply stained, large, flat, oval nuclei (fig. 18), with a well-marked nucleolus; enclosed by a cortex, for about two weeks after hatching. The cortex consists chiefly of its dorsal and ventral walls, and each of these is thickened at regular intervals by the above-mentioned fibrillæ. Each fibrilla runs the whole length of the myomere and is inserted into the intermuscular septa behind and in front. About a fortnight after the young Ammocœte is hatched, the substance of the fibrillæ increases at the expense of the medullary part, and this goes on until each plate of muscle-substance consists exclusively of fibrillar substance. The nuclei have increased in number, but instead of lying loose in medulla they become squeezed in between the fibrillæ, lose their regular shape and can only be recognised as small flattened bodies which stain deeply. The whole plate of muscle-substance now consists of fibrillar substance which stains uniformly with here and there a more deeply stained nucleus (fig. 29). The whole appears homogeneous, the fibrillæ cannot as a rule be recognised, though in some cases they are seen in transverse section as dots. Each "Kästchen" now resembles fundamentally the muscle-plate of the adult Lamprey; and it will be noticed that each is a development of what was a single cell.

The second variety of muscle-fibre met with in the Lamprey seems to be exclusively derived from the ventral unsegmented mesoblastic plate, and from the walls of the head cavities. The muscles with this origin are those which serve to move the lips, the velum and the other structures of the mouth, and certain muscles connected with the gill apparatus, and probably the muscles of the eye. These latter have the same histological structure, but owing to the fact that the eye does not develope until the Lamprey stage, no eye muscles appear till very late in the life of the Ammocœte and I have consequently been unable to follow their development.

The muscle-fibres of this second variety of muscle tissue, consist of long tubular cells, cylindrical in shape, with a medulla of clear substance which does not stain, and a cortex which is thickened at intervals by longitudinal rods. These give the cortex a beaded appearance in transverse section. The medulla contains the nucleus, which stains deeply. This is at first single, but subsequently divides until a row of nuclei occupy the axis of the muscle-fibre, in some cases so closely packed as almost to touch. It will be noticed that these muscle-fibres resemble in the minute structure the first stage in the development of the muscles forming the myomeres. These muscle-fibres are transverse striated.

The fibres of the heart belong to this second variety, and are developed from the same part of the mesoblast. They, however, possess certain peculiarities which will be described after the formation of the heart has been considered.

The Heart.

The first appearance of the body cavity as a space takes place in the region behind the posterior gill-cleft and in front of the liver. The part of the embryo lying in front of this region is at an early stage raised from the posterior half by the backward growth of the head fold, and the embryo lies within the egg-shell bent in half, the angle of the bend being just in that region where the heart is subsequently formed. By this means all those parts in front of the liver are free from the yolk-bearing cells, and the lining cells of the mesenteron all

become columnar. In this anterior region the mesoblast soon unites ventrally. In the posterior region the ventral union of the mesoblast is delayed, the lateral plates of mesoblast lying between the yolk-cells and the epiblast end in a free edge, and until these edges unite, the yolk-cells are in contact with the epidermis ventrally.

In the region between the liver and the last gill-slit the mesoblast splits at about the fifteenth day into a somatic and a splanchnic layer; between the two a well-developed body cavity appears. The former layer lines the body wall, the latter envelopes the alimentary canal. It forms a dorsal mesentery supporting that structure, and a well-marked ventral mesentery of considerable depth connecting the ventral wall of the intestine with the body wall. It is in this ventral mesentery that the heart is developed. The two layers forming the mesentery fuse dorsally and ventrally, but separate from one another in their middle, forming a cavity which is the lumen of the heart (fig. 24). Subsequently both the mesentery connecting the heart with the alimentary canal—the mesocardium—and the ventral one connecting the heart with the ventral body wall, atrophy and the heart lies as a tube unconnected with the surrounding structures (fig. 25).

From the fact mentioned above that the mesoblast behind the heart has not split into somatic and splanchnic layers nor united ventrally, it will be seen that the cavity of the heart communicates posteriorly with the space between the ventral yolk-cells and the epidermis. Such a space would be equivalent to part of the segmentation cavity. Soon after the heart is formed such a space arises, and at once becomes crowded with cells destined to form blood-corpuscles (fig. 26). At first I was inclined to think that these cells were budded off from the yolk-cells, but more careful observation has led me to believe that they originate from the free edge of the lateral plates of the mesoblast, which as I mentioned above are growing down between the yolk-cells and the epiblast. These corpuscles are oval with large nuclei, and they usually contain at first one or two yolk granules which they soon absorb.

The cavity in which the corpuscles lie in great numbers is subsequently shut off by the mesoblast as it grows downwards and becomes the subintestinal vein. It is from the first continuous with the posterior end of the heart, and the corpuscles soon pass from it into that organ. From the first appearance of the heart in the ventral mesentery its walls have been double; the splanchnopleure having split into two layers, of these the outer is at first much the thicker consisting of cubical cells; the inner layer is composed of comparatively flattened cells. The heart at first is a straight tube of the same length as the section of the body cavity in which it lies. Very soon, however, it increases in length, and thus becomes slightly twisted; at the same time two constrictions appear, dividing it into three chambers. The most posterior of these is the sinus venosus; it is directly continuous with the space in which the corpuscles are developing. By this time this space has acquired definite walls by the downgrowth of the mesoblast in this region, and it may now be spoken of as the subintestinal vein.

The liver which developes as a ventral outgrowth of the intestine first makes its appearance in this space, and when the latter gets closed off as a vein, the liver has become a branched gland projecting into it, so that the blood returning from the alimentary canal passes between the tubuli of the liver. Thus, from the very first an hepatic portal system is present. The tubuli of the liver do not appear to have any continuous mesoblastic coating, though here and there a flattened cell can be distinguished in the outside of a tubule.

The venous sinus communicates by a narrow opening with the auricle or second chamber of the heart. This in its turn opens by a similar narrow opening into the ventricle. This latter opening is guarded by a pair of valves, which appear by the tenth day after hatching; they effectually prevent any regurgitation of the blood into the auricle. The walls of the ventricle have undergone a considerable change. From the cells of the inner lining a number of branched muscle-cells have been developed (fig. 36). These cells stretch across the cavity of the ventricle from side to side, and fuse and anas-

tomose with one another in a very complex manner. They contain numerous nuclei, and show a longitudinal striation though not a transverse one. The centre of the ventricle is comparatively free from them, but at the sides they form a spongy reticulum in the meshes of which corpuscles abound.

The ventricle passes anteriorly into the ventral aorta, and at the point where the aorta passes into the solid tissue between the gills there is another pair of valves resembling the auriculo-ventricular ones. The ventral aorta, like the other vessels, arises by a split in the mesoblast which subsequently acquires a definite wall. It passes forward as a single vessel in the ventral median line until it reaches the thyroid gland, and here it splits in two branches. Each branch then passes forward on one side of this body, and ends in the most anterior gill vessel. From the single part of the ventral aorta three pairs of vessels are given off, passing in front of the fifth, sixth, and seventh gill-slits respectively. The posterior wall of the seventh cleft bears no gill filaments, and has no vessel. From each side of the double part of the ventral aorta five vessels are given off, the four posterior of these pass in front of the first, second, third, and fourth gill-slits. The most anterior is the vessel which in the earlier stages passes in front of a gill-slit which subsequently disappears. In the older embryos, when the mouth is fully formed it runs along the base of the velum.

The vessels after traversing the gills unite in the dorsal middle line to form the dorsal aorta; this runs backward to the posterior end of the body, lying just underneath the notochord. From its first appearance it gives off two transverse vessels in the neighbourhood of the pronephros; these supply the glomerulus. Anteriorly it gives off a pair of vessels to supply the upper lip, the carotids. In the older larvæ the aorta gives off a vessel which passes dorsally up one myotome, then along the dorsal surface of the myomere behind it, and hence the blood is collected by a vein which returns it to the posterior cardinal down the next myomere. The larvæ are fairly transparent, and in each myotome these two opposite

currents can be seen, and along the top of each myomere a backwardly directed stream. In the tail the aorta splits, and one branch passes each side of the cloaca; they unite ventrally, and are continued forwards as the subintestinal vein. Before it splits it gives off a vessel which runs back along the base of the notochord to supply the tail; this may be termed the caudal artery. The blood from this is returned by a caudal vein which soon splits into the two posterior cardinal veins. These large veins run forward, one each side of the aorta: the duct of the pronephros runs in their wall. Anteriorly they unite with the anterior cardinals, and form two ducts of Cuvier which open into the sinus venosus. The anterior cardinals bring back blood from the head. The tubuli of the pronephros lie in their cavity, so that the pronephros, like the kidney of the Amphibia, has a double blood supply. The cardinal veins do not appear till after the subintestinal vein, which for some little time is the only vein in the body. Later still a vessel appears in the right side of the intestine, opposite the subintestinal vein in the spinal fold; this, like the last named, passes through the liver. In my latest stages also there is an impaired vessel bringing blood back to the heart from the ventral region of the gills; this is mentioned by Balfour. The blood-corpuscles are of only one kind, large oval disc-like structures, with a well-marked nucleus. The protoplasm scarcely stains, but the nucleus assumes a deep colour.

Owing to the transparency of the larva, the circulation can be watched with great ease. The walls of the vessels at first possess no elasticity, hence great regurgitation takes place, and the blood advances by a series of jerks. The valves at the anterior end of the ventricle and between the auricle and the ventricle prevent this affecting the blood in the heart.

The heart begins to beat long before the cells exhibit any histological differentiation into muscles.

The Pronephros.

The first origin of the larval excretory system is by no means easy to make out, as it arises at a period when the embryo is

crowded with yolk. Scott has described it fully, and in most respects my observations confirm his. As he describes, the first structure to appear is the segmental duct which is at first solid. The cells forming this are derived from the mesoblast cells which lie between the already segmented dorsal part of the mesoderm and the ventral unsegmented portion. These cells form a solid cord lying between the mesoblast and the epiblast; the cord continues to grow backward by a differentiation of the cells in situ. A few hours later a lumen appears in the centre of the cord by the separation of the cells; this soon becomes elliptical in section (fig. 11). It opens into the posterior part of the alimentary canal.

