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STUDIES

FROM THE

MORPHOLOGICAL LABORATORY



IN THE

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EDITED BY

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THE last series of Studies which issued from the Morphological Laboratory of Cambridge was published under the superintendence of the late Professor Francis M. Balfour.

To his original impulse and to the inspiring influence of his memory is due whatever merit the present series may possess.

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† Reprinted from the *Proceedings of the Cambridge Philosophical Society*.

All the above are reprints from the *Quarterly Journal of Microscopical Science* with the exception indicated.

Note on the Early Development of *Lacerta Muralis*.

By

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With Plates I, II, and III.

THE following paper contains an account of some observations on the early stages in the development of *Lacerta muralis*, begun during the summer of this year at the zoological station at Naples and completed in the morphological laboratory at Cambridge. It relates chiefly to the mode of formation of the germinal layers and to the early development of the kidney.

On my return from Naples I found that in June last Professor C. K. Hoffmann¹ had published an account of the mode of formation of the germinal layers, and the results obtained by him agree generally with my own. As, however, Professor Hoffmann has published very few figures of the stages observed by him, and as my observations lead me to differ from him in one or two points of detail, it has seemed to me that it would not be useless to give a short account of my own results.

The segmentation, which conforms to the ordinary mero-

¹ C. K. Hoffmann, "Contribution à l'Histoire du Développement des Reptiles," 'Arch. Néerlandaises des Sciences exactes et Naturelles,' t. xvii.

blastic type, has already been fully described and figured by Kupffer and Benecke¹ and by Balfour.² Neither of these observers describes a segmentation cavity; but Hoffmann³ states that during the latter stages of segmentation a cavity is present, the floor of which is formed by the yolk, the roof by the lower layer cells. Towards the close of segmentation it disappears.

This cavity Hoffmann considers equivalent to the segmentation cavity of the Ichthyopsida.

I have observed cavities similar to that described by Hoffmann, but I have been unable to satisfy myself that they were not due to the action of the hardening reagents employed. The cavity described by Professor Hoffmann differs strikingly, as he himself points out, from the segmentation cavity of other vertebrates, in the fact that its floor is never formed of lower layer cells.

At the close of segmentation the blastoderm consists of a superficial layer of epiblast cells, which is generally stated to be a single cell thick; in my sections, however, the arrangement is very irregular, the epiblast being in some places two cells deep, in others more.

Beneath the epiblast is an irregular sheet of lower layer cells; this layer is in many places two or three cells deep, and the cells of which it is composed are large, irregular, loaded with yolk-granules, many having two or even more deeply-staining nuclei.

In the centre of the blastoderm the epiblast cells become more columnar than in the peripheral parts, and the lower layer cells become slightly more regular in their arrangement. An oval area pellucida is thus formed.

Hoffmann finds at this stage a marked thickening of the lower layer cells at the posterior extremity of the blastoderm.

The posterior region of the area pellucida now becomes dis-

¹ Kupffer u. Benecke, 'Die erste Entwicklung am Ei der Reptilien.' Königsberg, 1878.

² Balfour, "On the Early Development of the Lacertilia," 'Quart. Journ. Mic. Sci.,' vol. xix.

³ Loc. cit.

tinguished from the anterior by the presence of the primitive streak.

A median longitudinal section through an embryo with a commencing primitive streak is shown in fig. 1. Anteriorly the area pellucida is seen to be formed by an epiblastic layer of irregular columnar cells and a sheet of lower layer cells, the two layers being quite distinct. At a point (*bp*), however, the position of the future blastopore, these layers are replaced by a mass of closely-packed cells (*pr*), exhibiting no division into layers, and forming the primitive streak, which may in some cases at least extend backwards as far as the commencement of the area opaca.

The blastopore commences at the anterior end of this streak as a pit, open above, and closed below. This is shown in fig. 2.

The floor of this pit presently breaks up, and the blastopore assumes its normal condition, forming a communication between the archenteron and the exterior, its anterior wall forming a communication between the epiblast and the lower layer cells (see fig. 3).

From this time a change in the character of the lower layer cells takes place, beginning from the anterior wall of the blastopore, where they pass into the epiblast, and proceeding forwards. Instead of being large, irregular, full of yolk, as in the previous stages, they become columnar, lose their yolk, arrange themselves in a definite layer several cells deep, and take on the characters of normal hypoblast. A median longitudinal section through an embryo, in which about half the lower layer cells are thus converted, is seen in fig. 4.

This process is evidently an invagination comparable to that which takes place in an Elasmobranch. It especially resembles the process described by Scott and Osborne¹ in the newt.

The first traces of mesoblast appear at a stage slightly earlier than that represented in fig. 4. Fig. 5, which shows a portion of a lateral section from the same series as that to which

¹ Scott and Osborne, "On the Early Development of the Common Newt," 'Quart. Journ. Mic. Sci.,' vol. xix.

fig. 4 belongs, shows the condition of the mesoblast shortly after its origin.

The blastopore being funnel shaped, with its narrow opening directed downwards, it appears in a lateral longitudinal section as a pit, closed below, and from its closed extremity the mesoblast grows forwards as a solid cap, separate from epiblast and hypoblast.

Transverse sections show that the mesoblast is in connection not only with the walls of the blastopore, but also with the axial strip of invaginated hypoblast. Figs. 6—13 are selected from a series of transverse sections of an embryo slightly older than that represented in fig. 4, and show the relations of the mesoblast. The figures are arranged in order from behind forwards, fig. 6 being posterior. Figs. 6—9 pass through the blastopore, and a sheet of mesoblast, continuous with its walls, is seen growing out of each side. In figs. 10 and 11, which pass through the posterior embryonic region in front of the blastopore, each sheet of mesoblast is seen to be free laterally, but to be continuous near the middle line with the axial strip of hypoblast, the cells of which will give rise to the notochord, and are easily distinguishable from the more peripheral hypoblast cells by their more elongated forms and by being more than one layer deep.

This mode of origin of the mesoblast, however, only holds good for the posterior part of the embryo. Anteriorly (fig. 11) the mesoblastic sheet loses its connection with the axial hypoblast and finally disappears (fig. 12), being replaced by branched cells, which are budded off, partly from the axial, partly from the lateral hypoblast. This mode of origin of the anterior mesoblast has been overlooked by Hoffmann.

The account above given is obviously in complete accord with the observations of Balfour,¹ who described a stage a little later than that represented in figs. 6—13, with a widely-open, neuro-enteric canal, and a sheet of mesoblast on each

¹ Balfour, "On the Early Development of the Lacertilia," &c., 'Quart. Journ. Mic. Sci.,' xix.

side, which had separated from the axial hypoblast—all the layers being, however, still fused in front of the blastopore.

The statement of Kupffer,¹ that the blastoporic invagination gives rise to a closed sac, the walls of which become the allantois, is of course inconsistent with the truth of the above observations; but it has been already so abundantly disproved, first by Balfour and afterwards by Stahl and Hoffmann, that it is not necessary here to do more than refer to it in passing.

The actual mode of development of the allantois was first figured by Balfour,² a copy of whose drawing is reproduced in the woodcut. The details of the process were worked out by Strahl.³

I have nothing to add to the account given by these authors, but I would call attention to a consequence of it which neither observer has, to my knowledge, remarked.

It is obvious from the woodcut that, as has been shown in detail by Strahl,⁴ the allantois arises as a process of the primitive streak, which projects at first backwards into the body cavity.

Now, if this be the case, when the primitive streak is bent ventralwards during the establishment of the tail fold, the primitive streak must extend in the middle line from the posterior extremity of the medullary canal, round the end of the embryo, as far forwards as the point of connection of the allantoid stalk with the hind gut; and therefore the proctodæum, when it arises, must pass through the primitive streak.

Therefore, if we adopt the view of Balfour, that the primitive streak represents the position of the blastopore of other gastrulæ, we shall be forced to conclude that, at any rate in

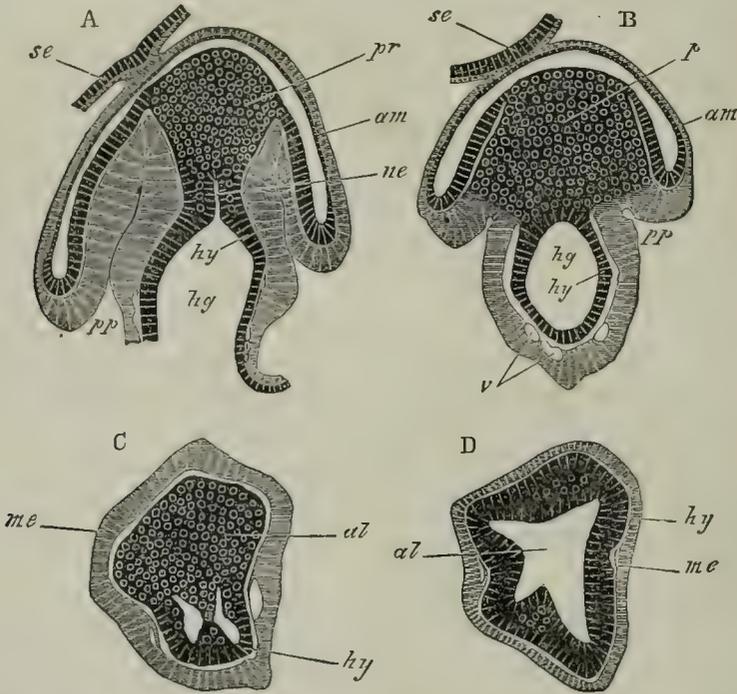
¹ Kupffer, "Die Gastrulation an den Meroblastischen Eiern der Wirbelthiere und die Bedeutung des Primitiv Streif," 'Arch. f. Anat. u. Phys.,' 1882.

² "On the Early Development of the Lacertilia," &c., 'Quart. Journ. Mic. Sci.,' xix.

³ Strahl, "Ueber die Entwicklung des Canalis Myelentericus und der Allantois der Eidechse," 'Archiv f. Anat. u. Phys.,' 1882.

⁴ Loc. cit.

this group of Craniata, the anus is in the position of a part of the blastopore—a supposition which simplifies our ideas as to the origin of the vertebrate anus in general.



FOUR TRANSVERSE SECTIONS THROUGH THE HINDER END OF A YOUNG EMBRYO OF *LACERTA MURALIS* (Balfour).

Sections A and B pass through the whole embryo, while C and D only pass through the allantois, which at this stage projects backwards into the section of the body cavity behind the primitive streak. *ne*. Neurenteric canal. *pr*. Primitive streak. *hg*. Hind gut. *hy*. Hypoblast. *pp*. Body cavity. *am*. Amnion. *se*. Serous envelope (outer limb of amnion fold not yet separated from the inner limb or true amnion). *al*. Allantois. *me*. Mesoblastic wall of allantois.

The development of the kidney has been described by Braun.¹ My observations lead me, however, to believe that his account of the mode of origin of the segmental tubules and of the Wolffian duct is erroneous.

¹ Braun, "Das Urogenitalsystem der einheimischen Reptilien," 'Arb. aus d. Zoolog. Inst. z. Würzburg,' Bd. iv, 1878.

According to him, the first part of the urino-genital system which appears is the Wolffian duct. He says: "In an embryo of *Lacerta agilis*, barely 5 mm. long, in sections just below the heart, I find the Wolffian duct lying close to the lateral mesoblast plates, in a region belonging neither to these nor to the protovertebræ, but lying between the two as a semicircular mass of cells, sharply defined towards the ectoderm, but passing gradually into the lateral mesoblast; in the middle of this cell mass is a lumen" which he considers to be the lumen of the Wolffian duct.

In the next stage described by Braun, a number of segmentally arranged vesicles are present, which are for a short time attached to the peritoneal epithelium, their cavities also opening for a short time into the body cavity, but which afterwards break away, form the well-known S-shaped tubes, and communicate with the Wolffian duct.

From this account it is evident that Braun has not investigated embryos less than 5 mm. long. I have been fortunate enough to obtain younger embryos, and have been led to somewhat different conclusions.

On the formation of the protovertebræ, each protovertebra does not at once become completely separated from the lateral mesoblast, but remains connected at a certain point with a continuous solid ridge of tissue, generally in early stages about two cells thick, which projects inwards from the peritoneal epithelium, thus forming an "intermediate cell mass" comparable with the structure so called in birds.

Figs. 15 and 16 show the characters of this ridge in an embryo of about seven protovertebræ; fig. 15 is taken from a vertebral region, and shows the ridge (*i. c. m.*) connecting the protovertebra with the peritoneal epithelium; fig. 16 is from the next intervertebral region, showing the ridge projecting freely inwards from the peritoneum. In fig. 16 traces of a prolongation of the body cavity into the intermediate cell mass may be observed. In an embryo with ten protovertebræ this cell mass, without losing its connection with the protovertebræ, swells up and becomes semicircular in section,

the convexity being directed outwards; this condition is shown for vertebral regions in fig. 17, for intervertebral in fig. 18.

At a stage with eleven protovertebræ, the vertebral portions of the intermediate cell mass, behind the fourth protovertebra, acquire a circular lumen, which is bounded by a single layer of columnar cells; this condition is seen in fig. 19. In fig. 20, which represents a section passing through the end of the same protovertebra as that from which fig. 19 is taken, the lumen is smaller; in the intervertebral region behind the lumen altogether vanishes, and the solid, swollen cell mass presents an appearance exactly like that seen in the preceding stage (fig. 18).

There is thus formed a series of cavities in the continuous intermediate cell mass, each situated opposite a protovertebra, and having its walls continuous both with the protovertebra and with the peritoneal epithelium. These cavities are separated from one another by the solid intervertebral parts of the intermediate cell mass.

In embryos with eleven protovertebræ there are five of these vesicles, opposite the fifth to the tenth protovertebra, the last two somites being as yet without them. In these last somites the intermediate cell mass is swollen and solid, as in the anterior region of an earlier embryo.

These cavities are, as will be seen from their subsequent history, the segmental vesicles described by Rathke and subsequent writers.

They have hitherto been described entirely separate from one another, and have been supposed (Braun., loc. cit.) to arise as invaginations of the peritoneal epithelium.

When twelve protovertebræ are present the Wolffian duct begins to appear as a solid cord of cells, splitting off in the intervertebral region only from the intermediate cell mass, and passing, in the region of each protovertebra, into the wall of a segmental vesicle.

Figs. 21—23 represent three sections through about the sixth and seventh somites of an embryo with twelve proto-

vertebræ. Fig. 21, the most anterior, passes through a vertebral region, and shows the segmental vesicle, with its lumen; the section passes through the attachment to the peritoneum (which in the vertebral regions is becoming smaller), but not through the connection with the protovertebra. The next section (fig. 22), through the commencement of the intervertebral region, shows the solid cell mass, with a few cells (*w. d.*) split off from its outer portion. These cells are the rudiment of the Wolffian duct. In the next protovertebral region this cord ceases to be visible. Fig. 23 shows a section through the commencement of the next protovertebra, passing through the solid wall of the corresponding vesicle, which has no trace of the duct.

These cords of cells are present at this stage in four intervertebral areas, behind protovertebræ five to eight inclusive.