From this account it will be seen that at first the segmental duct is between the mesoblast and epiblast; it, however, soon comes to occupy a deeper position by the growth of the surrounding tissue. So far we have only considered the duct in that part of its course where the body cavity is not yet developed; but in the region of the heart, where the body cavity has already appeared, its origin seems to be somewhat different. The lumen of the segmental duct here becomes continuous with a groove in the parietal peritoneum, lying near the angle where the somatopleure and splanchnopleure diverge. When this groove closes it leaves four or five openings which persist as the openings of the ciliated funnels. This account of the origin of the ciliated funnels agrees with that of Fürbringer, but differs from Scott's, who describes the funnels arising as blind projections of the segmental duct which acquire an opening into the body cavity. Each funnel soon acquires cilia, which extend for some distance down its lumen, and are usually directed downwards towards the tubuli. The funnel is composed of large cubical cells with a large nucleus, at its lip it passes suddenly over into the flat cells of the peritoneal epithelium. At its base it is continuous with a duct which soon becomes elongated and coiled, and ultimately joins the segmental duct. The walls of the tubuli are composed of large clear glandular cells. The posterior end of the segmental duct opens into the cloaca.

The segmental duct throughout its course runs in close connection with the post-cardinal vein, lying in contact with it, almost in its wall in the under and inner side. In the anterior region this vein has so grown round the pronephros that the tubuli really lie inside it (fig. 29). The tubuli are covered by a few flattened cells whose presence becomes more obvious about the twenty-fifth day by a deposit of dark brown pigment. The tubuli have thus a venous blood supply. The glomerulus on the other hand is supplied from the aorta. There is only one glomerulus on each side, stretching each side of the alimentary canal and extending through about the same space as the glandular part of the kidney. Each glomerulus is a diverticulum of the peritoneum, which generally becomes sacculated; it receives its blood by a single vessel on each side directly from the aorta.

Since the time of Bowman it has been known that the kidneys of Fishes, Frogs, and Snakes have a double blood supply, the tubuli uriniferi being surrounded by a capillary network of vessels which receive their blood from the renal portal veins, and the glomerulus which is supplied with blood from the aorta by the renal artery. It is an interesting fact to find that a similar blood supply is present from the very first in such a temporary organ as the pronephros of the Lamprey.

In the great majority of cases I found fine ciliated funnels in each pronephros. The whole gland did not extend over a greater space than that occupied by three myomeres, although in some cases the ciliated funnels, which were of some length, overlapped into a fourth myomere, but I was unable to confirm the relationship alleged to exist between the number of ciliated funnels and the number of somites through which the pronephros extended.

The Skeleton.

The skeletons of the oldest larva at my disposition consisted of the notochord derived from the endoderm, and of certain cartilages in the head and branchial region derived from the lateral mesoblast. The origin of the notochord has been completely

described by Calberla, Scott, and others, and I have nothing to add to their account. In the histological differentiation of the chord from a solid string of more or less cubical cells, to the vacuolated cylinder which forms the permanent notochord, there is a stage which is perhaps worth mentioning. In the early stages a transverse section of the chord shows portions of three or four cells, a little later these cells have pushed their way between one another and arranged themselves in such a way that they occupy the whole room inside the sheath of the notochord. Whilst in this condition vacuoles appear in the substance of the cells and for a day or two the notochord presents very much the same structure as the notochord of Amphioxus. This is, however, soon replaced by the vacuolated appearance characteristic of the notochordal tissue of the higher Vertebrata (figs. 18 and 23).

The posterior end of the notochord passes into the indifferent mass of tissue described in the tail. The anterior end is slightly curved downwards apparently by the increased vertical height of the brain. It ends just behind the infundibulum, its end being in contact with the posterior end of the nasal invagination. There is no trace that it has ever passed in front of this point, although in the young stages it reaches relatively almost as far forward as the nervous system. The relation of its anterior end to the brain hence appears to be due to the overgrowth of the nervous system anteriorly.

The cartilage which composes the rest of the skeleton is characterised by the small amount of intercellular substance. This stains very deeply. The cells are large with usually only one nucleus, though sometimes two. I have endeavoured to represent this structure in fig. 19. The branchial bases are the first part of the skeleton to appear. They arise about the twenty-fourth day as straight bars of cartilage lying external and slightly posterior to the branchial vessel. In their relation to the vessel they correspond with the extrabranchial bars of the Tadpole, and the Sharks. The true branchial bars run internal to the branchial vessel.

The bars run behind the gill-slit to which they belong, and

there is no bar in front of the first persistent cleft. They are slightly curved inwards towards the median line in the middle part of their course where they bend round the external opening of the cleft. About the thirtieth day they fuse with one another ventrally and so two rods are formed which lie close together in the posterior half of their course but diverge round the thyroid. About the same time each bar sends forward two processes, one above and the other below the opening of the gill to which it belongs; these ultimately fuse with the posterior edge of the gill bar next in front. The processes of the most anterior bar fuse with each other. Dorsally the last six of the bars also become continuous (fig. 42), and form two longitudinal bars which run parallel and close to the notochord. The most anterior bar does not join this rod but sends a process inwards, serving to support the auditory capsule, which lies just in front of it directly over the first persistent gill-cleft.

The first traces of the basi-cranial skeleton appear on the thirtieth day as two rods of cartilage, the trabeculæ (figs. 40). They lie close against the notochord for their posterior two thirds, anteriorly, however, they diverge and surround the pituitary space. About six days after their first appearance the trabeculæ send out laterally a transverse bar of cartilage which passes out on each side in front of the auditory capsule, lying between the ganglia of the fifth and seventh nerves. Professor Parker has identified this as the rudiments of the pedicle and pterygoid. They lie in the tissue of the bar which is in front of the first gill-cleft which has long ago disappeared.

Immediately beneath the trabeculæ the carotid artery runs forward as an anterior continuation of the dorsal aorta. The trabeculæ have become continuous with the dorsal end of the most anterior branchial bar, which is not united with the longitudinal bar formed from the fused dorsal end of the other six. The connection is very slight but is quite evident in sections. between this and the dorsal end of the second bar some little space exists, the latter when it commences lies at a slightly lower level than the trabeculæ.

The above description represents the condition in my oldest

larva, fifty-two days (fig. 43). The further development of the Lamprey's skull has been described by Professor Parker in his great work on 'The Skeleton of the Massipobranch Fishes.'

The Mesenteron.

The cavity of the alimentary is formed by the invagination of the endoderm described in the first section of this article, when once found it does not disappear again, although in the region of the intestine it may be reduced to a slit by the pressure of the surrounding yolk-cells.

The most anterior section, including the branchial region and that part of the intestine in front of the liver, is now separated from the rest by the raising of the head and neck from the remaining part of the embryo. The lining cells of this portion at once assume a columnar character; the hypoblastic cells in the region of the blastopore, or as it may now be termed the anus, also assume a similar form. But the cells in the middle part of the intestine still retain the features of the yolk-cells, those forming the roof of the enteron being however, rather more columnar than those of the floor and sides.

In the head region almost the whole of the space inside the epiblast is taken up with the brain, which has a great depth, and with the notochord and the alimentary canal, which ends blindly in front. A small band of mesoblast lies on each side of the nervous system and notochord. This segments dorsally into a series of myomeres, the first lying close behind the ear. Ventrally the mesoblast has not grown down between the endoderm, so that along the sides and under surface the hypoblast and epiblast are in contact. The first gill-slit appears, as Scott has described, about the twelfth or thirteenth day, the others arise during the next three or four days, the most posterior being the last formed. The gill-slits appear to me to be the result of the ventral downgrowth of mesoblast taking place only at certain places, these forming the gill-bars. Between each downgrowth the hypoblastic lining of the alimentary

canal remains in contact with the epiblast, and here the gill opening subsequently appears about the twenty-second day.

Huxley was the first to point out that the embryo Lamprey possesses eight gill-slits, and his account has been confirmed by Scott and Dohrn, who, however, point out that the first slit remains closed, and does not open to the exterior, as Huxley described. Dohrn has further shown that the first or rudimentary gill-slit becomes converted in the ciliated groove encircling the mouth, which was first described by Anton Schneider in Ammocœtes.

Fig. 27 represents a longitudinal horizontal section of the head of a twenty-one days' old embryo. The eight primitive gill-slits are here shown lined by columnar epithelium, which in the posterior seven is most flattened at those points where the opening will subsequently appear. The corresponding area in the first cleft, however, will be seen to be lined with very high columnar cells. These cells afterwards acquire cilia and come to lie in a deep groove.

The branchial vessels have only appeared in the first gill-bars, but the cells which will be converted into the cartilaginous gill arches have already become distinct (*br. b.*). About the twenty-second day a process begins to grow backward from the middle of each gill-bar into the gill-slit behind. This reduces the slit to a ⋖-shaped opening. After the opening to the exterior has been established the gill-bars overlap each other, the passage from the cavity of the mouth to the exterior being directed outwards and backwards. Each gill-bar acquires a few gill filaments, into which the blood courses. The whole is covered by a layer of thick columnar epithelium continuous with that lining the rest of the mouth, except certain small areas, mostly at the end of the short filaments, where the epithelium has become suddenly thin, thus putting the blood into closer communication with the surrounding water.

The columnar glandular-looking cells which line so much of the cavity of the mouth contain a number of very fine gran-

ules, which stain deeply with hæmatoxylin, giving the cell a very characteristic appearance. I have been unable to form any opinion as to the nature or fate of these granules.

The ciliated ring mentioned above is shown in section in fig. 41, *c. g.* It lies close in front of the most anterior gill-bar; ventrally its two halves converge and run back as two parallel grooves to the opening of the thyroid gland in the ventral median line. The grooves here unite, and after receiving the opening of the thyroid they continue as a single groove running in the ventral median line as far as the most posterior gill arch. Dorsally the grooves unite and become continuous with a median dorsal ridge, which is covered by high columnar cells, also ciliated. This ridge extends from the first gill arch to the commencement of the œsophagus. Anton Schneider describes a band of cilia running from this dorsal ridge on each side along each gill arch. This is not present in my oldest larva, but is no doubt formed later.

Dohrn (23) has recently described the development of the thyroid so fully, and his paper is so beautifully illustrated, that it appears to me to be superfluous to describe again the origin of this organ. I can only confirm his results. He deals at length with the homology of the thyroid of Ammocœtes, with the endostyle of Ascidians, and the hypobranchial ridge in Amphioxus. And the homology of the circumoral ciliated ring in Ammocœtes and Ascidians is also pointed out. To these homologies we may add, I think, that of the dorsal ciliated ridge of the young larval Lamprey to the dorsal lamella of Ascidians, and the hyperpharyngeal groove of Amphioxus. It is a curious fact, however, that in the last animal the form of the structure is reversed. We find ventrally a ridge and dorsally a groove, whereas in Ammocœtes and Ascidians we have the ridge dorsal and the groove ventral. In spite of this, I think Dohrn's arguments fully support the homology of the ventral organs, and the same reasoning holds good for the dorsal.

The alimentary canal behind the branchial region may be divided into three sections. Langerhans has termed these

the stomach, mid-gut, and hind-gut, but as the most anterior of these is the narrowest part of the whole intestine, it would perhaps be better to call it œsophagus. This part of the alimentary canal lies entirely in front of the yolk, and is, with the anterior region which subsequently bears the gills, raised from the rest of the egg when the head is folded off. In my later larvæ it is composed of a single layer of very high columnar cells, and is ciliated throughout. Round this is a thin layer of cells, which, I imagine, give rise to the muscular coats. The whole is supported by a dorsal mesentery, each side of which lies the head kidney (fig. 25). The ciliated columnar cells are directly continuous with those covering the dorsal ridge of the branchial region, but not with those of the ventral groove; this later connection must arise subsequently, as Anton Schneider describes it in the fully-grown Ammocœte.

The mid-gut which follows the œsophagus is, in the younger stages, crowded with yolk granules. The cells of the roof soon acquire a columnar shape, whilst the ventral part consists of a mass of cubical cells, each crowded with yolk. By degrees the yolk is absorbed, and the cells assume the same character as those lining the œsophagus. The lumen of the mid-gut is very much larger than that of the œsophagus, the alimentary canal expanding suddenly at the commencement of the former. The absorption of yolk takes place from before backward, so that lumen and walls of the fore part of the mid-gut assume their permanent size and form, whilst the posterior half is choked with yolk. The lining high columnar cells are ciliated and quite continous with those of the œsophagus.

By the time the yolk is all absorbed a longitudinal invagination of the wall of the mid-gut takes place. This occurs anteriorly on the left side, but twisting through a quarter of circle it comes to lie in the ventral side posteriorly. The ridge thus formed reduces the lumen of the alimentary canal from a round to a reniform shape in section. In this ridge or spiral valve runs the subintestinal vein, which has become quite small and has lost its median ventral position. Around

this vessel, filling up the space between the two sides of the spiral valve, is a quantity of fatty tissue. The cilia on the inner face of the spiral valve are very evident.

The lumen of the mid-gut is so large that almost the whole of the body cavity in that region of the Ammocœte is taken up by this part of the intestine; consequently the liver, the only gland opening into the mid-gut, is pushed forward and lies on each side and below the œsophagus. This gland has its origin at a very early stage, about the fourteenth day, as an evagination of the mid-gut, whilst the latter is still crowded with yolk. The diverticulum thus produced grows out in the ventral side of the alimentary canal into that space between the hypoblast and epiblast which was mentioned above as being crowded with blood-corpuscles. This space subsequently becomes enclosed by definite walls by the downgrowth of the mesoblast in this region. It becomes the subintestinal vein which still continues to supply the liver with venous blood. The single diverticulum soon begins to branch, and at an early stage one of the branches becomes differentiated from the others, acquires a large lumen, and forms the gall-bladder. The cells forming the liver are cubical with large nuclei, they do not appear to have a definite outer layer of flattened cells, though occasionally such a cell is present. In the older larvæ the gall-bladder has a great relative size. It lies embedded in the liver on the right side of the œsophagus. The bile-duct runs from it above the mid-gut, bending down to enter the mid-gut in the spiral valve on the left side.

The hind-gut is smaller than the mid-gut, its anterior limit is marked by the termination of the spinal valve, which does not extend into this region. The two segmental ducts open into it just where it turns ventrally to open to the exterior by a median ventral anus. Its walls are in this region slightly puckered. The cells lining it are not so high as in the other parts of the intestine, but more cubical.

Its lumen is from an early stage lined with cells which have lost their yolk, and it is in wide communication with the exterior from the first. This condition seems to be, as Scott

suggests, connected with the openings of the ducts of the pronephros, for this gland is completed and seems capable of functioning long before any food could find its way through the mid-gut, or indeed before the stomodæum has opened.

The stomodæum has a very early origin; it commences on the fifteenth day as an invagination of ectoderm against the blind anterior end of the fore-gut. This gradually deepens and attains a very large size, partly due to great development of the upper lip, which grows forward and downward to constitute the large hooded structure which is so characteristic of the Ammocœte. The greater part of this hood consists of simple muscle-fibres which interlace and cross one another in a diagonal direction. The lower lip does not reach so far forward as the upper (figs. 34 and 35). About the twentieth day the velum begins to appear in the posterior angle of the stomodæum. This structure is formed by two grooves which gradually deepen and cut off a flap of tissue on each side of the middle line. These two grooves, shown in fig. 27, are not very deep. The tissue between them is broken through the next day so that the two lateral folds that remain are covered on their anterior face by epiblast, and on the greater part of their posterior face by hypoblast (fig. 28). Subsequently the mesoblast in these two flaps develope into muscle-fibres, and in the young larva a constant current is kept up by them, passing in at the mouth and out at the gill-clefts. This current is easily demonstrated by the aid of a little Indian ink suspended in the water.

On the twenty-third day two tentacles begin to grow out from the under surface of the upper lip, one each side of the middle line; a little later two more appear on the sides, but placed more posteriorly; later still two more appear behind the level of the last; these are situated at the junction of the lower lip with the upper. Finally, a median tentacle appears in the ventral middle line. This last is far longer than the others and from its base a ridge, which is at first low, but increases in height posteriorly, extends back between the ventral portion of the ciliated ring (figs. 40 and 41). The number of tentacles

is afterwards increased by a pair of new ones arising between each of those already formed. The tentacles subsequently become branched (fig. 39).

With regard to the mesoblast of the head I have little to add to the descriptions of Balfour and Scott. The area over which the gills extend at their first appearance extends to the posterior boundary of the sixth myomere. The most anterior myomere is situated close behind the ear, and the ear lies above the hyobranchial or first persistent gill-cleft. So that at their first appearance the six posterior gill-clefts correspond in their extent with the six anterior myomeres. As the larva grows the gill region appears to elongate with relation to the muscular myomeres, so in my latest larva there are about nine myomeres over the area of the six gills (fig. 43). These anterior myomeres become V-shaped with the open angles directed forwards; turned the opposite way to those of Amphioxus.

The mesoblast between the gills arranges itself into head cavities (fig. 21), and as Balfour and Scott have already shown, there are two head cavities in front of the hyomandibular cleft. These are at first continuous, but with the formation of the stomodæum they separate. One becomes præoral and obviously corresponding with the præmandibular head cavity of Elasmobranchs; the other with the mandibular (fig. 21). The walls of these cavities ultimately form the skeleton of the gill arches, the muscles of which are all of the tubular kind. Owing to the rudimentary condition of the eye in Ammocœtes, no eye-muscles are present and consequently it is impossible to say whether or no they are derived from the walls of the head cavities, but the researches of Stannius and Langerhans have shown that they possess the same histological characters as the muscles of the gills and upper lip.

The Central Nervous System.

The development of the central nervous system has been described above up to the stage when the central canal has

first appeared. The lumen is at first circular in outline, and the walls of the canal of uniform thickness (fig. 11). Ultimately in the region of the body the lumen becomes elongated and slit like (fig. 24); in the anterior end the lumen widens into the variously shaped cavities which form the ventricles of the brain. The cells forming the walls of the canal are primarily more or less cubical, but they soon become spindle shaped, except those which form the roof and the floor of the central canal. These are formed of a single layer of short columnar cells. The canal is in the youngest stages proportionately very much larger than in the later; its size is diminished and its form altered by the thickenings which take place in different parts of the brain.

The white matter first makes its appearance on the eighteenth day as two thin bands, one on each side of the brain and spinal cord (fig. 37). Later these unite in the ventral side and form an anterior commissure. After the appearance of the white matter the ganglion cells lose their spindle-shaped outline and become again circular.

The cranial flexure is very slight; the anterior end of the brain is, however, slightly bent down, and with it the anterior end of the notochord (fig. 23).

About the sixteenth day considerable changes take place in the brain; from the anterior and ventro-lateral angles of the fore-brain two diverticula are given off; these are the optic vesicles (fig. 30). They continue to grow upwards and backwards till their blind end reaches a position behind and above the anterior end of the notochord.

At the blind end of the diverticulum a knob is formed by the outer face proliferating cells, which form a multicellular retinal layer. The posterior face later on developes pigment in its cells. The lens is budded off from the inside of the single layer of epidermis, and lies as a flattened mass of cells close against the retinal layer (fig. 40). The stalk of the primary vesicle becomes solid by its walls coalescing on all sides, and forms the optic nerves. At their origin these nerves form a commissure projecting into the cavity of the fore-brain

on its ventral side; by the twenty-second day this optic chiasma is covered in by a single layer of ganglion cells. It is this body that Dohrn has by mistake figured as the Tuber cinereum (21). The commissure is shown in transverse section in fig. 39; the lumen of the infundibulum is seen below it, the cavity of the fore-brain above.

About the same time that the optic vesicles commence to be given off from the anterior end of the brain a median dorsal evagination also appears. It was mentioned above that in the median line, both dorsally, ventrally, and in front, the central canal is enclosed by a single layer of more or less columnar cells, whilst the lateral walls are thick. This single layer is interrupted ventrally by the formation of the optic chiasma. Dorsally it is produced on the sixteenth day by the evagination in question, which is the rudiment of the pineal gland (fig. 31). The walls of the pineal gland then consist at first of a single layer of cells forming a hollow sac which pushes its way between the brain and the epidermis, spreading out on all sides (fig. 31). At first its lumen is continuous with that of the fore-brain, but ultimately, by the folding of its walls, its cavity is obliterated and the communication with the lumen of the fore-brain is shut off.

The eighteenth day, two days after the first appearance of the optic vesicles and the pineal gland, is the earliest date on which I have been able to recognise the appearance of any division into fore-, mid-, and hind-brain. On this day the single layer of cells roofing the central canal becomes folded in the manner indicated in fig. 23. This takes place at about the level of the attachment of the velum, a little in front of the ear. In larva of fifty-two days, this groove has not changed its form, but has become deeper.

The division between the fore- and hind-brain is by no means so well marked; indeed, I have been unable to find any external groove, although it has been described by previous writers. Longitudinal horizontal sections through the brain show, however, that just behind the infundibulum and pineal gland the walls thin out so that the lumen appears diamond

shaped. This thin wall I conclude makes the division between the optic thalami and the crura cerebri.