With the formation of the thirteenth protovertebra the solid rudiment of the Wolffian duct becomes more distinctly split off in the intervertebral regions, while opposite the protovertebræ it appears as a solid appendage of the wall of the segmental vesicles, with which it is perfectly continuous.

At the same time it extends backwards into the ninth intervertebral region.

In an embryo with fourteen protovertebræ there are eight segmental vesicles with a lumen opposite the protovertebræ five to twelve inclusive. All these have the Wolffian duct as a solid knob on their outer wall, while in the corresponding intervertebral regions there appears a distinct lumen in the duct, which is more or less completely split off from the rest of the intermediate cell mass.

The relations of the duct and vesicle in an embryo with fourteen somites are shown in fig. 24, from the second segmental vesicle of such an embryo. In this figure the segmental vesicle (*s. v.*) is seen to have a large lumen, and the solid Wolffian duct (*w. d.*) appears attached to its outer wall.

In fig. 25, from the next intervertebral region behind fig. 24, the Wolffian duct has a large lumen, and is attached to the solid intervertebral cell mass.

A section through the next protovertebra would repeat the features shown in fig. 24.

On the appearance of the fifteenth protovertebra the lumen of the Wolffian duct becomes continuous throughout the region of the first eight segments, and at the same time it acquires a communication with the cavity of each segmental vesicle in its course.

The first eight segmental tubules are therefore differentiated, continuously with the Wolffian duct, from a ridge of cells, continuous at first along its entire length with the peritoneal epithelium, and at certain points with the adjacent protovertebræ.

With regard to the tubules behind the eighth, they are developed from the intermediate cell mass in exactly the same way as those in front; but the Wolffian duct, instead of arising continuously with them, grows backwards as a free projection of the above-described portion, without coming into relation with adjacent structures. It is at first solid, but afterwards acquires a lumen, and becomes connected with the segmental vesicles in order from before backwards.

On the subsequent behaviour of the tubules and on the development of the metanephros I have no observations.

The most interesting feature in the preceding account of the early development of the lacertilian kidney is the close resemblance which it shows to exist between the process of development in that group and the process which has been shown by Sedgwick¹ to exist in birds and Elasmobranchs. In both these groups Sedgwick has shown that the segmental tubules arise from a continuous cell mass connected with the peritoneal epithelium and with the mesoblastic somites, which cell mass is present from the very beginning of the process of mesoblastic segmentation.

In the anterior part of the Wolffian body of the chick

¹ Sedgwick, "Development of the Kidney in its relation to the Wolffian Body in the Chick," this Journal, vol. xx; and "Early Development of the Wolffian Duct and anterior Wolffian Tubules in the Chick," &c., 'Quart. Journ. Mic. Sci.,' vol. xxi.

Sedgwick has shown that the Wolffian duct and segmental tubules arise continuously by differentiation of the cell mass. In the chick, as in the lizard, the independent origin of duct and tubules in the posterior region is probably a secondary character.

In conclusion, I wish to express my gratitude to the authorities of the Zoological Station at Naples for their kindness to me during my visit, and to Mr. Sedgwick for the advice and assistance which he has given me since my return to Cambridge.

EXPLANATION OF PLATES I, II, & III.

Illustrating Mr. W. F. R. Weldon's Note on the "Early Development of *Lacerta muralis*."

In all figures:—*pr.* Primitive streak. *bl.* Blastopore. *ep.* Epiblast. *hy.* Hypoblast. *ch.* Notochord. *me.* Primary mesoblast. *me'*. Secondary mesoblast, budded off from lower layer cells. *pp. c.* Body cavity. *i. c. m.* Intermediate cell mass. *s. v.* Segmental vesicle. *w. d.* Wolffian duct.

FIG. 1.—Median longitudinal section through a blastoderm with a primitive streak, but no blastopore.

FIG. 2.—Median longitudinal section through a blastoderm with a blastopore visible as a pit at the anterior end of the primitive streak.

FIG. 3.—An embryo in which the blastopore has just broken through. Median longitudinal section.

FIG. 4.—From an embryo slightly older than that shown in Fig. 3.

FIG. 5. Lateral longitudinal section through the same embryo as that from which Fig. 4 is taken, showing the forward growth of mesoblast from the lips of the blastopore.

FIGS. 6—13.—A series of transverse sections through an embryo slightly older than that shown in Figs. 4 and 5. The sections are in order from behind forwards.

FIG. 14.—Longitudinal section through the posterior region of an embryo in which the mesoblastic split is complete, to show the median posterior body-cavity into which the allantois will project.

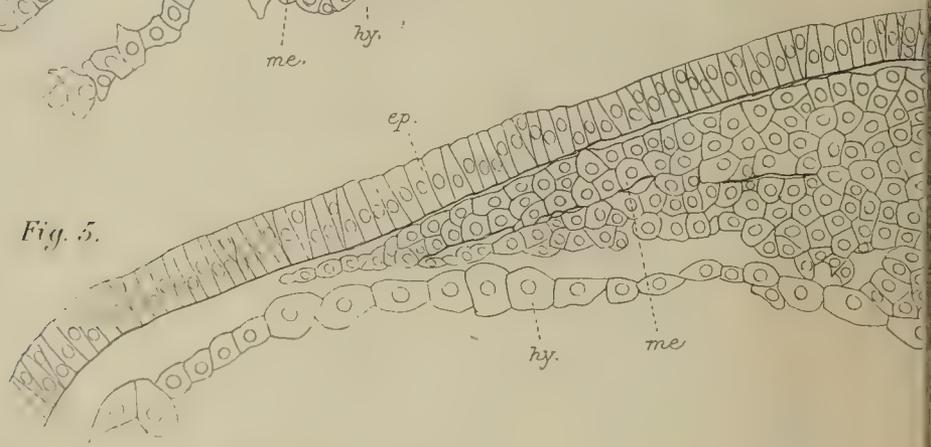
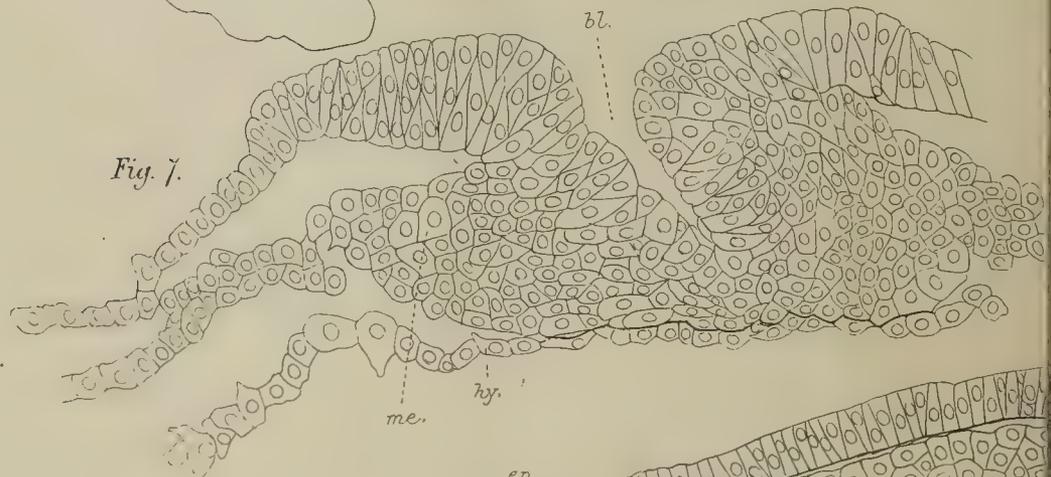
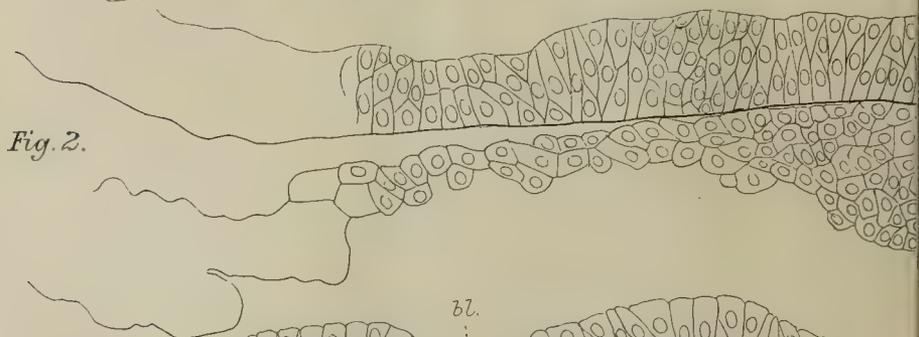
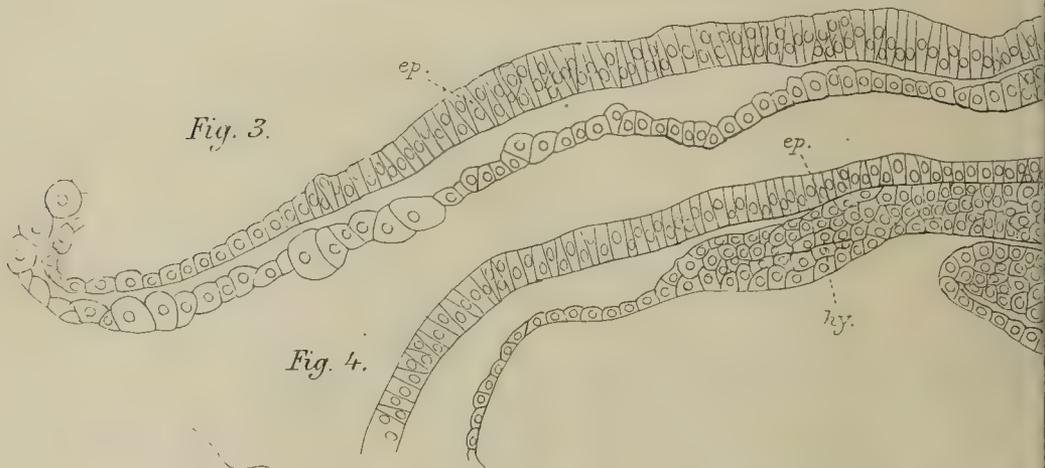
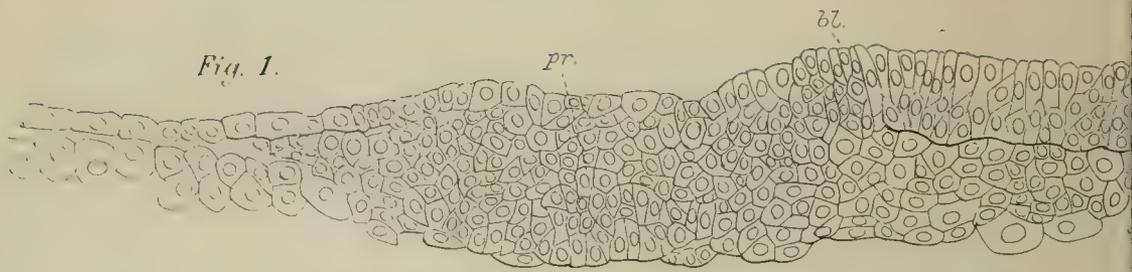
FIGS. 15 and 16.—Transverse sections through an embryo with seven protovertebræ. Fig. 15, from the fifth protovertebra; Fig. 16, from the intervertebral region behind.

FIGS. 17 and 18.—From an embryo with ten protovertebræ. Fig. 17, vertebra; Fig. 18, from the next intervertebra.

FIGS. 19 and 20.—From an embryo with eleven protovertebræ. Fig. 19, vertebra; Fig. 20, from the next intervertebra.

FIGS. 21—23.—From an embryo with twelve protovertebræ. The first of these sections passes through a protovertebra; the second through the next intervertebral region behind the first; and the third through the commencement of the following protovertebra.

FIGS. 24 and 25.—From an embryo with fourteen protovertebræ. Fig. 24, from a protovertebra; Fig. 25, from the next intervertebra.



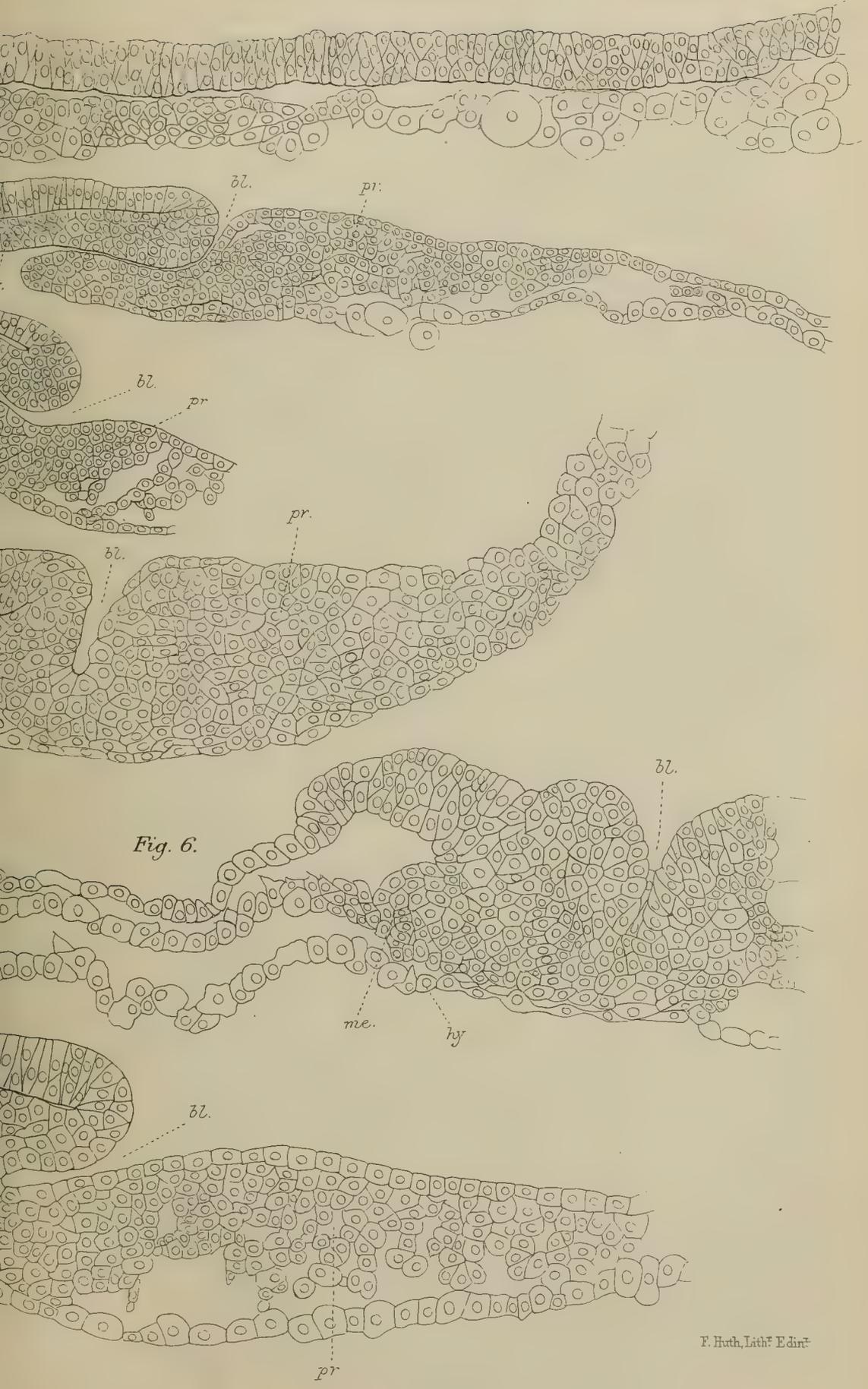


Fig. 6.

Fig. 8.



Fig. 10.

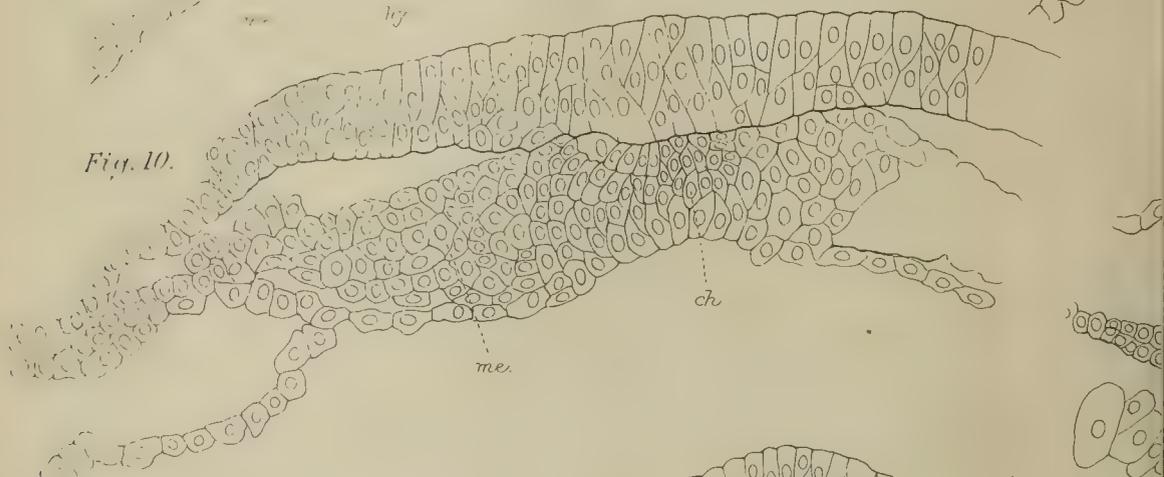


Fig. 12.

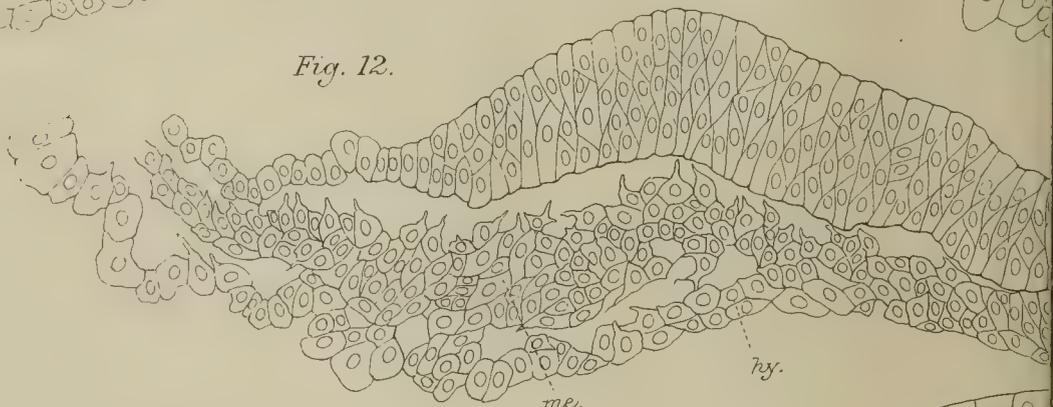


Fig. 13.

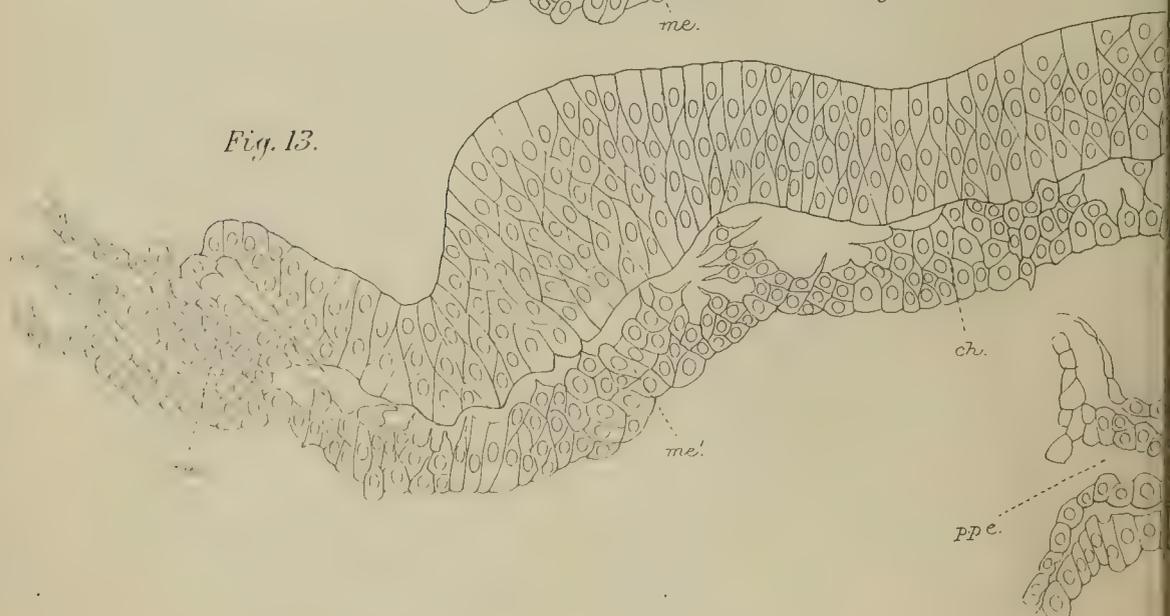




Fig. 9.

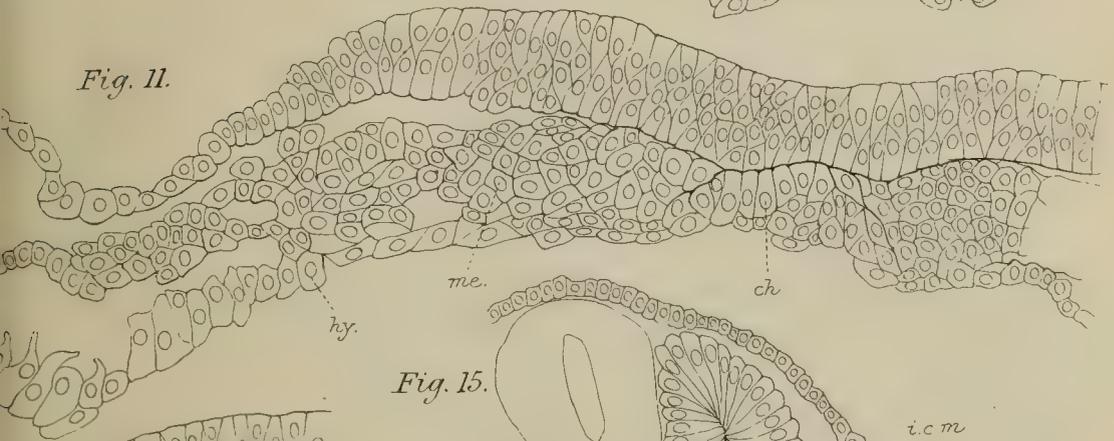


Fig. 11.

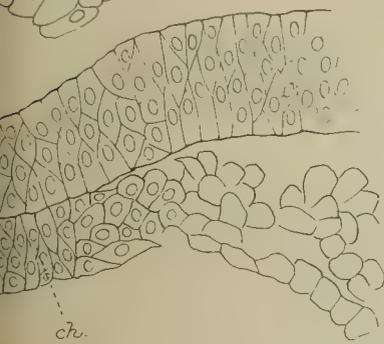


Fig. 15.

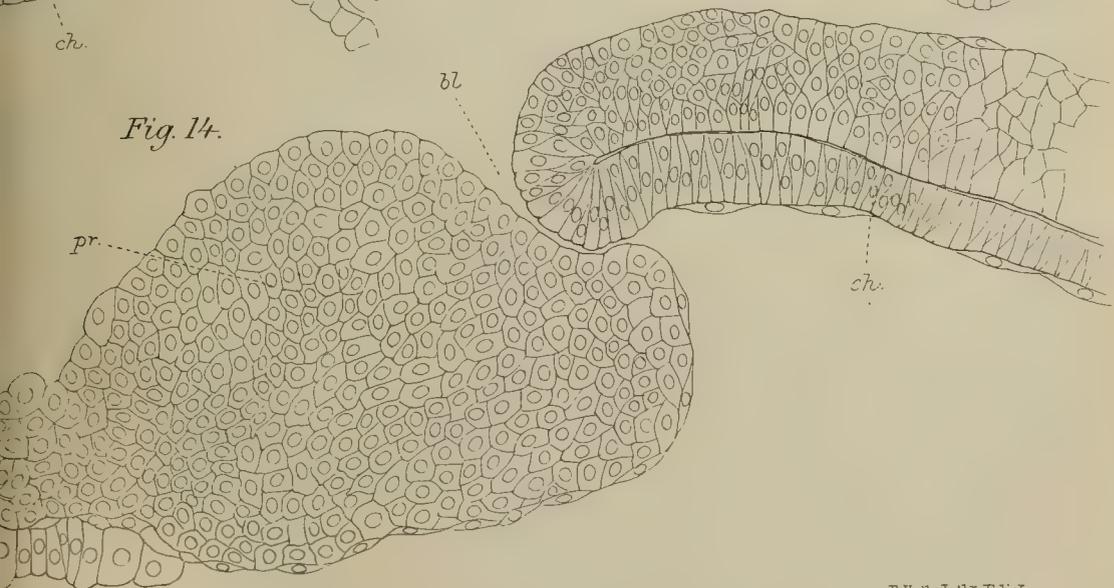
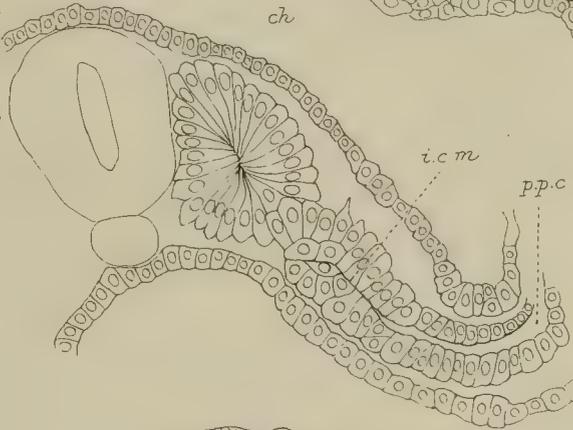


Fig. 14.

Fig. 16.

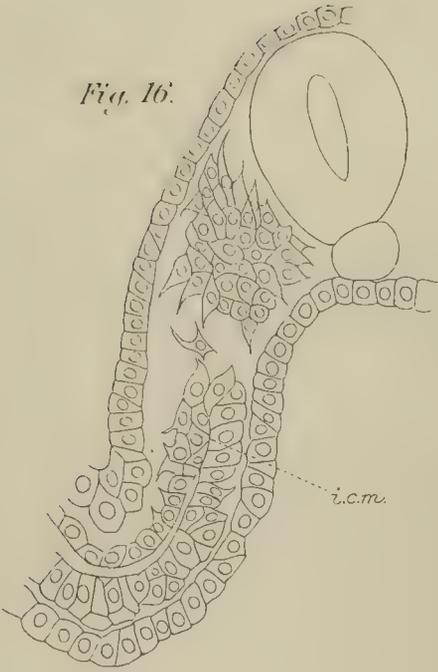


Fig. 17.

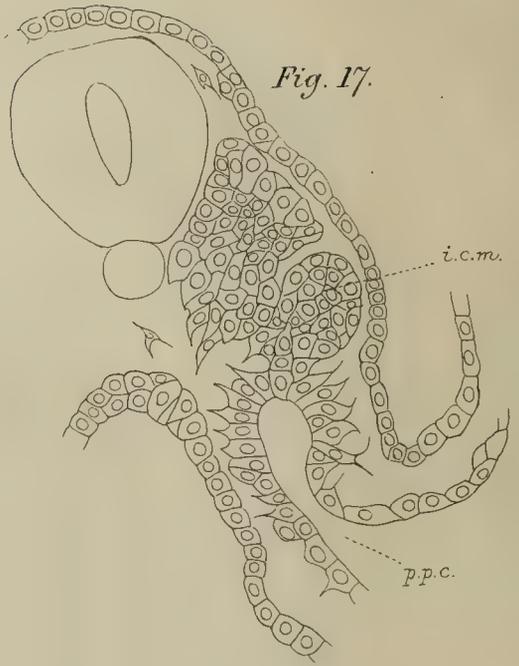


Fig. 20.

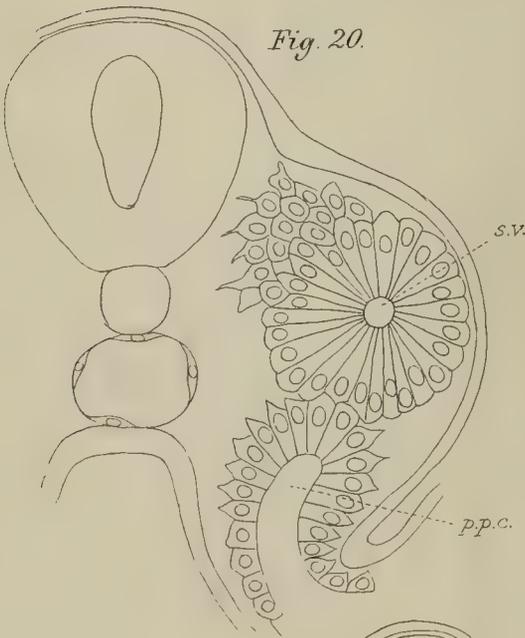


Fig. 22.

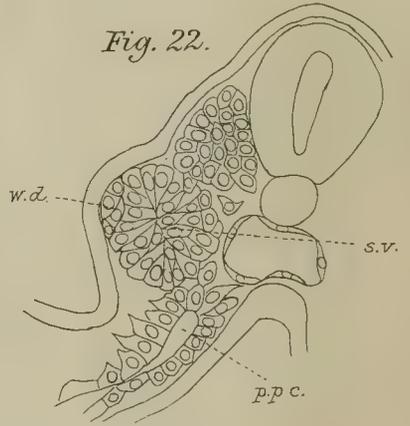


Fig. 21.