The hind-brain and mid-brain resemble each other closely in structure, the mid-brain being only a trifle larger. Their cavity, which is at first slit like, becomes triangular by the lateral growth of the roof which pushes the side walls apart dorsally (figs. 40 and 41). This thin roof extends back as far as the second gill-cleft, after which it disappears and the nervous system has the structure represented in fig. 42.

About the forty-fifth day a median longitudinal fold appears in the thin roof; this is the first of the numerous folds found in the roof of the mid- and hind-brain of the adult (fig. 41).

The fore-brain still has its thick side walls, the optic thalami. Just in front of the stalk of the pineal gland a commissure of transverse fibres is found which runs from side to side on about the twenty-third day. This commissure corresponds with the Commissura tenuisima, described by Ahlborn in his exhaustive work on the brain of the adult Lamprey. It also probably corresponds with the commissure found by Balfour in Scyllium situated just in front of the base of the pineal gland. Osborn has recently described a similar commissure in the brain of the Amphibia, Menopoma, Menobranchus, Amphiuma, and Rana, and I have adopted the name he proposes for it, the Superior Commissure. The commissure of the pineal stalk in the Mammalian brain seems to occupy the same relative position. This superior commissure is at first covered with but a few ganglion cells, but these afterwards increase until two bodies are formed, the Ganglia Habenulæ. The left one is very small (fig. 39), but the right is a structure of considerable size, projecting downwards and backwards, and reducing the lumen of the fore-brain to a Y-shaped slit. These bodies have been fully described by Ahlborn in the adult; it is interesting to note that the curious asymmetry they possess is present from their first appearance. No other commissure has made its appearance by the fifty-second day.

The cerebral hemispheres show some signs of appearing as lateral outgrowths in my oldest larvæ, but no trace of paired

lateral ventricles are to be seen. The lateral outgrowths of the hemispheres embrace between them a mass of tissue formed at the back of the olfactory pit, which resembles in every way nerve matter. This structure is shown in figs. 33, 34, and 35, drawn from a series of sections taken through the head of a fifty-two days' larva. This tissue in question appears to consist of ganglion cells. It is traversed by a canal which ends blindly behind and opens by the median nasal pit in front. Posteriorly it is continuous with a sheet of tissue which is described by Dohrn and Scott as giving rise to the pituitary body (fig. 39). Unfortunately my larvæ were not sufficiently old to enable me to determine whether this mass of tissue comes into closer relation with the brain and forms the olfactory lobes, or whether, as seems more probable from what we know of the development of these structures in other animals, it forms only the peripheral portion of the olfactory apparatus.

About the twenty-fifth day some of the ganglion cells in the postero lateral angle of the grey matter become much larger than the surrounding ones. These cells are particularly frequent in that part of the hind-brain lying between the auditory capsule. They probably develope into the "outer large cells" of Reissner.

With regard to the development of the cranial nerves, I have no observations on the origin of the olfactory nerve, as this apparently does not arise till a much later stage than that attained by my oldest larvæ. The origin of the optic nerve as an outgrowth of the brain has been described above. Owing to the rudimentary condition of the eye, the muscles of that organ are not developed, and consequently the third, fourth, and sixth nerves do not arise till a much later stage. This leaves the fifth, seventh, eighth, ninth, and tenth nerves to be considered.

The origin of these nerves is much obscured by the yolk which crowds the cells of the embryo at the time they first appear. On the seventeenth day the first origin of the ganglia in the fifth and seventh nerve is seen. The ganglia arise as proliferations of the epiblast. By this means a knob of cells

is formed, which arises at about the level of the notochord (fig. 32). This heap of cells arises close behind the lens of the eye, but seems to be distinct from it. It is divided into a larger anterior part, which belongs to the fifth nerve, and a smaller posterior portion, which forms the ganglion of the seventh. The roots of the nerves seem to me—though it is difficult to be certain on this point—to arise as outgrowths from a neural ridge in the lateral surface of the brain; these grow down and fuse with epiblastic thickening. This origin of the roots of the nerves corresponds with that described by Balfour, Marshall, Van Wijhe, and Beard, in the Elasmobranchs, and differs from what occurs in the Amphibia as described by Spencer, where the nerve also is derived from the inner layer of epiblast. As Spencer suggests, this is probably due to the presence of a double layer of epiblast, the epidermic and nervous, in the Amphibia.

By the nineteenth day the ganglion of the fifth nerve has completely separated off from the skin. It has now divided into two portions, which have, however, a common root taking its origin from the hind-brain just in front of the ear. The most anterior part forms a large ganglion on the root of a nerve which runs over the eye (fig. 22). This is the ophthalmic ganglion, and the nerve is the ophthalmic branch of the trigeminus; it probably corresponds with the portio-profunda of the ophthalmicus superficialis of the Elasmobranchs. Immediately behind the ophthalmic ganglion, but quite distinct from it, lies the ganglion of the other half of the fifth nerve. From this a mandibular nerve proceeds to run close behind the mouth, and later a maxillary branch appears præorally. In the angle between these ganglia the eye lies. The nerve connecting the ophthalmic with the main ganglion of the fifth nerve, described by Ahlborn in the adult, is not found at this stage, and both the ganglia are of approximately equal size.

The seventh nerve arises behind the fifth and enters its ganglion, which, when separated off from the epiblast, lies close in front of the ear capsule (fig. 38). In early stages

whilst the most anterior gill-cleft—spiracle—is still present, the nerve can be seen passing from the ganglia between the rudimentary gill-cleft and the first persistent one—the hyobranchial. Later on the ganglion increases in size, and extends round the under and inner face of the auditory sac towards the ganglion of the ninth nerve, but it never quite reaches it, and the connection between the ganglion of the seventh and of the tenth nerves must be of later origin. Neither does the ganglion of the seventh fuse with that of the fifth, though they are close together, and the root of the seventh does not enter the ear capsule to leave it again, as is the case in the adult. After the appearance of the ciliated ring in the place of the first gill-cleft, the seventh nerve supplies this structure.

A few fibres from the brain enter the recessus labyrinthi of the ear; these arise close to the root of the seventh, and constitute the eighth nerve.

The ganglia of the ninth and tenth nerves would seem to arise from a mass of cells split off from the epiblast close behind the ear. At a little later stage the ninth nerve has its ganglion lying close against the posterior boundary of the ear; the nerve is continued along the posterior wall of the first persistent cleft, the hyobranchial. The ganglion seems to be still connected with the ganglion of the tenth nerve. This is a very large structure; it lies more dorsally than the others and it is in close connection with the mid-brain, having as yet developed no root. Behind it and connected with it lies a ganglion which is situated dorsally above the second persistent gill-cleft; from this chord the main branch of the vagus is continued backward, lying just external to the anterior cardinal vein (fig. 42). In front of each remaining cleft the chord bears a large ganglion, so that, counting the first, there are six distinct ganglia borne on the vagus. I have not been able to trace the fibres of this nerve beyond the last gill-cleft, but my friend Mr. Ransom, of Trinity College, tells me he has traced the vagus into the heart in the adult Petromyzon. Each of the ganglia in the vagus supplies the gill-cleft behind which it lies.

There is no trace of the ramus lateralis of the vagus even in my oldest larvæ.

The ganglion on the ninth nerve lies in front of the first myomere, between that and the ear, whilst that of the vagus lies between the first and second. The first dorsal root of the spinal nerves with its ganglion lies between the third and fourth myomere. Behind this there is a dorsal ganglion lying opposite each myotome.

Sagemehl (17) has described very correctly the origin of the spinal nerves. The dorsal roots with their ganglia arise from a neural ridge which is at first of the same size all along. From this the ganglia begin to grow out about the eighteenth day, intersegmentally, that is opposite the myotomes. The ganglia are in connection with one another for some time by a longitudinal commissure. This commissure appears to consist of the remains of the neural ridge; it ultimately disappears, as in Elasmobranchs. The dorsal nerves, after leaving the ganglia, run into the myotomes and eventually, I believe, reach the skin, though on this point I cannot be quite certain. On the other hand the ventral roots consist of nerve-fibres only, and run straight into the myomeres. They appear, according to Sagemehl, very soon after the first appearance of white matter in the chord, and they never have any connection with the dorsal roots. The resemblance between the distribution of the spinal nerves of this larva with those of Amphioxus as described by Rohon is very striking.

The ear is formed, as Scott has described, from an invagination of the epiblast. This appears very early about the fourteenth day. It soon deepens and becomes completely shut off, consisting then of an oval vesicle with a dorsally placed stalk, the recessus labyrinthi. This last is the remains of the duct leading to the exterior. The ear is in the same condition in my oldest larvæ. No signs of the semicircular canals have appeared. The epithelium lining the vesicle is high and columnar; about the twenty-second day certain patches of the epithelium become higher than the others and the cells develope each a very large cilium which projects into the cavity and

bears a knob at its free end (fig. 41). About the same time a number of small concretions appear in the ear. These form the numerous spherical otoliths.

Summary.

I have now described the structure of the chief organs in my oldest larva, and I propose to conclude this paper by a brief summary of the results obtained.

In the first place the mesoblast is not completed ventrally by a layer of cells split off from the hypoblastic yolk-cells, as Scott has described. But the ventral mesoblast is formed by the downgrowth of the mesoblastic plates, which ultimataly meet and unite in the ventral middle line.

The blastopore does not close up, as later observers have maintained, but, as Max Schultze described thirty years ago, it persists as the anus. There is no neurenteric canal, though a solid strand of tissue proceeds back from the alimentary canal and fuses with an indifferentiated mass of cells, into which the nervous system and mesoblast also pass.

The lumen of the alimentary canal is that of the mesenteron; it does not become obliterated during larval life. In its anterior end the hypoblast remains in connection with the epiblast at certain points, and here the gill-clefts arise; between these the mesoblast grows down and forms the gill-bars. The origin of the ciliated ring and the hypopharyngeal groove and hyperpharyngeal bar are also described, and the ciliated condition of the œsophagus and stomach.

The "muscle-plates," whose structure is so peculiar in the Lamprey, arise each from a single cell of the mesoblastic somites. This increases in size, slides in between the neighbouring cells, and ultimately occupies the whole of the space between two myotomes. Its nucleus divides until each cell contains several nuclei. Striated fibrils then appear and increases till the whole "muscle-plate" consists of little else besides these fibrils, squeezing between them a few nuclei. These "muscle-plates" arise from the segmental half of the mesoblast; the muscles of the gills, lips, and probably of the eye,

have a different structure and arise from the ventral unsegmented part.