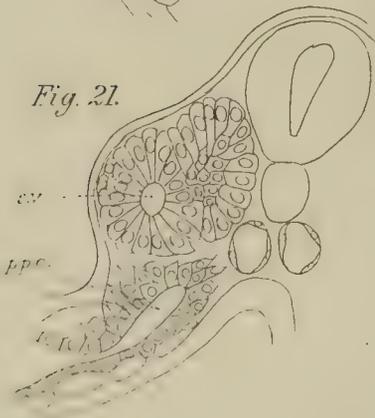
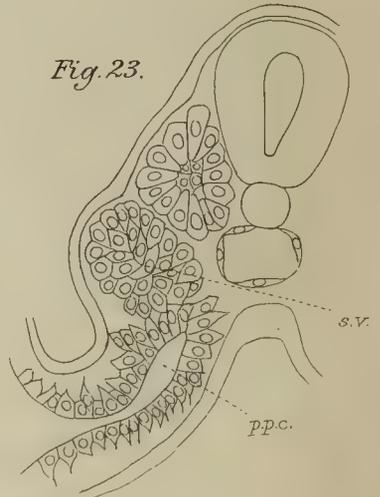


Fig. 23.



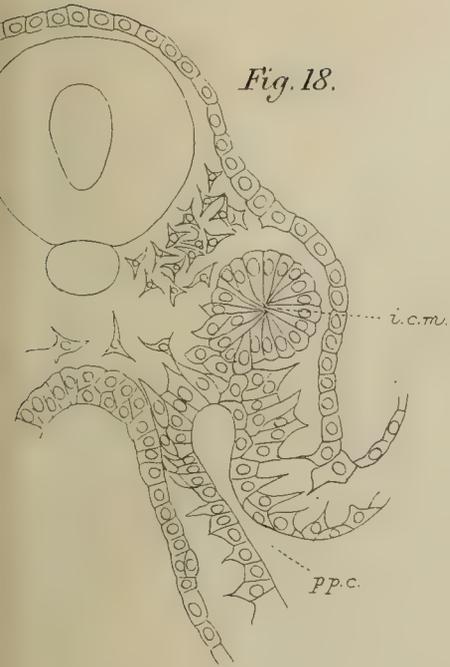


Fig. 18.

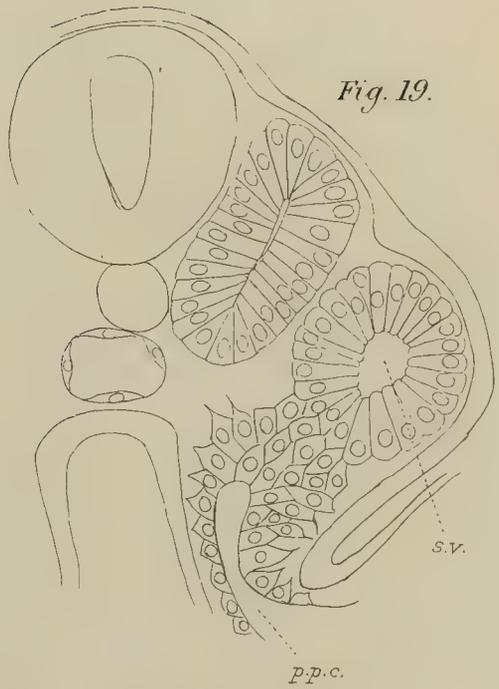


Fig. 19.

Fig. 24.

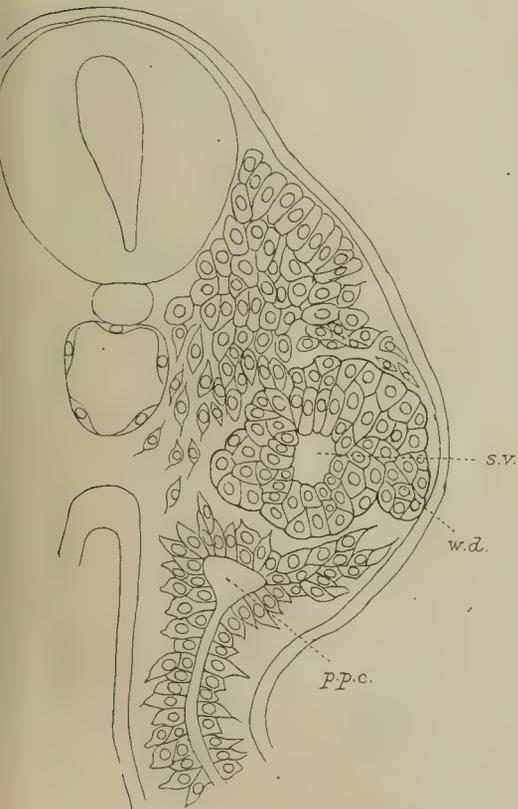
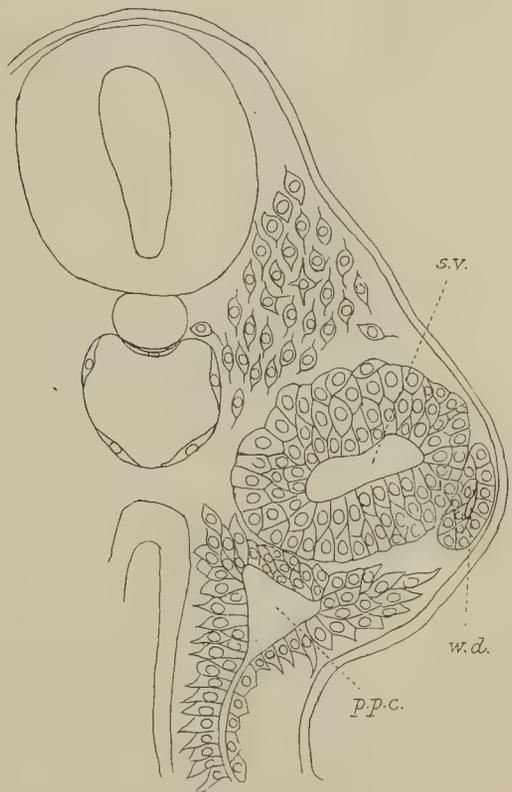


Fig. 25.



On the Development of the Pelvic Girdle and Skeleton of the Hind Limb in the Chick.

By

Alice Johnson,
Newnham College, Cambridge.

With Plates IV & V.

THE investigations described below were undertaken at the suggestion of the late Professor Balfour, with a view to finding out, through a study of the development of the pelvic girdle in the chick, what are the homologies of the pubis in birds with that of other Vertebrata.

In connection with this question one or two other points, which appear to me of some importance, have presented themselves.

The histological development may be briefly considered at the outset. On the fourth day of incubation the limb is merely a local exaggeration of the Wolffian ridge, consisting, like it, of a mass of rounded mesoblastic cells, very closely aggregated together. The epiblast forms a thickened cap round the free end of the limb. No differentiation into cartilage or muscle is yet visible.

The first trace of the skeletal parts appears on the fifth day. The mesoblastic tissue of the limb is now differentiated into an axial, or more condensed, and surrounding, or less condensed, region. Both parts consist of the same rounded cells as before. They only differ in the degree of concentration of the cells. These features are shown diagrammatically in fig. 1.

The differentiation of tissue goes on more rapidly in the

skeleton of the limb than in the girdle, and more rapidly in the axial than in the superficial regions of both skeletons. Its main features are almost the same as those described by Strasser¹ in the developing cartilage of the newt. On the sixth day, or thereabouts, the cells begin to be compressed in the direction of the long axis of the cartilages. This happens especially in the tibia and fibula. Dark irregularly-shaped masses—the “prochondral elements” of Strasser—appear among the cells. They are apparently derived from the metamorphosed cells, for one occasionally meets with forms that appear intermediate, in which the protoplasm has become opaque and stains deeply, while the nucleus is still visible. I take the prochondral elements to be cells which have retrograded still further and lost their nuclei.

Rather later, on the sixth or seventh day, the prochondral elements have almost disappeared from the central part of the cartilage. Their place is taken by a homogeneous, slightly-staining matrix, by means of which the cells gradually become widely separated from one another. Still later the cells take on the crescent shape of adult cartilage cells.

Morphology.—Since chicks of the same day vary so much in their degree of development I have taken the length of the hind limb as the standard of their age. The following table shows roughly to what number of days of incubations these lengths correspond :

Length of hind limb.	Number of days of incubation.
0·06 in.—0·1 in.	5—6
0·12 in.—0·2 in.	6—7
0·2 in.—0·25 in.	7—8
0·25 in.—0·3 in.	8—9
0·5 in.—0·8 in.	9—10
1·5 in.—3· in.	14—20

The Pelvic Girdle on its first appearance (length of hind limb 0·06 in.—see fig. 1) is seen in transverse sections to form one mass with the skeleton of the limb. It consists of two slight

¹ H. Strasser, “Zur Entwicklung der Extremitätenknochen bei Salamandern und Tritonen,” ‘Morph. Jahrbuch,’ Band v, 1879.

outgrowths of the proximal part of the femur, one being directed upwards, the other inwards, each, however, hardly extending beyond the limb itself. The future cartilage is only just distinguishable from its surroundings of indifferent mesoblastic cells, since the two tissues pass quite gradually into one another.

The next stage (length of hind limb 0·12 in.) is seen in longitudinal section in fig. 2. The series of sections shows the same perfect continuity of the girdle and femur that existed at first. We can distinguish in the girdle a blunt dorsal prolongation—the beginning of the ilium—an acetabular region behind the obturator nerve and a downward process in front of it, which is obviously to become the pubis. As we go inwards in the series of sections these two outgrowths, the ilium and the pubis, disappear, and the central or acetabular region is prolonged a little way inwards, being bounded in front by the obturator nerve. The nerve does not appear in the same sections with the pubis and ilium, but in the figure it is represented as viewed from the outside, the girdle being supposed to be transparent. At this stage the nerves are remarkable for their large size in proportion to the skeletal parts. The obturator nerve coming off from the crural plexus is at this time by far the most important of its distal branches.

In the next stage (length of hind limb 0·15 in.—see fig. 3) we can clearly distinguish three elements in the girdle, meeting in the broad acetabular region, which passes on without a break into the femur. The region of its junction with the latter is shown diagrammatically in the figure, but the cartilage of the femur is continuous with that of the girdle, as are the three elements of the girdle with one another. The ilium has grown forwards, arching over the crural nerve, and has given off a slenderer pointed process backwards. The ischium is directed almost vertically downwards, but also slightly inwards, being, as a whole, situated nearer to the middle line of the body than are the other elements. The main point of interest is the double nature of the pubis, the anterior branch of which points directly forwards and slightly outwards, while the posterior is directed

downwards and slightly forwards. The obturator nerve passes between the posterior branch of the pubis and the ischium. A series of twelve longitudinal sections has been combined to produce the figure, which is therefore diagrammatic only in so far as it represents as existing in two dimensions what really exists in three. The other figures, in which the whole girdle is represented, were drawn in the same way.

The study of the stages described above shows that the early development of the Pelvic Girdle of the Chick is similar to that of the limb-girdles of Elasmobranchs¹ in two points: (1) the skeleton of the limb is developed continuously with the girdle; (2) the parts of the girdle which are in the immediate neighbourhood of the skeleton of the limb are first developed, and the dorsal and ventral outgrowths appear later.

In the next stage (length of hind limb 0.17 in., see fig. 4) the posterior branch of the pubis has grown more than the anterior, and is curved backwards. Its proximal half, however, retains the direction which the whole posterior branch had in the earlier stage, and from this we may conclude that the change of form results from a growth, rather than from a rotation backwards of the whole cartilage.

A transverse section (see fig. 5) of about the same stage shows that the girdle is still continuous with the femur. In the latter, the cartilaginous matrix has begun to be formed internally, while the peripheral parts (a region of which is cut through in the middle of the limb) and the girdle still consist of the condensed tissue described above.

A later stage is shown in fig. 6 (length of hind limb 0.2 in.). The most striking feature here compared with the preceding stage is the large development of the posterior part of the ilium. The ischium has become distally expanded, and the posterior branch of the pubis is larger still in proportion to the anterior branch.

About this time the femur begins to be separated from the girdle by an intervening tract of tissue which has not gone so

¹ F. M. Balfour, "On the development of the skeleton of the paired fins of Elasmobranchii," 'Proc. of Zoological Society,' 1881.

far on the way to becoming adult cartilage. At first the whole structure progresses uniformly, except that the girdle always lags a little behind the femur, but passes off gradually into it. The "prochondral elements" and a small quantity of cartilaginous matrix exist across the future line of division, which, however, develops no further, but retrogrades into the fibrous tissue of the joint.

Fig. 7 represents a further advance of the girdle towards the adult form.

In later stages, no important changes take place. The anterior branch of the pubis, which is always rather behind the rest of the girdle in histological development, becomes more and more proportionately insignificant, and forms at last the pectineal process of the pubis. The posterior branch of the pubis becomes very slender. Both it and the ischium grow more and more backwards, passing through the stage permanent in such forms as *Apteryx* (where they are much curved, and their long axes are inclined at an acute angle to the long axis of the ilium) to the stage found in the adult fowl, where the pubis and ischium—except the most proximal portions of them—are straight, and point directly backwards, so that the long axes of all three bones are parallel to one another.

Ossification begins comparatively late, i. e. later than in the limb. For a long time there is a cartilaginous continuity of the three elements round the acetabulum. The bones gradually grow up to and surround the acetabulum. Cartilage remains also at the free ends of the bones for a long time. A day or two before hatching (see fig. 18), the acetabulum is surrounded by bone, except for a small region of its front wall, continuous with the likewise cartilaginous anterior branch of the pubis. The position and relations of this latter element, together with the fact of its remaining cartilaginous so long, remind one to some extent of the cartilage found in a similar situation in the *Crocodile* embryo after the rest of the girdle has ossified. According to Hoffmann,¹ this cartilage is homo-

¹ C. K. Hoffmann, "Beiträge zur Kenntniss des Beckens der Amphibien und Reptilien," 'Nied. Archiv f. Zoologie,' Band iii, 1876.

logous with the Pubis, while he calls the bone generally known as the pubis the epi-pubis. But since the acetabular regions of each bone always remain cartilaginous longer than the other parts, and since this cartilage is replaced in the adult by a bony process of the Ischium shutting out the pubis (Epi-pubis of Hoffman) from the acetabulum, I should be more inclined to agree with the older view that Hoffmann's pubis is merely a part of the ischium. This seems to me quite consistent with his own account of the ossification. He says:—(loc. cit. p. 186) “Die Verknöcherung dieses vorderen Acetabularfortsatzes des Sitzbeines fangt zuerst an der dem Sitzbein angrenzenden Partie an und schreitet so allmählig dem vorderen Fortsatz des Iliums zu, erreicht diesen aber erst bei ganz ausgewachsenen alten Thieren.” The fact that the pubis is moveable in the crocodile is quite sufficient to account for its being shut out from the acetabulum.