The blood-corpuscles arise from the ventral free edges of the mesoblast, before they unite in the ventral middle line, they collect in a large sinus just behind the heart. The heart appears in the ventral mesentery, formed by the union of the lateral mesoblastic plates; at first its lumen is continuous with the sinus just mentioned. This sinus lies between the hypoblastic yolk-cells and the epiblast; it subsequently acquires walls and forms part of the subintestinal vein.

The ciliated funnels of the pronephros are left as apertures by the segmental duct which in its anterior end is formed from a groove. The groove closes up at intervals, leaving four or five openings which become the funnels. They do not arise as blind projections from the duct, which subsequently, acquire ciliated openings. From the first the pronephros has a double blood supply, pure blood from the aorta passing to the glomerulus, and impure blood in the cardinal veins surrounding the tubuli.

The early development of the skeleton is described up to the stage where Professor Parker commenced his researches.

The canal of the central nervous system developes after the neural chord has separated off from the epidermis; it does not appear to be lined by any invaginated epidermis, as Calberla and Scott maintained.

The first sign of differentiation of the parts of the brain is the formation on the sixteenth day of the optic vesicles and pineal gland. The division into fore-, mid-, and hind-brain appears soon after, but the fore- and mid-brain are not separated by any well-marked groove. The first transverse commissure to appear is situated just in front of the stalk of the pineal gland. It forms the superior commissure of Osborn. Afterwards the ganglion cells thicken round it and form the asymmetrical ganglia habenulæ.

The ganglia on the fifth, seventh, ninth, and tenth nerves are derived from epiblastic thickenings. Their roots probably arise as outgrowths from the neural ridge. The ganglion of the

fifth divides into two parts, the ophthalmic and mandibular; these have a common root.

The seventh nerve at its first appearance supplies the first or spiracular gill-cleft; when this is converted into the ciliated ring it continues to be supplied by the seventh nerve.

The connection between the fifth, seventh, and tenth nerve ganglia does not exist and must be of later origin.

The tenth nerve has a large ganglion on its root and bears a ganglion above each of the last six gill-clefts. No trace of the ramus lateralis is to be seen.

The origin of the ganglia on the cranial nerves has no relation to the sense-organs of the skin; these have not appeared even in my oldest larva.

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EXPLANATION OF PLATES XVIII, XIX, XX, and XXI,

Illustrating Mr. Arthur E. Shipley's Paper on "Some Points in the Development of Petromyzon fluviatilis."

Reference Letters.

a. Anus. *a. c.* Anterior cardinal. *ao.* Aorta. *au.* Ear. *aur.* Auricle. *b. c.* Body cavity. *bl. c.* Blood-corpuscles. *bp.* Blastopore. *br.*^{1}-*br.*^{8} First to eighth gill-clefts. *br. b.* Skeleton of branchial bars. *br. v.* Vessels of branchial bars. *c.* Cerebral hemispheres. *c. g.* Ciliated groove. *d. f.* Dorsal fin. *d. l.* Dorsal lamella. *d. m.* Dorsal mesentery. *e.* Eye. *e. g.* Egg membrane. *ep.* Epiblast. *f. b.* Fore-brain. *f. g.* Fore-gut. *g.* Groove between mid- and hind-brain. *g. h. l.* Left ganglion habenulæ. *g. h. r.* Right ganglion habenulæ. *gl.* Glomerulus. *g. n.* Ganglion cells at base of olfactory invagination. *h.* Heart. *hd.* Head. *hd. c.* Head-cavities. *h. b.* Hind-brain. *hy.* Hypoblast. *i.* Iter a tertio ad quartum venticulum. *inf.* Infundibulum. *li. t.* Liver tubules. *l. l.* Lower lip. *l. t.* Lamina terminalis. *m.* Mesenteron. *m. b.* Mid-brain. *m. br.* Muscle of branchial bar. *mes.* Unsegmented mesoblast. *mes. som.* Mesoblastic somites. *m. f.* Muscle-fibre of heart. *m. g.* Mid-gut. *m.* Muscle-plate. *my.* Myomere. *n.* Notochord. *na.* Olfactory invagination. *n. r.* Neural ridge. *nu.* Nucleus of muscle-plate. *o. e.* Ciliated epithelium lining nasal invagination. *op. ch.* Optic chiasma. *oph.* Ophthalmic ganglion. *op. th.* Optic thalami. *op. v.* Optic vesicle. *p. g.* Postanal gut. *pin.* Pineal gland. *pit.* Pituitary body. *pr.* Primitive streak. *r. l.* Recessus labyrinthi. *s. c.* Segmentation cavity. *s. cm.* Superior commissure. *s. d.* Segmental duct. *sm. pl.* Somatopleure. *sp. c.* Spinal cord. *sp. gl.* Spinal ganglion. *sp. pl.* Splanchnopleure. *st.* Stomodæum. *s. v.* Sinus venosus. *t.* Tentacles. *th.* Thyroid gland. *tr.* Trabeculæ. *tub.* Tubule of pronephros. *u. l.* Upper lip. *v.* Velum. *v. ao.* Ventral aorta. *ven.* Ventricle. *v. f. b.* Cavity of fore-brain. *v. h. b.* Cavity of hind-brain. *v. r.* Ventral ridge in mouth. *v. v.* Valves of the heart. *y. c.* Yolk-cells. *V. g.* Ganglion of fifth nerve. *V. g. e.* Epiblastic ingrowth to form ganglion of fifth nerve. *VII. g.* Ganglion of seventh nerve. *X. g.* Ganglion of tenth nerve.

PLATE XVIII.

Fig. 1.—Spermatozoa of Petromyzon fluviatilis.

Fig. 2.—Segmenting ovum at the completion of the third or equatorial furrow. *e. g.* Egg membrane.

Fig. 3.—Segmenting ovum, showing the next two vertical furrows which have divided the upper cells and are extending into the lower.

FIG. 4.—Ovum after the invagination is complete, twelve days old, showing the blastopore, *bp.*, at posterior end of the neural ridge, *n. r.*

FIG. 5.—Transverse section through ovum of thirty-six hours. *ep.* Epiblast. *s. c.* Segmentation cavity. *y. c.* Yolk-cells.

FIG. 6.—Transverse section through ovum of forty-eight hours. *s. c.* Segmentation cavity. *ep.* Epiblast. *y. c.* Yolk-cells.

FIG. 7.—Transverse section through ovum of sixty-seven hours.

FIG. 8.—Transverse section through ovum of eighty-six hours, showing epiblast gradually thinning out.

FIG. 9.—Longitudinal section through commencing gastrula, 136 hours. *bp.* Blastopore. *hy.* Hypoblast. *y. c.* Yolk-cells. *m.* Mesenteron. *s. c.* Segmentation cavity.

FIG. 10.—Section through embryo of about the same stage as Fig. 4. *bp.* Blatsopore. *y. c.* Yolk-cells. *hd.* Head.

FIG. 11.—Transverse section through the body of an embryo just before hatching, seventeenth day. *sp. c.* Spinal cord. *n.* Notochord. *m.* Mesenteron. *mes.* Mesoblast. *s. d.* Segmental duct. Zeiss's A, oc. 2, cam. luc.

FIG. 12.—Transverse section through embryo of thirteenth day. *sp. c.* Spinal cord. *n.* Notochord. *mes.* Mesoblast. *m.* Mesenteron. *y. c.* Yolk-cells. Zeiss's A, oc. 2, cam. luc.

FIG. 13.—Transverse section through embryo of fourteen days. Letters as in Fig. 12. Zeiss's A, oc. 2, cam. luc.

FIG. 14.—Transverse section through tail of larva twenty days old. *sp. c.* Spinal cord. *n.* Notochord. *p. g.* Solid postanal gut. *mes.* Mesoblast. *bp.* Blastopore. *d. f.* Dorsal fin. Zeiss's A, oc. 3, cam. luc.

FIG. 15.—Transverse section from the same series as Fig. 14, but posterior to blastopore. *d. f.* Dorsal fin. *mes.* Mesoblast. *pr.* Fused tissue of notochord, spinal cord, and postanal gut, or primitive streak. Zeiss's A, oc. 3, cam. luc.

FIG. 16.—Transverse section of embryo just before hatching, seventeen days, through region of blastopore. *bp.* Blastopore. *sp. c.* Spinal cord. *n.* Notochord. *y. c.* Yolk-cells.

FIG. 17.—Longitudinal section of embryo, showing formation of somites. *n.* Notochord. *mes. som.* Mesoblastic somites. *sp. c.* Spinal cord. *d. f.* dorsal fin.

FIG. 18.—Longitudinal section of embryo just before hatching. *sp. c.* Spinal cord. *my.* Myomere. *sm. pl.* Somatopleuric layer of somite. *sp. pl.* Splanchnopleuric layer. *n.* Notochord. Zeiss's A, oc. 3, cam. luc.

FIG. 19.—A piece of the cartilage of a branchial bar.

FIG. 20.—A longitudinal vertical section through the tail of a larva twenty-

one days old. *a.* Anus. *p. g.* Solid postanal gut. *n.* Notochord. *sp. c.* Spinal cord. *pr.* Primitive streak. *y. c.* Yolk-cells.

Fig. 21.—A longitudinal section through side of head of seventeen days' embryo, showing the first three evaginations to form gill-clefts, and the true head-cavities. *au.* Ear. *hd. c'.* and *hd. c''.* The first and second head-cavity. br^1., br^2., and br^3. The first rudiments of gill-clefts. *br. v.* The vessels of gills. *st.* Stomodæum. Zeiss's A, oc. 3.

PLATE XIX.

Fig. 22.—A longitudinal section through side of head of a larva twenty-one days old. *au.* Ear. *e.* Eye. br^1., br^2., br^3., br^4. The first to fourth primary gill-clefts. *h. b.* Hind-brain. *oph.* Ophthalmic ganglion. *V. g.* Ganglion in main branch of fifth nerve.

Fig. 23.—A median longitudinal section through the head of a larva twenty-one days old. *pin.* Pineal gland. *op. ch.* Optic chiasma. *inf.* Infundibulum. *n.* Notochord. *st.* Stomodæum. br^2. Second primitive gill-cleft. *th.* Thyroid gland. *na.* Olfactory invagination. *pit.* Pituitary invagination. *m. b.* Mid-brain. *h. b.* Hind-brain. *g.* Groove between mid- and hind-brain. *l. t.* Lamina terminalis.

Fig. 24.—Transverse section through the body of a larva of twenty days. *sp. c.* Spinal cord. *f. g.* Fore-gut. *n.* Notochord. *som. pl.* Somatopleure. *sp. pl.* Splanchnopleuric layers of myomeres. *b. c.* Body cavity. *h.* Heart. *c. f.* Ciliated funnel. *s. d.* Segmental duct. Zeiss's A, oc. 3, cam. luc.

Fig. 25.—Transverse section through trunk of larva about twenty-four days. Letters as in Fig. 24, and *ao.* Aorta. *a. c.* Anterior cardinal. *d. m.* Dorsal mesentery. *sp. gl.* Spinal ganglion. *gl.* Glomerulus. Zeiss's C, oc. 1, cam. luc.

Fig. 26.—Section through embryo, one day before hatching, seventeen days old, cut whilst in the egg-shell. *h.* Heart. *sp. pl.* Splanchnopleure. *sm. pl.* Somatopleure. br^7. and br^8. Seventh and eighth gill-clefts. *hd.* Head-cavities behind these. *y. c.* Yolk-cells. *m. g.* Mid-gut. *b. c.* Body cavity. Zeiss's A, oc. 3, cam. luc.

Fig. 27.—Longitudinal horizontal section through a larva about twenty-two days. br^1.—br^8. The eight primary gill-clefts. *br. v.* Vessels of gills. *br. b.* Branchial bars. *f. g.* Fore-gut. *tub.* Tubule of pronephros. *st.* Stomodæum. *v.* Velum. *g. n.* Ganglion cells at base of nasal invagination. *op. ch.* Optic chiasma. *inf.* Infundibulum. *v. f. b.* Cavity of fore-brain. *n.* Notochord. Zeiss's B, oc. 1, cam. luc.

Fig. 28.—Longitudinal horizontal section through larva of thirty-six days. *u. l.* Upper lip. *v.* Velum. *th.* Thyroid gland. *v. ao.* Ventral aorta. *ven.*

Ventricle. *aur.* Auricle. *vv.* Valves. *s. v.* Sinus venosus. *li. t.* Liver tubules. *m. g.* Mid-gut. *br. b.* Branchial bars. *v. r.* Ventral ridge. *my.* Myomere. Zeiss's A, oc. 1, cam. luc.

Fig. 29.—Transverse section through pronephros of larva of forty-seven days. *n.* Notochord. *m. p.* Muscle-plates. *nu.* Nucleus. *ao.* Aorta. *a. c.* Anterior cardinal. *gl.* Glomerulus. *tub.* Tubules. *s. d.* Segmental duct. *bl. c.* Blood-corpuscles. *f. g.* Fore-gut. *d. m.* Dorsal mesentery. Zeiss's D, oc. 1, cam. luc.

Fig. 30.—Transverse section through fore-brain of embryo, seventeen days. *na.* Olfactory epithelium. *op. v.* Optic vesicle. *v. f. b.* Cavity of fore-brain.

Fig. 31.—Transverse section through thalamencephalon of larva of eighteen days. *pin.* Pineal gland. *op. th.* Optic thalmi. *v. f. b.* Cavity of fore-brain. *na.* Olfactory epithelium.

Fig. 32.—Transverse section through region of mid-brain of larva of sixteen days. *st.* Stomodial epithelium. *V. g. e.* Epiblastic origin of ganglion of fifth nerve. *n.* Notochord. *m. b.* Mid-brain.

Figs. 33, 34, and 35.—A series of sections through the anterior end of head of a larva fifty-two days old, to show the ganglia cells at base of olfactory epithelium. *u. l.* Upper lip. *l. l.* Lower lip. *t.* Tentacles. *g. n.* Ganglion cells at base of nasal invagination. *o. e.* Ciliated epithelium lining nasal invagination. *c.* Cerebral hemispheres. *v. f. b.* Cavity of fore-brain.

PLATE XX.

Fig. 36.—Branched muscle-fibres of heart of larva forty-nine days old. *bl. c.* Blood-corpuscles. *m. f.* Muscle-fibre cut across.

Fig. 37.—Transverse section through the hind-brain, showing appearance of white matter and ganglion of fifth nerve. *h. b.* Hind-brain. *st.* Stomodæum. *V. g.* Ganglion of fifth nerve. This section is rather oblique.

Fig. 38.—Transverse section through hind-brain, showing origin of ganglion of seventh nerve from epiblastic ingrowth. *VII. g.* Ganglion of seventh nerve. *au.* Auditory vesicle. *f. g.* Fore-gut.

Fig. 39.—Transverse section through fore-brain of larva forty-nine days old, to show superior commissure. *pin.* Pineal gland. *v. f. b.* Cavity of fore-brain. *s. cm.* Superior commissure. *g. h. l.* Left ganglion habenulæ. *g. h. r.* Right ganglion habenulæ. *op. ch.* Optic chiasma. *pit.* Pituitary body. *inf.* cavity of infundibulum. *u. l.* Upper lip. *l. l.* Lower lip. *t.* Tentacles. Zeiss's C, oc. 1, cam. luc.

Fig. 40.—Transverse section through mid-brain of larva of forty-nine days. *i.* Iter a tertio ad quartum ventriculum. *e.* Eye. *tr.* Trabeculæ. *v. r.* Ventral ridge. Zeiss's C, oc. 1, cam. luc.

FIG. 41.—Transverse section through hind-brain of larva of fifty-two days. *v. h. b.* Cavity of hind-brain. *au.* Ear. *r. l.* Recessus labyrinthi. *VII. g.* Ganglion of seventh nerve. *d. l.* Dorsal lamella. *c. g.* Ciliated groove. *v. r.* Ventral ridge. *v.* Velum. *ao.* Aorta. *br. v.* Branchial vessels. Zeiss's A, oc. 3, cam. luc.

FIG. 42.—Transverse section through region of sixth gill-bar of fifty-two days' larva. *br*^{6}. Sixth gill-bar. *sp. gl.* Spinal ganglion. *ao.* Aorta. *a. c.* Anterior cardinal. *br. v.* Branchial vessels. *ao. v.* Ventral aorta. *X. g.* Ganglion in tenth nerve. *d. l.* Dorsal lamella. *br. b.* Skeleton of branchia bars. *m. br.* Branchial muscles.

PLATE XXI.

FIG. 43.—Drawing of larva of fifty-two days. The notch in the liver, behind the heart, is due to the large gall-bladder, through whose walls the œsophagus is seen. This drawing was made by Mr. E. Wilson from the living specimen.

Fig. 1

Fig. 2

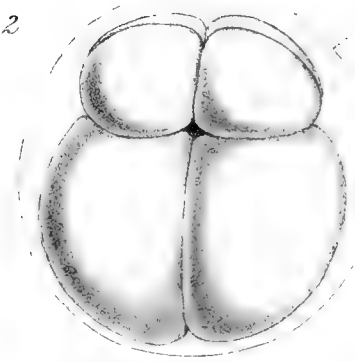


Fig. 6.

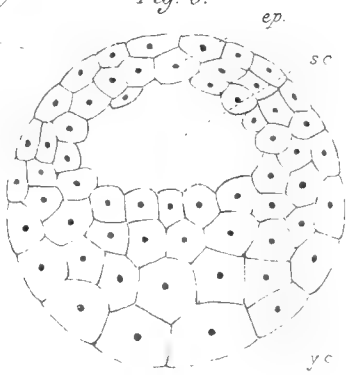
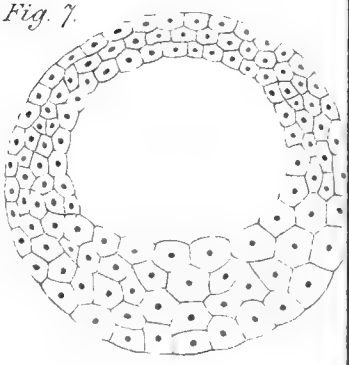


Fig. 7.



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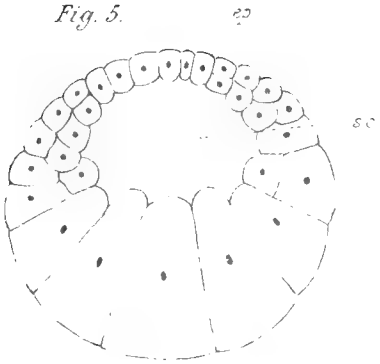

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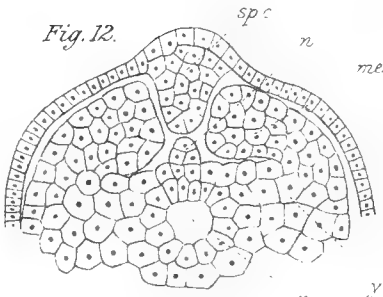
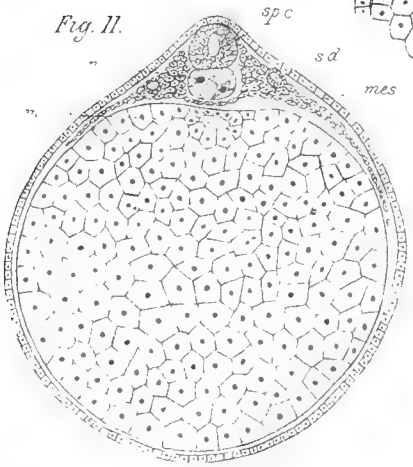


Fig. 13.

Fig. 11.



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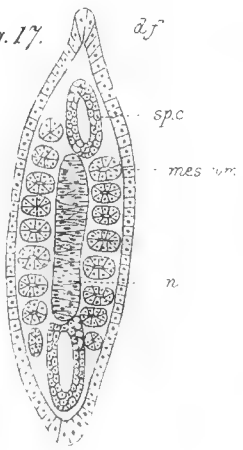

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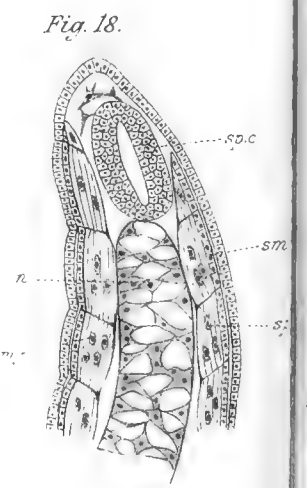

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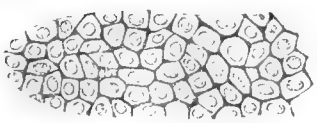

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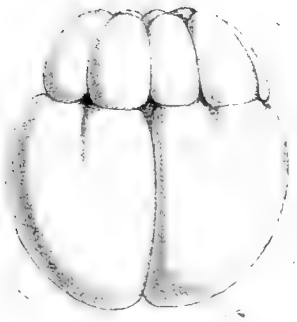
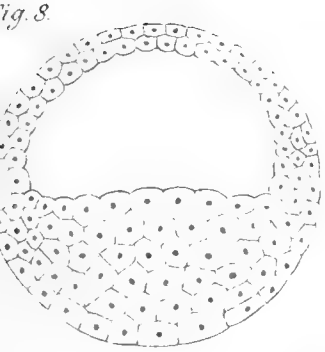


Fig. 4

Fig. 8.