So far as I know, the only literature bearing directly on the subject of the development of the pelvic girdle in birds is a paper by Bunge.¹ According to him, the pubis and ischium are at first situated with their long axes in a position vertical to the vertebral column, and later become rotated backwards, thus taking on the adult form. This statement has been generally accepted, but I am unable to agree with Bunge's other conclusions. He has omitted to mention the primary continuity of the femur and girdle and the existence in the embryo of an anterior branch of the pubis which becomes the pectineal process. Speaking of the pectineal process in the adult, he only says that his account of the development proves that it is a part of the ilium, and he therefore retains the name “Spina iliaca” given it by the older anatomists. He also concludes that the avian pubis is homologous with the pubis of Reptiles. He describes the pubis as originating independently of the other elements of the girdle and beginning to fuse with them about on the eighth day. I find that the pubis is absolutely continuous with the girdle at the earliest,

¹ A. Bunge, “Untersuchungen zur Entwicklungsgeschichte des Beckengürtels der Amphibien, Reptilien, und Vögel,” Dorpat, 1880.

and all other cartilaginous stages. But, since it lies in a somewhat different plane from the rest of the girdle, their junction is only visible in a few sections. In most, the region of junction is not cut through, as appears in fig. 10, which represents a section taken from the series out of which fig. 4 was compounded. I think that Bunge must have been misled by the frequent occurrence of such sections, and so have overlooked the few in each series in which the junction is really visible, such as that represented in fig. 11. Fig. 12 again represents a single section, showing the complete continuity of pubis and girdle. It is the ossification alone which gives rise to any want of continuity in any part of the girdle.

Homologies of the pubis in the different Vertebrate groups.—In the pelvic girdle of *Ornithorhynchus* (see fig. 17) a large process, whose length is about three quarters of that of the pubis, projects forwards from the region in front of the acetabulum, in bony continuity with the pubis. The same process is found in a somewhat reduced form in *Echidna*, and is still more reduced in many Marsupials and higher Mammals. Sometimes it is entirely absent. In embryo birds (see fig. 15), the process is found in about the same proportionate condition of development as in *Ornithorhynchus*. In the adult, it becomes much reduced, or is absent. Sometimes, as in the Ostrich, the ilium takes a small share in its formation, but this appears to be a secondary condition. It is the pectineal process of the pubis.

In the Dinosaurs, as described first by Marsh,¹ the embryonic condition of birds and the adult form of *Ornithorhynchus* is preserved in the almost equal development of the two branches of the pubis, the anterior being shorter and more massive, and the posterior longer and more slender (fig. 16).

The homologies in these cases seem clear, and have been generally recognised.

Turning to the reptiles, it is easy to compare the pubis of

¹ O. C. Marsh, "Principal characters of American Jurassic Dinosaurs," 'American Journal of Science and Arts' (Silliman), vols. xvi and xvii, 1878 and 1879.

Lizards with that of Chelonia. In both Lizards and Chelonia the pubes are directed forwards from the acetabulum, and form a symphysis. The angle at the symphysis is generally much greater than that in Mammals. In some Chelonia it is even greater than 180° . In both Lizards and Chelonia a process is given off from the outer side of the pubis. In the latter group it is often very large (see fig. 13), and is directed forwards, outwards, and somewhat downwards. In Lizards it is not so large, but still considerable, or it may be absent. It is generally directed outwards and downwards; but in some forms, such as *Cyclodus* (see fig. 14), it curves backwards and slightly inwards. In this case we could hardly compare it with the process found in Chelonia were it not for the many intermediate forms existing between these two extreme types. The process in question is the *processus lateralis pubis*. In Crocodiles it is absent.

In the Urodela the pubes are generally represented by an unpaired cartilaginous plate, not clearly marked off from the ischium, which is often ossified. Rarely the pubis itself has a superficial ossification. The pubic cartilage in *Cryptobranchus* is oblong, with a median process in front bearing the epipubis, and the anterior angles of the oblong are slightly produced. In *Salamandra maculosa* these angles form short broad processes, which may be compared with the *processus lateralis pubis* of Chelonia.

We have, then, in reptiles two branches of the pubis—the *processus lateralis* and the main body of the pubis—which two branches it is possible to derive from the condition found in Urodela. Also in Dinosaurs, Birds, and Mammals we have the pectineal process and the main body of the pubis. The splitting of the pubis into two branches is more complete—i. e. it approaches nearer to the acetabulum—in the higher forms.

There is every probability that the two branches correspond in some way in all these types. Two theories on the subject are obviously possible. Either (1) the *processus lateralis* of reptiles is the pectineal process of the pubis in Dinosaurs,

birds, and Mammals, and the pubis itself is in both cases homologous, or (2) the pubis of reptiles is the pectineal process, and the processus lateralis is the pubis of the higher forms. The first is the view apparently assumed by Huxley.¹ Supposing it to be true, the processus lateralis, in becoming the pectineal process, has retained the forward and outward direction which it has in the Chelonia. In Dinosaurs the downward direction is also seen. The pubis itself has become rotated backwards. The mere fact of its pointing forwards in reptiles and backwards in Dinosaurs, Birds, and Mammals, is no reason whatever against the theory of its being homologous in the two cases, for it is generally believed that the whole girdle has rotated in Mammals through an angle of about 90° from the position it occupies in reptiles. This would completely account for the altered position of the pubis. The fact that the angle formed at the symphysis of the pubes has generally become more acute in Mammals is a natural consequence of the transition from the crawling flat-bodied reptiles to the higher walking forms, in which the body is more laterally compressed.

In birds the case is somewhat different. The fact of the two primary sacral vertebræ being situated, as Gegenbaur² has shown, at a very short distance behind the acetabulum may indicate that the girdle has been rotated backwards to some extent from the reptilian position. The pubis may thus have come to point vertically downwards or very slightly backwards, as in the embryo bird. The adductor muscles passing from the pubis and ischium to the femur in reptiles are to a great extent replaced in birds by large muscles, which act as flexors of the thigh and adductors of the leg. It is evidently advantageous for these muscles to arise high up, and for their points of origin to be as rigid as possible. These advantages are attained by the disposition of the bones in the adult bird's pelvic girdle, the

¹ Huxley, "On the Pelvis in Mammalia," 'Proceedings of Royal Society,' vol. xxviii, 1879.

² Gegenbaur, "Beiträge zur Kenntniss des Beckens der Vögel," 'Jenaische Zeitschrift,' Band vi, 1871.

form of which, therefore, may be accounted for in this way. The pubis, being placed lowest, loses its functional importance as a point of support for muscles and becomes very slender. Its middle portion may even abort altogether, as sometimes happens in ducks and other swimming birds.

So far it appears quite possible to explain the facts by the first theory.

Turning to the second, we have to imagine a somewhat different process. The *processus lateralis* of reptiles, in becoming the pubis of the higher forms, has retained the position which it had already come to occupy in some Lizards (see fig. 14), and has increased in extent and functional importance. In Mammals it goes so far as to form a new symphysis, while in birds the backward direction of the bone is very much exaggerated. The part corresponding to the reptilian pubis at first retains its original situation and almost its original dimensions, as the anterior branch of the pubis in Dinosaurs, the embryo bird, and *Ornithorhynchus*. It gradually dwindles into the subordinate position of the pectineal process.

This theory again, accounts for all the known facts, and it agrees, better than does the former view, with the relations of the pubis in Dinosaurs. Marsh¹ found that the anterior branch of the pubis in the *Stegosauria* and *Ornithopoda*, e. g. *Laosaurus*, passed forwards and inwards, ending in a broad spatulate free extremity. In the *Theropoda* and *Sauropoda*, e. g. *Atlantosaurus*, no posterior branch of the pubis existed, but the bone which evidently corresponded to the anterior branch in *Laosaurus* formed the symphysis. Judging from this fact there seems no doubt that the anterior branch is homologous to the reptilian pubis. I think there can also be no doubt of the homology between it and the anterior branch, which, however, no longer forms the symphysis in birds and mammals.

These conclusions may be tabulated as follows :

¹ O. C. Marsh, "Classification of Dinosaurs," 'American Journal of Science' (Silliman), 1882.

Reptiles.	Dinosaurs.	Embryo Bird.	Birds.	Mammals.
1. Pubis	Anterior branch of pubis ('pubis' of Marsh)	Anterior branch of pubis	Pectinal process of pubis	Pectinal process of pubis.
2. Processus lateralis pubis	Pubis ('post-pubis' of Marsh)	Posterior branch of pubis	Pubis	Pubis.

The development of the skeleton of the limb has been described by Gegenbaur.¹ Rosenberg,² has supplemented Gegenbaur's accounts by his discovery of the fifth metatarsal, and quite recently Baur³ has published a paper on the Tarsus of Birds and Dinosaurs. The results of my work on the development of the bird's tarsus agree with Baur's in almost every detail, so that I will give only a short account of it.

In a five days' chick (see fig. 1) the tissue of the limb is condensed axially into a single mass, about three times as long as it is broad, and extending through the proximal half of the limb. The skeleton is produced by the subsequent elongation and segmentation of this mass.

On the sixth day (length of hind limb 0.14 in., see fig. 8) we can recognise all the chief elements of the skeleton, though they are completely continuous. The tarsus forms a broad transverse band, continuous with the tibia and fibula above, and with the metatarsals below. Five metatarsals are present, the first and second being rather closely united. The third is the longest and the fifth the shortest. No cartilaginous

¹ C. Gegenbaur, "Vergleich.-Aant. Bemerkungen über das Fuss skelet der Vögel," 'Archiv für Anat. und Phys.,' 1863; and "Untersuchungen zur vergleichenden Anatomie der Wirbelthiere," i Heft, 'Carpus und Tarsus,' 1864.

² A. Rosenberg, "Ueber die Entwicklung des Extremitäten-Skelets bei einigen Wirbelthieren," 'Zeitschrift f. wiss. Zoologie,' 1873.

³ G. Baur, "Der Tarsus der Vögel und Dinosaurier," 'Morphologisches Jahrbuch,' Band viii, Heft iii, 1882.

matrix has yet appeared, but the "prochondral elements" are visible in the femur, tibia, and fibula.

Soon after—when the limb is 0.17 in. long—separate elements begin to appear in the tarsus. Of these there are three, two in the proximal row and one in the distal. The tarsus is still continuous throughout and continuous also with the tibia, fibula, and metatarsals. But in these three centres, as well as in the tibia, fibula, and metatarsals, the differentiation of tissue has gone further. The outlines of the various parts are indistinct. They all pass gradually into one another by means of the general groundwork of condensed tissue formed by the tarsus. The knee-joint is, however, developed at this stage.

A little after this stage, the first metatarsal, which does not keep step with the others in histological development, begins to split off from the tarsus and soon lies at some little distance from it. Baur describes the first metatarsal as originating quite independently and never coming into any connection with the tarsus.

The phalanges next begin to appear. When the limb is about 0.2 in. long, they are marked off by constrictions from the metatarsals, but are cartilaginously continuous with them. Later, when the limb is 0.3 in. long, the phalanges are marked off by intervening tracts of condensed tissue with no matrix in it. The tip of each toe at this period and for some time to come consists of a mass of condensed tissue such as always precedes cartilage (see fig. 9). This appears to be the growing point of the cartilage. From these facts it seems that the phalanges are produced by a lengthening and subsequent segmentation of the original distal cartilages of the limb, so that these cartilages represent the skeleton of the digits as well as the metatarsals.

On the eighth day (length of hind limb 0.27 in.—see fig. 9) all the elements of the tarsus are at their most distinct and independent stage, though they are still united with one another, with the tibia and fibula, and with the metatarsals by the condensed tissue of the groundwork of the tarsus.

Later, the distal and proximal parts of the tarsus become

separated, and the two proximal elements fuse together. Next, the proximal part begins to fuse with the tibia, which has grown more than the fibula, so that the latter no longer reaches the tarsus. The posterior lower edge of the tibia first becomes continuous with the proximal tarsal cartilage, while the anterior face of the latter gives off an upward process, the so-called "ascending process of the astragalus," which fits into a groove in the tibia, and remains for a long time separate from it. At about the same time the distal part of the tarsus fuses with the metatarsals, first with the second, next with the fourth, and lastly with the third. All these processes take place while the tarsus is still cartilaginous.

Morse¹ describes, in the tarsus of the embryo bird, an intermedium, which at first projects upwards between the distal ends of the tibia and fibula. Later, the tibiale and fibulare fuse behind it, while the tibia extends so as to cover the whole proximal surface of the tarsus, and the intermedium remains fitting into a groove on the anterior face of the tibia. It has a separate centre of ossification, but becomes ankylosed with the tibiale and fibulare, forming what is called the ascending process of the astragalus.

Both Baur and myself fail to find a separate origin for the intermedium. Baur describes the ascending process as an outgrowth from the tibiale, in which view I am inclined to concur. But the deviation of our views from that of Morse may, perhaps, be explained by the fact that while Baur worked only at the chick, duck, sparrow, pigeon, and blackbird, and I only at the chick, Morse investigated some aquatic birds—the tern, penguin, petrel, gull, &c.

In conclusion, I have to thank Dr. Gadow for his kindness in giving me help and advice during the course of my work.

E. S. Morse, "On the Identity of the Ascending Process of the Astragalus in Birds with the Intermedium," 'Anniversary Memoirs of Boston Society of Natural History,' 1880.

EXPLANATION OF PLATES IV & V,

Illustrating Miss Johnson's paper on the "Development of the Pelvic Girdle and Hind Limb of the Chick."

List of Reference Letters.

Il. Ilium. *Is.* Ischium. *pb.* Pubis. *p. p.* Pectineal process. *F.* Femur. *Cr.* Crural nerves. *Ob.* Obturator nerve. *Is. n.* Ischiatic nerves. *m.* Muscles. *W. b.* Wolffian body. *m. p.* Muscle plate. *n. a.* Neural arch. *Sp. c.* Spinal cord. *n. c.* Notochord. *d. a.* Dorsal aorta. *int.* Intestine. *sp. n.* Spinal nerve. *Fb.* Fibula. *Fbe.* Fibulare. *Tb.* Tibia. *Tbe.* Tibiale. I, II, III, IV, v. Skeleton of digits. *pl.* Processus lateralis. *Ac.* Acetabulum. *Tar.* Tarsalia.

FIG. 1.—Transverse section through hind limb and adjoining parts of five days' chick; length of whose hind limb was 0·06 inch.

FIG. 2.—Diagrammatic longitudinal section of early six days' chick; length of hind limb 0·12 in.

FIG. 3.—Diagrammatic longitudinal section of six days' chick; length of hind limb 0·15 in.

FIG. 4.—Diagrammatic longitudinal section of six days' chick; length of hind limb 0·17 in.

FIG. 5.—Transverse section of seven days' chick.

FIG. 6.—Diagrammatic longitudinal section of six days' chick; length of hind limb 0·2 in.

FIG. 7.—Diagrammatic longitudinal section—more advanced stage.

FIG. 8.—Longitudinal section through cartilages of hind limb of six days' chick; length of hind limb 0·14 in.

FIG. 9.—Longitudinal section through cartilages of limb; length of limb 0·27 in. Eighth day.

FIG. 10.—Single longitudinal section, in which junction of ilium and pubis does not appear; length of hind limb 0·17 in.