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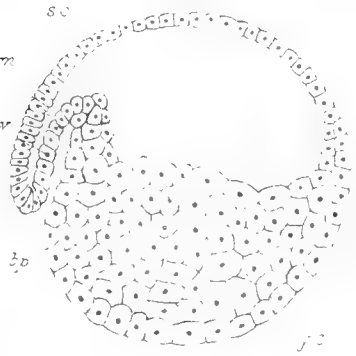

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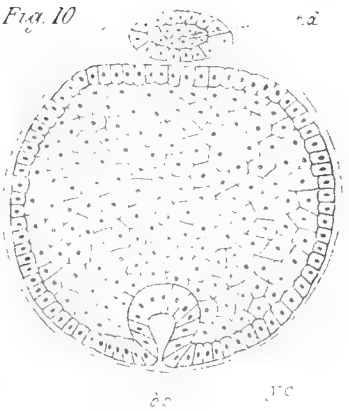

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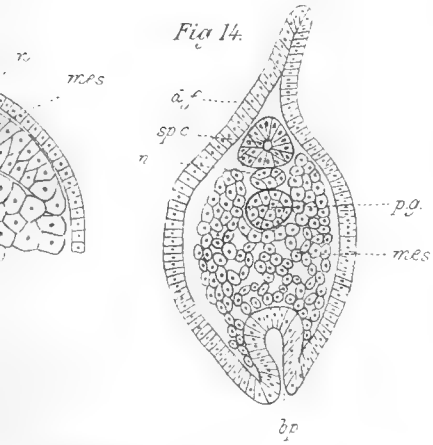

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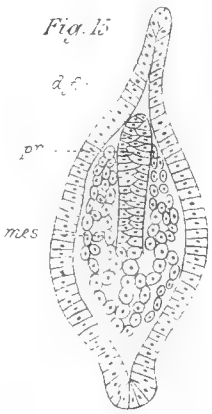

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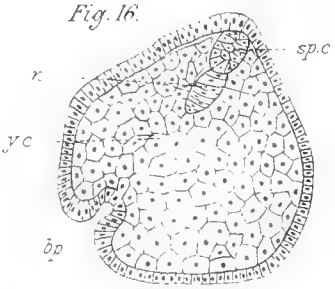

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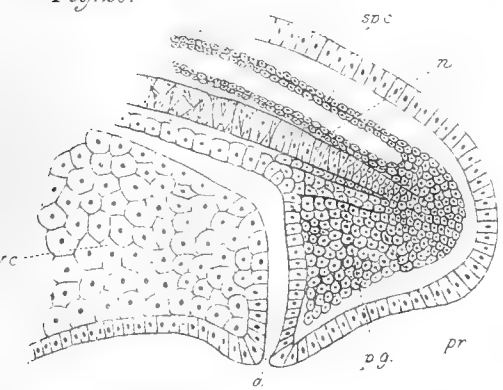

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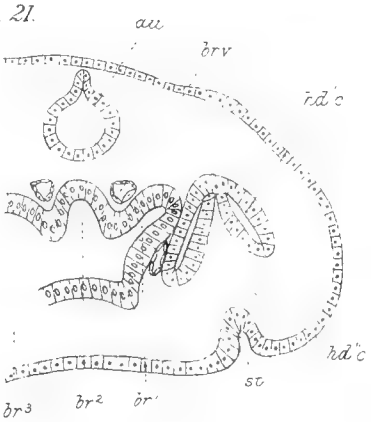

F Huth, Lith^r Edin^r

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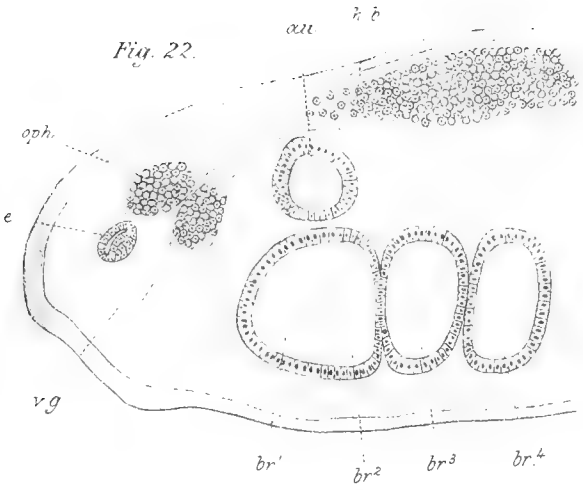

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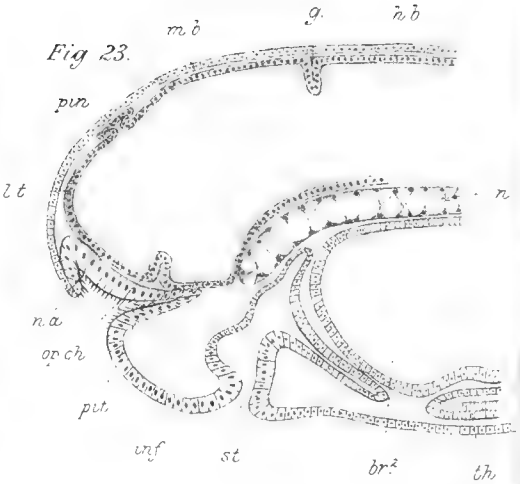

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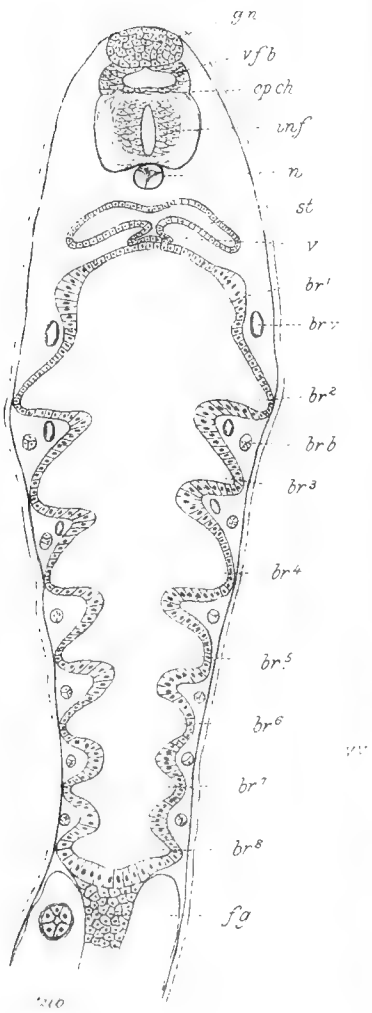

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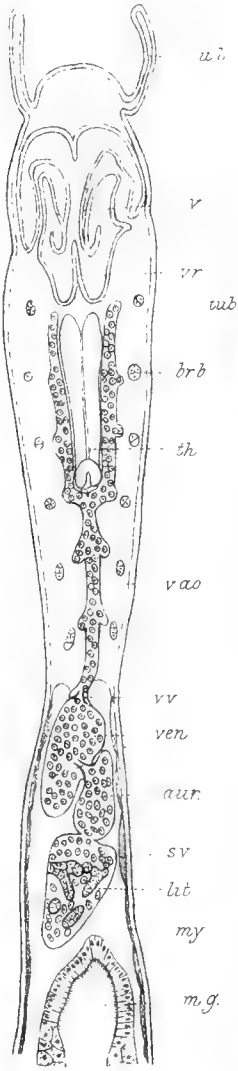

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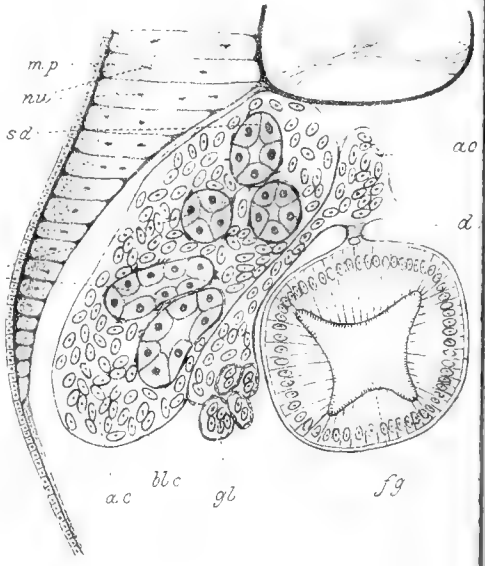

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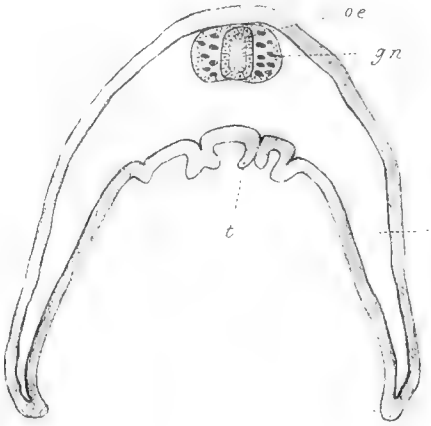

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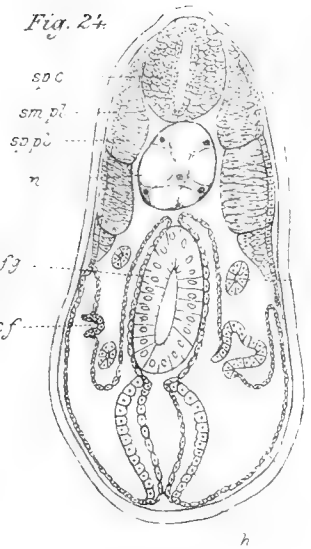

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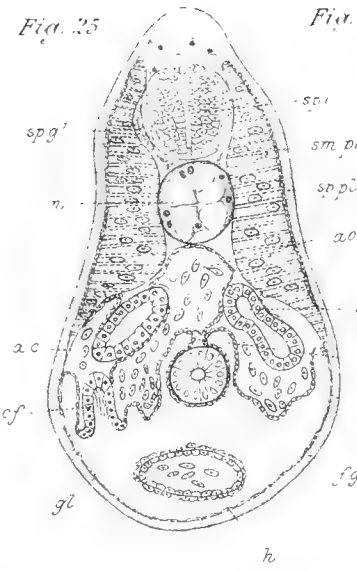

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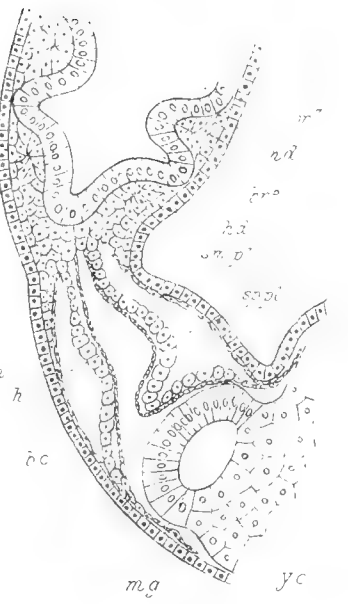

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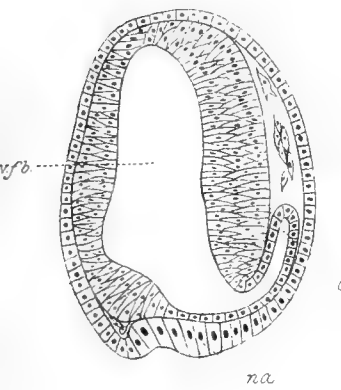

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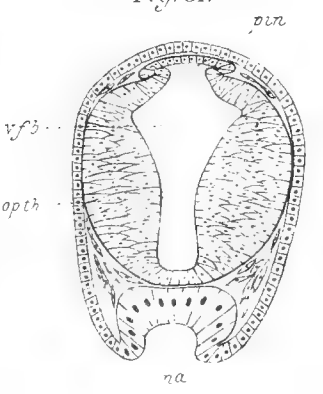

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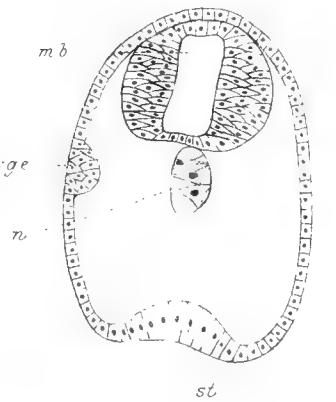

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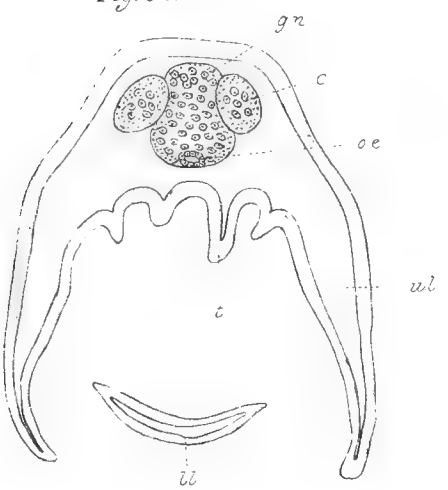

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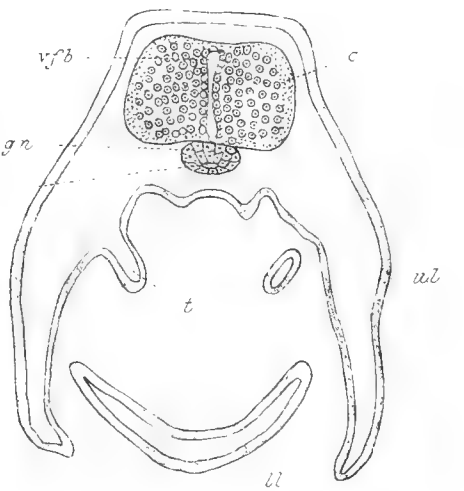

F Huth, Lith^r Edin^r

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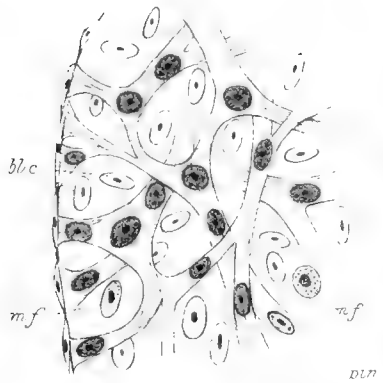

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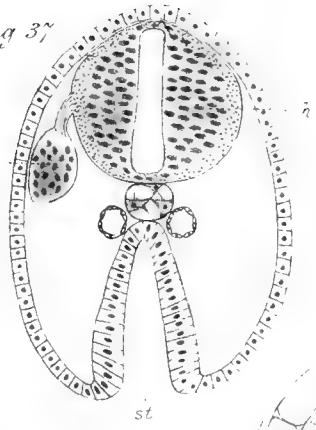

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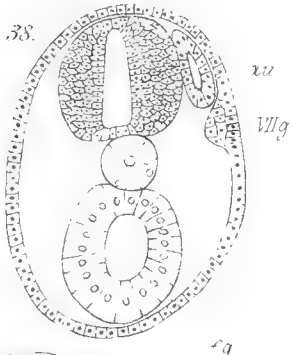

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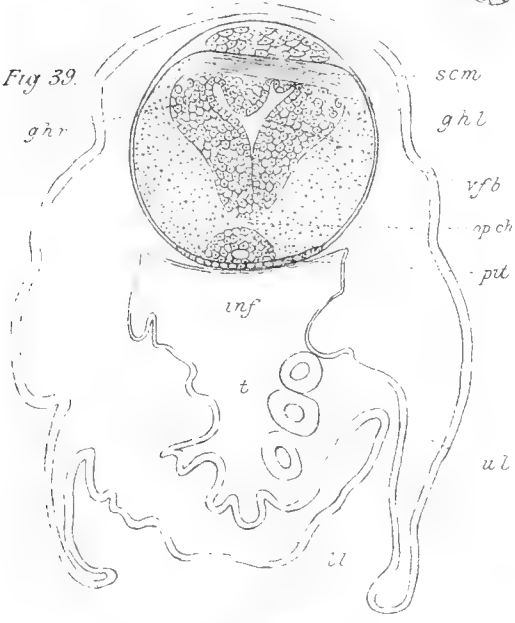

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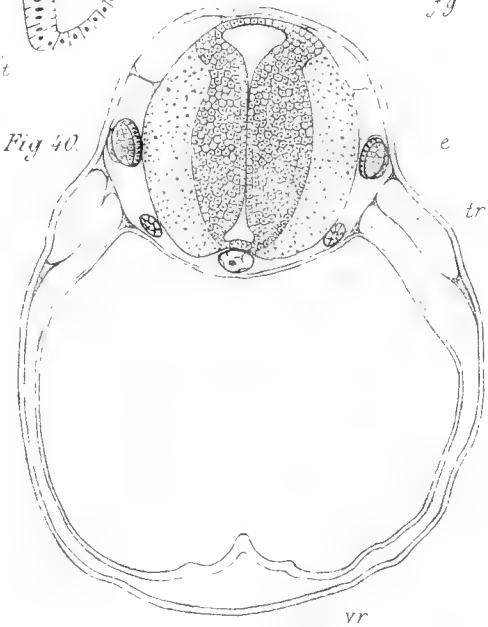

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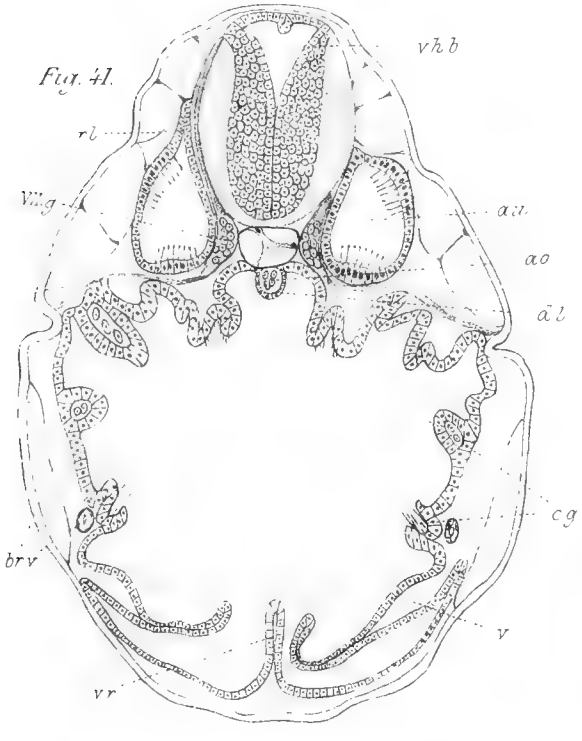

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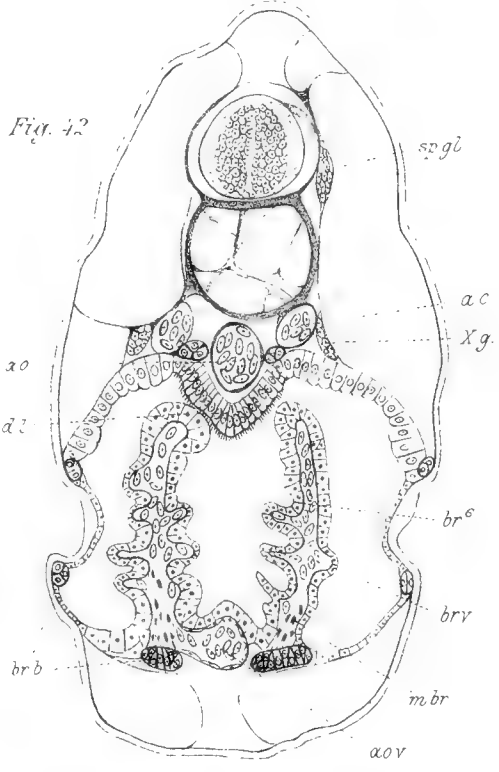

F Huth, Lith^r Edin^r

Fig. 43.

F Huth, Lith^r Edin^r

Sidney F. Harmer

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