FIG. 11.—Single longitudinal section, showing junction of ilium and pubis. Same specimen as that from which fig. 10 was taken.

Fig. 1

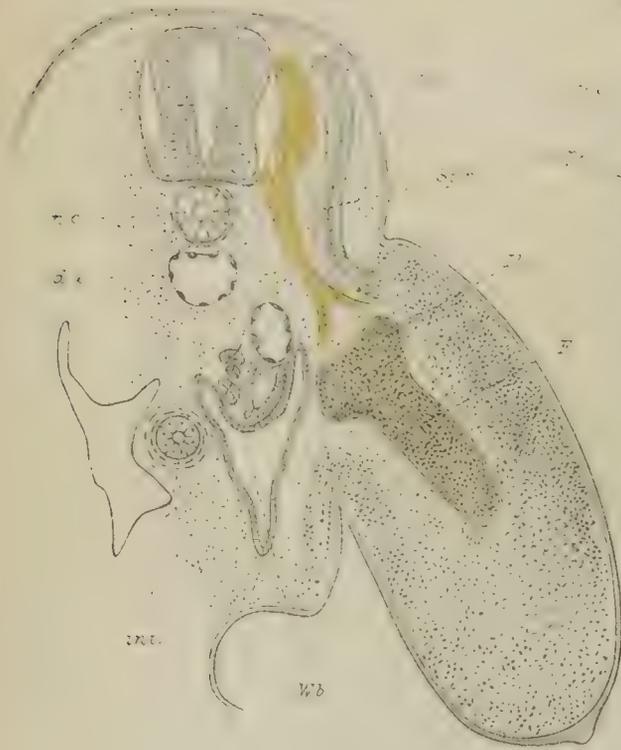


Fig. 2

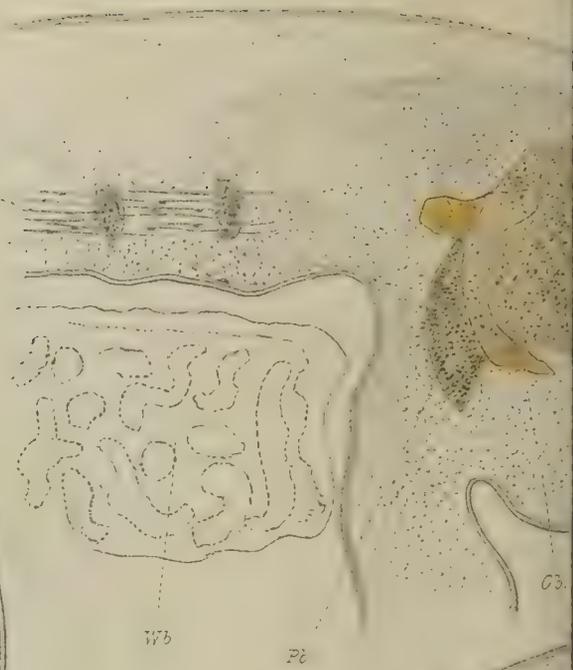


Fig. 6.

Fig. 4.

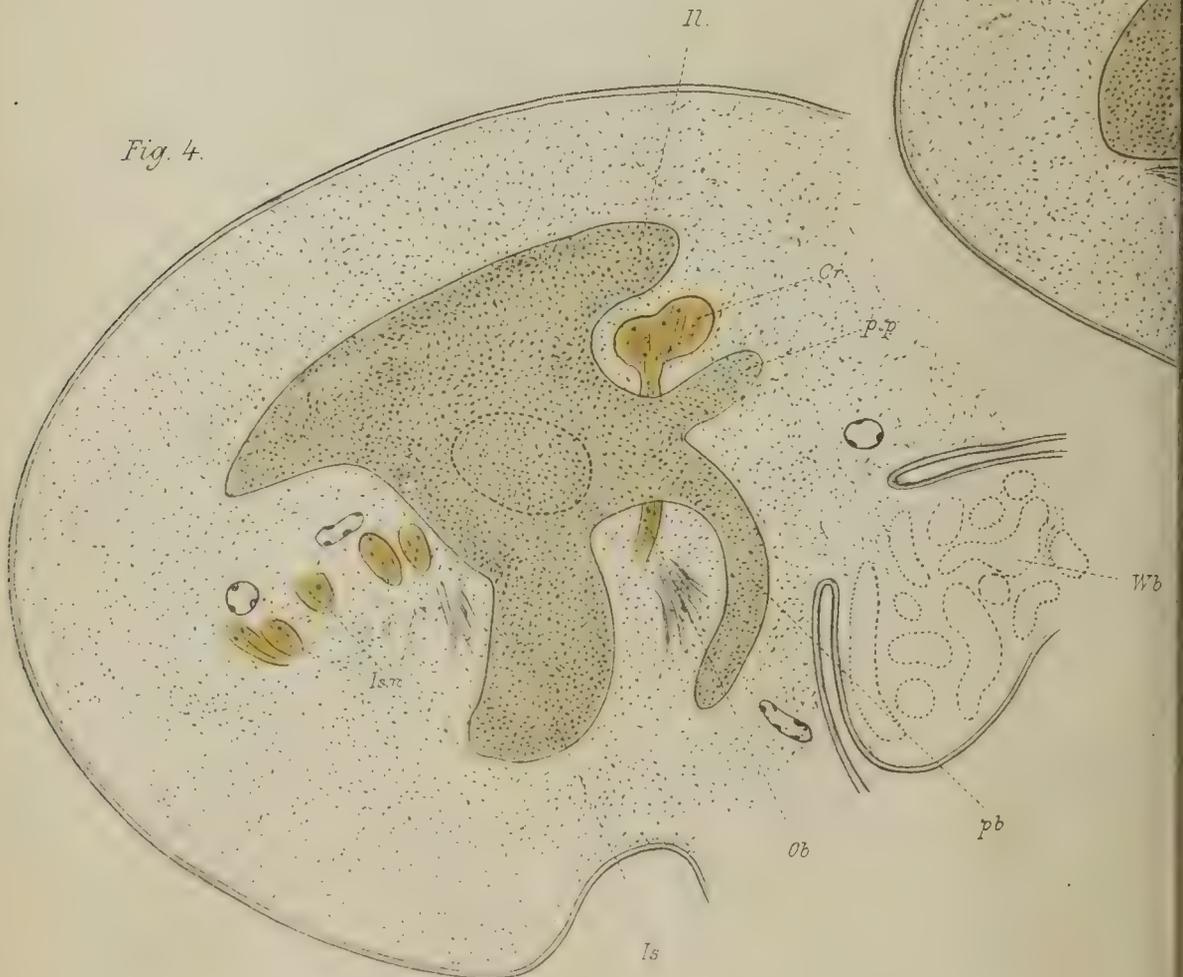


Fig. 3.

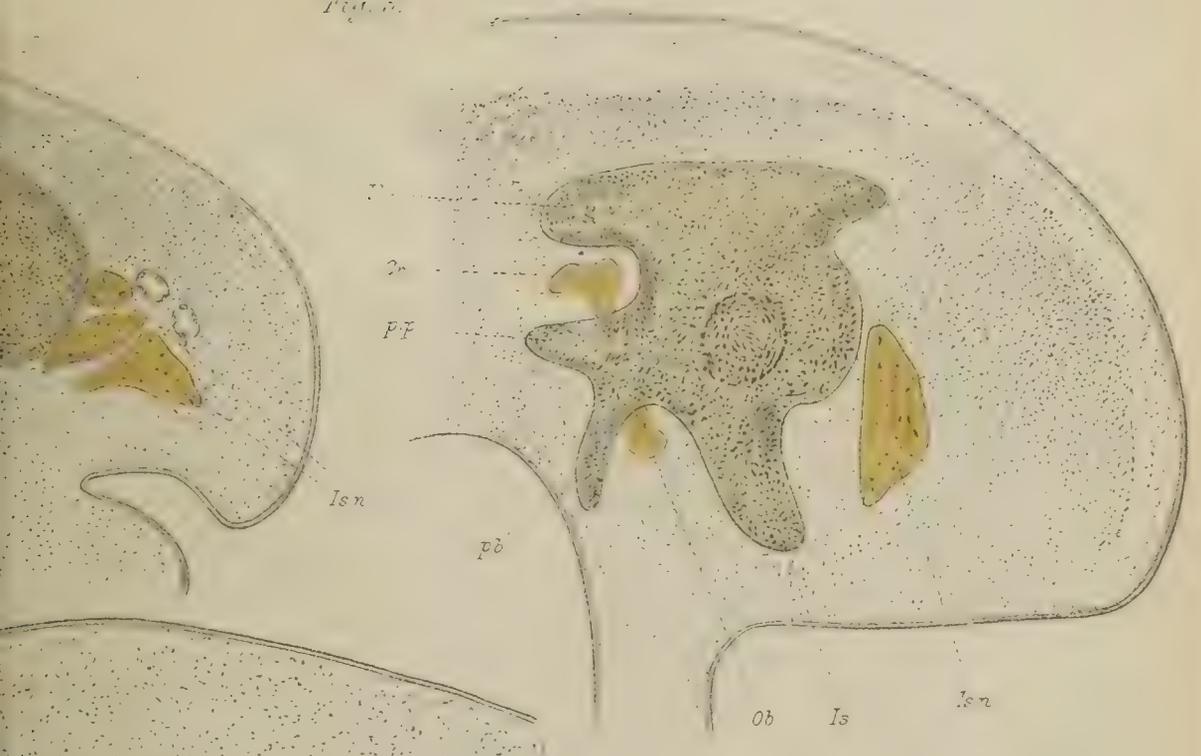


Fig. 5.



Fig. 7.

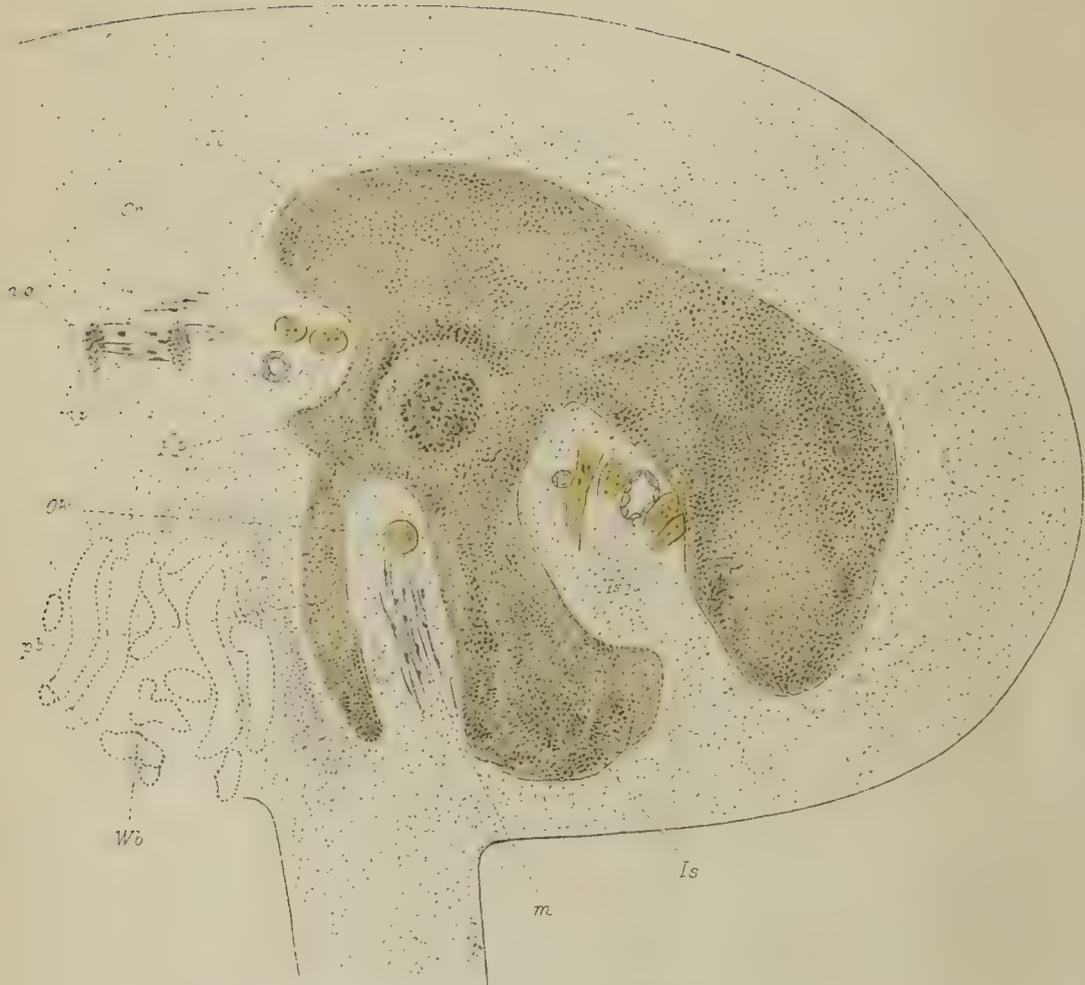


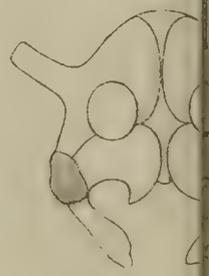
Fig. 10.



Fig. 12.



Fig. 13.

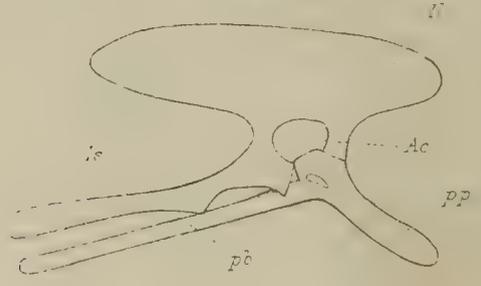


Emys eur

Fig. 11.



Fig. 16.



Laosaurus.

Fig. 8.

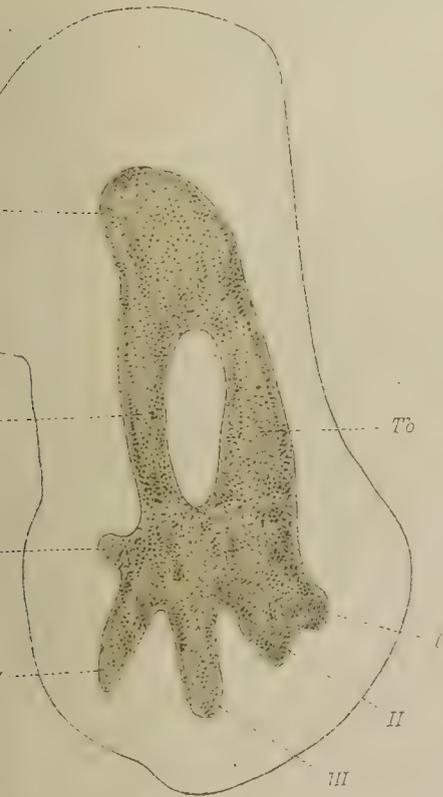


Fig. 9.

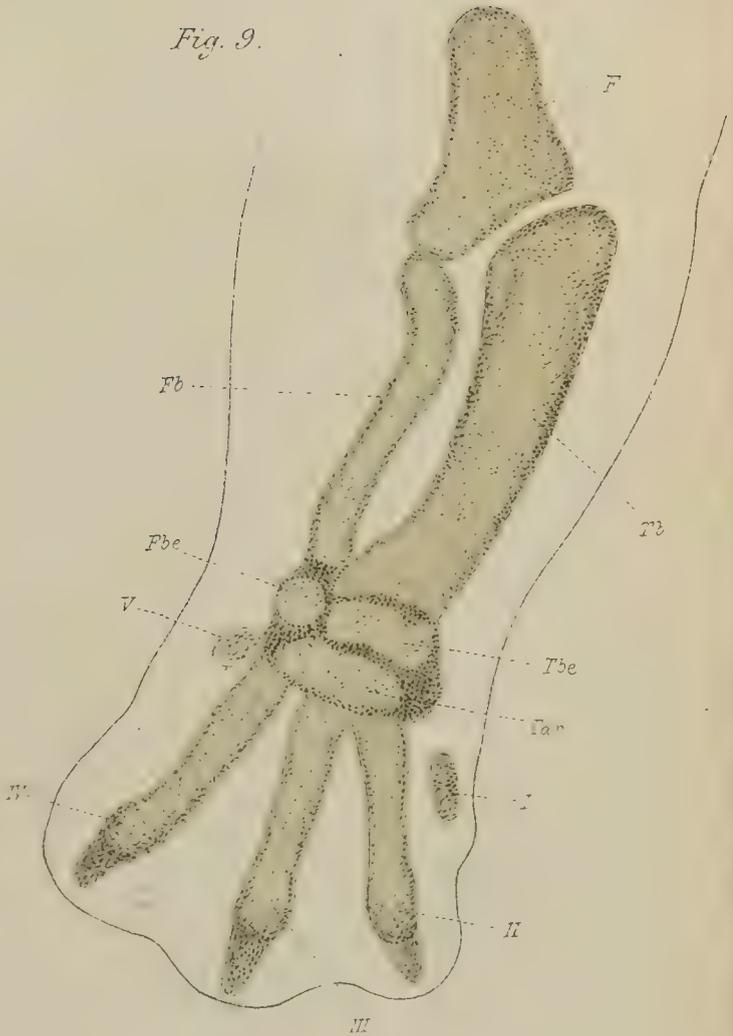
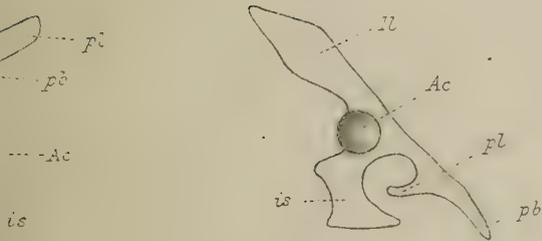
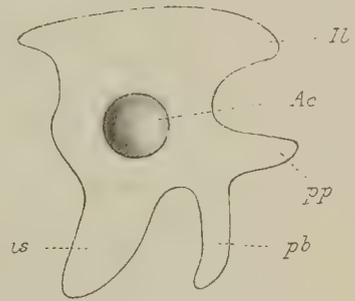


Fig. 14.



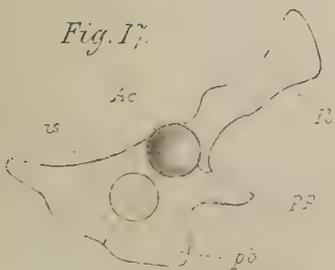
Cyclodus gigas.

Fig. 15.



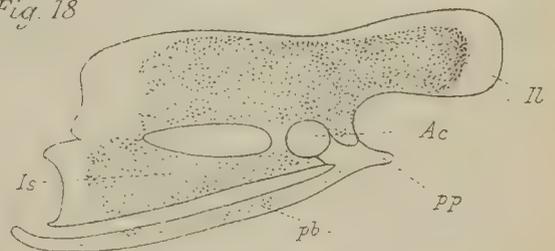
Very young stage of Chick.

Fig. 17.



Ornithorhynchus.

Fig. 18.



Chick 20 days

EXPLANATION OF PLATES IV & V—continued.

FIG. 12.—Single longitudinal section, showing junction of ilium, pubis, and pectineal process; six days' chick; length of hind limb 0·2 in.

FIG. 13.—Pelvic girdle of *Emys europæa* (from Hoffmann).

FIG. 14.— „ *Cyclodus gigas*; right side.

FIG. 15.— „ chick on sixth day of incubation.

FIG. 16.— „ *Laosaurus* (from Marsh).

FIG. 17.— „ *Ornithorhynchus*; right side.

FIG. 18.— „ chick; twentieth day of incubation.

(The shading denotes ossification; the unshaded parts are still cartilaginous.)

The Development of the Mole (*Talpa Europea*).
The Formation of the Germinal Layers, and
Early Development of the Medullary Groove
and Notochord.

By

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With Plates VI, VII, VIII, IX.

IN the following paper I propose to commence with a description of the fully-segmented ovum, leaving the details of the segmentation for a future communication; thence to trace the growth of the blastodermic vesicle and the ultimate formation of the hypoblast, epiblast, and mesoblast of the embryo; to follow the early stages of the development of the medullary canal and notochord; and finally to touch upon the phenomenon of the inversion of the layers in certain mammals, and to endeavour to show that in the mole there exists in development an intermediate condition between the inverted type, of which the guinea-pig is an example, and the normal type as exemplified by the rabbit.

Owing to the difficulty of keeping moles alive and the still greater difficulty of observing their breeding habits when in captivity, I have found it impossible to determine the exact age of any embryos, and am obliged to fix their relative age in accordance with their size, and what appears to me to be the course of their development.

Under these circumstances it will be convenient to divide

embryonic life into periods which I propose shall be regulated by the following conditions:

Stage A. The period of the development of an ovarian into a fully-segmented ovum.

Stage B. The further development of the fully-segmented ovum prior to the formation of mesoblast. That is to say, the stage in which the hypoblast and epiblast are definitely formed.

Stage C. The formation of the mesoblast; and,

Stage D. The formation of the medullary groove and notochord, and the structure of the neurenteric canal.

Stage A.

I have been hitherto unable to satisfy myself as to the details of the process of segmentation in the ovum of the mole, but have been fortunate enough to obtain a fully-segmented ovum. It was found at the upper end of the uterus, and its structure is as follows;

It is formed of a number of distinct cells, each surrounded by a cell-wall, and containing a nucleus. The cells are arranged in two layers (vide fig. 1), which I propose to name (1) the outer layer (*o. l.*), and (2) the inner mass (*i. m.*). The cells of the outer layer are more or less cubical in form, and are placed side by side in a single row. The main portion of each cell is composed of hyaline protoplasm, but along its inner border the protoplasm is finely granulated.

The cells of the inner mass are slightly smaller than those of the outer layer. They are irregularly polygonal in shape, and the protoplasm of which they consist is filled with large and small granules, rendering the cells opaque and dense.

The single row of outer layer cells closely invests and completely surrounds—except at one point—the inner mass of segments. At this point (*bl.* of B.), however, there is a break in the continuity of the outer layer, and here one of the granular cells of the inner mass projects on to the surface.

The ovum is surrounded by a thick membrane, the zona radiata (*z.*), which is in its turn enclosed in an irregular layer of hyaline gelatinous material (*m. c.*) derived from the uterus.

The zona is radially striated, and its outer edge has a granular appearance, which I have reason to believe, from an examination of ovarian ova, is due to the irregularity of its surface caused by the pressure of the follicular epithelium upon it while still in the ovary.

There is no albumen deposited round the ovum during its passage down the Fallopian tube, as is the case with the rabbit's ovum.

The ovum within the zona measures $\cdot 15$ by $\cdot 17$ mm., while the inner mass measures $\cdot 1$ by $\cdot 12$ mm. in diameter; the outer layer being about $\cdot 05$ mm. thick. The zona is $\cdot 01$ mm. and the outer coat $\cdot 014$ mm. in thickness.

The size of segmenting ova vary somewhat, but as a rule, while in the Fallopian tube, they measure between $\cdot 08$ and $\cdot 1$ mm. in diameter. This fully-segmented ovum shows a considerable increase on that size, and this is probably due to the absorption by it of nutritive material present in the uterus. It was examined first of all while fresh, but the details of its structure were rendered more apparent by treatment with silver nitrate. The figure was drawn after treatment.

The structure of the fully-segmented mole's ovum as described above is identical with that of the fully-segmented ovum of the rabbit which van Beneden has described (Nos. 4 and 5). According to this author the result of the first division of the ovum of the rabbit is the formation of two cells, the one of which is smaller and more granular than the other. The product of these two cells can be distinguished from one another throughout the process of segmentation, and Beneden finds the cells derived from the granular segment become involuted within those derived from the larger hyaline segment, and two layers are thus formed which he terms "entoderm" and "ectoderm" respectively, according to what he considers is their respective fate.

Further, the point where the involution took place remains open in the fully-segmented ovum, and gives rise to the gap in the outer layer, which is called by Beneden the "blasto-

pore," and compared by him to the blastopore of other Vertebrata.

The fully-segmented ovum is therefore considered by this author to be comparable to the gastrula stage of other vertebrata.

I have hitherto been unable to confirm the account given by Beneden of the segmentation, but am by no means therefore disposed to conclude his careful descriptions are inaccurate. At the same time it appears to me obvious, from the subsequent development of the mole, that his views of the homologies both of the two layers of segments and of the "blastopore" are incorrect.

In the first place, the so-called "entoderm" segments will be found to give rise to the greater part of the epiblast of the embryo; and in the second place, the structure of the primitive streak will be seen strongly to confirm Balfour's opinions (vide Nos. 1 and 3) of the homology of that organ with the blastopore of lower types.

Had Beneden's interpretations been correct, however, and had the inner mass really been entodermic, the fully-segmented mammalian ovum could not even then be compared to the gastrula condition of *Amphioxus*; for whereas the enteric cavity of the latter is within the entoderm cell mass, that of the former is eventually found to be outside those cells, and between them and the ectoderm.

Up to this point in the development no differentiation of the segmentation spheres into epiblast and hypoblast has yet taken place, and there is indeed, as I will show later, no evidence of any differentiation until some considerable time after the completion of segmentation.

The structure of the mammalian ovum at the close of Stage A is therefore seen to be, as far as we can now tell, entirely unlike that of any other animal; and until we have some knowledge of the steps by which mammals were evolved, it appears to me useless to attempt to draw any homologies.

It may be interesting to note that although the earliest conditions of mammalian development cannot be compared with

those of other animals, yet the further development proceeds (up to a certain point) the more strikingly similar these conditions become, and the usual rule that embryos of various animals differ from one another less in their earlier than in their later stages of development is therefore here reversed.

In concluding this section I would draw attention to the facts treated fully below (1) that the central position of the inner mass of segmentation spheres in both the rabbit and the mole is merely temporary, and that subsequently these cells, with the exception of a very small number, form a portion of the wall of a vesicle, the "blastodermic vesicle."

(2) That the so-called blastopore (Beneden) cannot be similar to the blastopore present in *Amphioxus*, and has merely a secondary origin, its existence being caused by the temporary involution of a portion of the wall of the blastodermic vesicle.

Stage B.

The Blastodermic Vesicle and the Formation of the Hypoblast and Epiblast of the Embryo.

The conversion of the fully-segmented ovum into the so-called blastodermic vesicle takes place shortly after the appearance of the ovum in the uterus. It is due partially to a flattening-out of the cells of the outer layer, and partially to the conversion of certain of the cells of the inner mass into outer layer cells.

The result of these changes is a vesicle the wall of which is composed of, for the most part, a single row of flattened cells, the much attenuated zona radiata surrounding the whole.

In the course of its growth the vesicle becomes so large that the wall of the uterus in the region where it is placed is distinctly swollen.

It is clearly impossible for the delicate-walled ovum to expand in the form of a vesicle, and distend the uterine walls by virtue of the growth of its cells; it must be therefore concluded that it obtains some support. This support is rendered from within.

The vesicle contains a transparent fluid, the nature of which I am only sufficiently conversant with to say that, after treatment with alcohol a white precipitate is present in the vesicle.

It is equally evident that this fluid can only have been obtained from the uterus, and that it is present within the vesicle at a very considerably greater pressure than in the uterus itself. Such a condition is caused by means of the cells of the wall of the vesicle; they secrete the fluid within the vesicle, this function being performed against a pressure which is greater on their inner than on their outer side, exactly as the cells of the salivary glands are known to act.

The uterine fluid is secreted by glands, present in great numbers in the uterine tissue, and is poured through their open mouths into the cavity of the uterus (*vide* fig. 51). There is every probability it has nutritive qualities, since it is thence taken up into the cavity of the embryonic vesicle, which eventually functions as a yolk-sac, in the walls of which embryonic blood-vessels ramify.

A specimen showing an early condition of this change of the segmented ovum into a vesicle has been drawn in optical section in fig. 2. It differs mainly from fig. 1 in that a crescent-shaped cavity (*bl. cav.*) exists between the inner mass and outer layer, this being the cavity of the blastodermic vesicle.

Van Beneden's blastopore has entirely disappeared, and I have no evidence to offer as to the position which it originally occupied; although there is good reason to believe, from a comparison of the development of the rabbit and mole with animals which exhibit the phenomena attending the inversion of the layers, that Beneden's statement is correct, *viz.* that the inner mass remains attached to that side of the outer layer where the gap was originally placed.

The appearance of the cells has altered but little; the outer layer cells are slightly more granular, while the cells of the inner mass are somewhat smaller and less granular than were those of the fully-segmented ovum.

The size of the two ova are different, the specimen from which

fig. 2 was drawn being smaller than the fully-segmented ovum and not larger, as would have been expected.

I can only attribute this condition either to the variation in size of different ova of the same age, of which fact I have abundant evidence, or to the effect of the preserving fluid, although in both instances the objects were treated with silver nitrate and preserved in glycerine.

However that may be—and this is the point which I wish to emphasise—the size of the inner mass in fig. 2 is relatively smaller than that in fig. 1, the diameter of the ovum (fig. 2) being $\cdot 12$ mm., and that of the inner mass $\cdot 06$ mm.

The ovum rapidly enlarges, and in fig. 3 the relation in size of the whole vesicle to the remnant of the inner mass is represented in an early stage of the development of the blastodermic vesicle.

The vesicle in this specimen is $\cdot 31$ mm. and the inner mass $\cdot 04$ mm. in diameter.

This increase in size is due to some extent, without doubt, to the flattening out and multiplication of outer layer cells (vide figs. 16—19); but I believe that up to this point in stage B the cells of the inner mass also contribute to that end.

I have been unable clearly to substantiate this opinion by means of sections, but the size of the inner mass in this specimen bears out my views; it is $\cdot 02$ mm. less in diameter than the inner mass in the specimen figured in fig. 2, and $\cdot 07$ mm. less than the inner mass of the fully-segmented ovum. Further, I have made measurements of a considerable number of specimens of a similar age, and have found this ratio to be almost uniformly constant.

The structure of the wall of the vesicle and of the inner mass at this stage is seen in figs. 16—19.

The vesicle wall is formed of much flattened polygonal cells closely attached to the zona radiata, which bounds them on their outer side.

The cells contain a large nucleus situated in the centre, and causing it to bulge towards the cavity of the vesicle.

The nucleus in section appears to be of oval form, while in

a surface view (vide fig. 4) it is seen to be rounded. The oval shape in section is due to it being flattened out, and it is for this reason also that the nuclei of the outer layer appear in a surface view larger than those of the inner mass (fig. 4).

The inner mass is solid, more or less rounded in form, and is attached on one side to the wall of the vesicle. The cells of which it is made up are always, after treatment with picric acid, closely adherent to one another, and are sharply marked off from the cavity of the vesicle (vide figs. 17 and 18).

The specimen drawn in fig. 19, however, was treated with silver nitrate and preserved in weak glycerine, afterwards being transferred to spirit, embedded, and cut into sections; in it the cells are much more loosely held together, and in another specimen I have, which was similarly preserved, the same appearance presents itself.

The irregularly-rounded cells of the inner mass, which are very considerably smaller than either the cells of the inner mass in the fully-segmented ovum, or of the specimen drawn in fig. 2, are composed of granular protoplasm, and many of their nuclei exhibit the modifications attending cell division.

As the vesicle continues to enlarge the inner mass also now increases in size, changes its shape, and becomes flattened out along the side where it adjoins the outer layer; and further, the cells of which it is now composed become differentiated into two layers.

The differentiation occurs in the following manner:—Certain of the cells bordering the blastodermic cavity become separated off from the main portion of the inner mass, and form a single layer of cells bounding the mass on its inner side.

This layer is the hypoblast.

The hypoblast is, therefore, derived from cells which result from the multiplication of the inner cell mass present in the fully-segmented ovum.

Figs. 20 to 23 adequately represent these changes as they take place; the cells here and there along the lower border of the inner mass become more flattened than their fellows, and stain more deeply with hæmatoxylin (fig. 20); gradually a

continuous layer exhibits these phenomena (fig. 21), and then becomes separated from the remainder of the mass (fig. 22).

The remaining cells of the inner mass increase in number, and assume a columnar form, at the same time becoming separated by a narrow cavity in the centre from the outer layer. The cells of the latter layer in the region of the cavity also increase in number and become thicker than their fellows (fig. 23).

The further development of the hypoblast may be stated in a few words; it extends laterally by virtue of the multiplication of its cells, which at the same time become considerably flattened. Later on, as may be seen in fig. 28, the cells are again more rounded, and, indeed, at different stages during the formation of the layers, they assume various proportions. It is to be noted that this layer, after being once completely separated off from the inner mass (vide fig. 23), remains separate until the mesoblast is formed, and increases, therefore, wholly by the division of its own cells.

The hypoblast eventually completely surrounds the whole of the blastodermic vesicle.

The changes which take place in the remaining portion of the inner mass and in the outer layer adjoining it are somewhat more complicated.

1. The inner mass increases in size and its columnar cells, arranged in a double row, form an hemispherical plate, the edge of which rests upon and is continuous with the cells of the outer layer. In consequence of this the narrow cavity mentioned above assumes considerably greater proportions; it is bounded below and at the sides by the plate, and above by the outer layer; at the same time it becomes partially filled up by branched stellate cells, which are derived from the cells of the outer layer.

This cavity may be termed the secondary cavity of the blastodermic vesicle in contradistinction to the cavity which is formed at the close of stage A, and which also arises, although at the opposite side, between the outer layer and inner mass.

Fig. 5 is a drawing of an ovum at this stage of growth.

The opaque inner mass is seen attached to the wall of the vesicle, and in the centre of the mass a lighter coloured space indicates the presence of the secondary cavity.

The relations of these parts are, however, more clearly seen in fig. 24, which represents a section through the centre of the inner mass of the ovum drawn in fig. 5. The single row of long columnar cells (fig. 23) has given place to a double row of more cubical and broader cells which are continuous with the cells of the outer layer at the circumference of the plate.

The hypoblast lies free below the inner mass and stretches out laterally beyond the area of the latter.

The cells filling up the secondary cavity are stellate, and are connected with both the outer layer and inner mass by means of protoplasmic processes; the size and general appearance of the cells and of their nuclei, however, as well as the manner in which they stain with hæmatoxylin, leaves little room for doubt in my mind that they are derived from the former (outer) layer.

2. The plate of cells now changes its form and becomes flattened out and applied closely to the zona above, the stellate cells within the secondary cavity and the outer layer cells above uniting with it, and the secondary cavity is obliterated. The structure resulting from these changes is the epiblast plate of the embryonic area.

Reference to figs. 7, 25, 26 and 27, will, I think, substantiate this view.

Fig. 7 is a surface view of the inner mass represented in section in fig. 25. The section is cut along the line of the greatest diameter of the mass, and shows the commencement of the process of the flattening out of the plate.

The flattening occurs in the first place along one side, the secondary cavity being there much shallower, while elsewhere it is as deep as before. This arrangement gives rise to the appearance seen in fig. 7, in which the light, crescent-shaped area at one side of the inner mass is the deeper portion of the cavity (compare figs. 7 and 25).

In all the sections of this inner mass only a few cells were to

be seen in the secondary cavity, and in the section here figured there were none present. I have, however, never found this to be the case in any other specimen, and imagine they must have been displaced during the process I then used of cutting and mounting sections.

The flattening process afterwards extends all round the edge of the inner mass and the cavity is throughout much shallowed (fig. 26), cells are present within the secondary cavity, and are seen in this section becoming incorporated with the plate of columnar cells. The edge of the plate, as I before mentioned, is continuous with the cells of the outer layer, and at a slightly later stage, when the plate is completely flattened out, it occupies the position until then held by that portion of the outer layer which overlay the inner mass.

At this stage the two layers are indistinguishable from one another, but wedge-shaped cells can be observed in the upper portion of the plate (fig. 27 *t c*) which on account of their shape, the direction of the long axis of their oval nucleus, and the position they occupy appear to me without doubt to have been derived from the cells of the outer layer.¹

Up to this point in the development the blastodermic vesicle lies free within the cavity of the uterus, and can be obtained therefrom without difficulty by merely slitting up the uterus with scissors and transferring the ovum upon the point of a scalpel to a watch-glass containing the hardening reagent. This method is, however, no longer possible when the ovum attains a very slightly older stage. It then becomes still further enlarged and its walls project into the widely open mouths of the uterine glands. I find no actual attachment between the two, and have not been able to distinguish any outgrowths from the zona such as Bischoff described for the rabbit and dog (Nos. 6 and 7).

The only method which I have found to enable me to obtain the fresh vesicle entire, is to sink the uterus, after being cut open, with the ovum in *sitû*, slowly in a vessel of salt so-

In support of this view see below, an account of a stage in the formation of the epiblast of the rabbit.

lution, it then becomes possible to separate the two without damage.

Figs. 8 and 9 represent very faithfully the appearance of ova of this age obtained by the above method.

The wall of the vesicle is bulged out here and there into papillæ where it projected into the mouths of the glands. The elongated condition is due to the fact that when the uterus was slit open the vesicle fell into that shape in which it received the greatest amount of support from the surrounding tissue.

If it is not desired to examine the vesicle in a fresh state it will be found advantageous to harden the embryo within the uterus, and to dissect it out afterwards which is an easy matter.

In order to show the position and condition of the uterine glands, I have drawn in fig. 51 a transverse section through that region of the uterus from which the ovum represented in fig. 8 was obtained.

It will be seen that only on the free non-mesometric side of the uterus are there any widely open mouths of glands; while upon reference to figs. 8 and 9, only that side of the ovum around the embryonic area is seen to be prolonged into papillæ-form projections, and as the embryonic area lies against the non-mesometric side of the uterus in the mole we may conclude the projections lie in the mouths of the glands. A portion of the epithelium of the uterus abstracted from the latter is drawn in fig. 52; it is seen to be prolonged into hollow finger-like processes which line the uterine glands.

Transverse sections of the embryonic area of this embryo (fig. 28) show that it is formed throughout of two layers of cells, epiblast and hypoblast.

One of the prolongations of the vesicle wall has been cut at one side of the embryonic area (*pl*) and another is shown in fig. 29. They are seen to be formed wholly of epiblast, the hypoblast not being extended into them.

Of the hypoblast layer in the area I have nothing to add to the account already given; the epiblast, however, has under-

gone a slight change since we last examined it, inasmuch as it now consists for the most part of a single row of columnar cells, which at the sides of the area gradually become less and less columnar and eventually merge into the flattened epiblast cells of the wall of the vesicle. This change is, however, temporary, since in sections of older embryonic areas the epiblast is again two layers deep (figs. 33, &c.).

Fig. 30 is a transverse section of the area drawn in fig. 10; it is very similar to fig. 28; but the edges of the embryonic area in this case appear to end abruptly, the wall of the vesicle having been torn away owing to its close attachment to the uterus.

The condition of the ovum is now considerably changed from what it was when the blastodermic cavity first appeared; it may be divided into two areas, the embryonic and non-embryonic areas. The embryonic area is throughout composed of an outer thickened layer of columnar epiblast cells which has been derived partially from a portion of the inner mass and partially from outer layer cells, and an inner layer of somewhat rounded hypoblast cells derived entirely from cells of the inner mass.

The non-embryonic portion of the ovum may in its turn be divided into two regions. First, the region immediately surrounding the embryonic area which is formed of two layers, an outer of flattened outer layer cells now known as epiblast cells, continuous with the epiblast of the area, and an inner of flattened hypoblast cells continuous with the same layer in the embryonic portion of the ovum.

Secondly, the region situated at the opposite pole of the ovum to the embryonic area, where a single row of flat epiblast alone exists.

Historical.—The details attending the formation of the epiblast has given rise to a considerable amount of discussion. According to Edward van Beneden (No. 5) the fully-segmented ovum of the rabbit develops into the blastodermic vesicle by a multiplication and a flattening of the outer layer cells, the inner

mass remaining the same in size, and attached to the outer layer in the region of the now-closed "blastopore." Subsequently the inner mass flattens out and splits up into two layers, the lower of which forms the hypoblast and the upper the mesoblast of the embryonic area, the epiblast being formed solely by the multiplication of the outer layer cells, which become at the same time columnar and arranged in a single row.

Beneden, therefore, has not found a stage in which two layers only exist throughout the area.

Rauber (No. 21), in a previously written paper, finds three layers present in the embryonic area of a rabbit before the formation of the primitive streak; the outer of these (my outer layer) he calls the "Deckschicht," and states that it early disappears, while the middle layer alone forms the epiblast and the lower the hypoblast of the area; a two-layered area being thus formed.

Kölliker, in a recent elaborate paper (No. 16), traces the fate of the three layers, which he also finds in common with Rauber and Beneden, and declares, in accordance with the views of the former author, that the outer layer gradually disappears, the middle forming the epiblast and the lower the hypoblast of the embryo. The details of the gradual disappearance of the Deckschicht occupy much of this paper. Professor Kölliker has never seen the cells of this layer assume a columnar form, as Beneden asserts is the case, and by means of nitrate of silver staining he satisfies himself they gradually become broken up, and eventually disappear altogether.

Lieberkühn (No. 19) gives an account of the formation of the epiblast in the dog and mole which is very similar to my own, in that he considers it is formed of the greater portion of the inner mass, together with that portion of the outer layer cells which originally overlaid it. He also draws attention to the cavity which appears, according to him, within the inner mass of cells in the mole, and which he suggests may be comparable to the segmentation cavity of other animals.

Hensen (No. 12) for the rabbit, and Schäfer (No. 25) for the

cat, also describe a two-layered stage of the embryonic area prior to the formation of the primitive streak.

Summary.—With regard to my own work I hold that the blastodermic vesicle increases in size, not merely on account of the increase in number and the flattening of the outer layer cells, as Beneden believes, but by the migration of inner mass cells to the exterior. This view is supported by the fact that the inner mass decreases in size during the early development of the vesicle. I have also satisfied myself of the existence, both in the mole and rabbit, of a stage in which the embryonic area is composed of only two layers, the epiblast and hypoblast.

The hypoblast I have shown to be derived from the cells of the inner mass—a fact which all the observers above mentioned are agreed upon.

The epiblast I believe to be formed, as does Lieberkühn, of the remaining portion of the inner mass, after the hypoblast has been detached, together with that portion of the outer layer which overlies the inner mass.

In the mole this includes also certain cells which we have seen are derived from the outer layer, and which at one time lie in a cavity between that layer and the inner mass.¹ In the rabbit, however, no such cells exist, and I believe that the epiblast is formed of inner and outer layer cells.

With reference to the development of the epiblast in the rabbit I may say that since working at the question under the supervision of the late Professor Balfour (No. 3), I have examined more embryos, and have been fortunate enough to obtain good sections of the embryonic area of a rabbit embryo of six days four hours old, which appear to me to be conclusively in favour of the view we were then inclined to accept. Fig. 49 represents a section through this area; in it the epiblast plate is seen to be composed of two entirely different kinds of cells—(1) a lower more or less columnar or rounded cell, and (2) an upper flattened or wedge-shaped cell. The latter cells invariably occupy a position on the outer side of the plate, across

¹ In a previous paper (No. 11) I erroneously described these cells as being derived from the inner mass.

which they form an almost continuous layer, and they are distinctly darker stained than are the deeper placed, more columnar cells. They are generally wide at the top, ending below in a wedge-shaped base, which grows downwards between two of the columnar cells lying beneath. Some of the cells are, however, more flattened, possessing no downward prolongation, and some are more columnar, having little or no expanded upper surface; indeed, there are cells in all stages of transition, between the flattened outer layer cells of the previous stage and the columnar cells of the future epiblast plate (vide fig. 49, *t. c.*).

Kölliker's valuable paper contains most careful descriptions and drawings, which, however, appear to me to be capable of a very different interpretation from that put forward by him; in fact, they appear to me to be strongly confirmatory of my own views. He states that the large nucleated plates which are visible in surface views of young areas split up in older embryos into small polygonal areas without nuclei. Now, I would venture to suggest that the disappearance of the nuclei of these large outer layer plates can be fully accounted for by their migration downwards among the cells of the inner mass (vide fig. 49): and the apparent breaking up of the large cells may be explained by the actual appearance on the surface of the epiblast plate, of the polygonal ends of the columnar cells of which it is now composed.

Stage C.

The Formation of the Mesoblast.

The middle germinal layer has two distinct sources: in the first place it arises from the epiblast and hypoblast at the hind end of the embryonic area, in the structure known as the primitive streak; and, secondly, from the hypoblast alone in the anterior region of the area in front of the primitive streak.

The Primitive Streak Mesoblast.

The primitive streak originally appears at the hind end of an area similar to the one represented in fig. 10, its presence being shown in surface view by a slight opacity.

Fig. 31 is a longitudinal section through such an area, along the middle line. The anterior portion of the area consists of a layer of columnar epiblast, and a somewhat flattened layer of hypoblast: at the hind end, however, a passage perforates the blastoderm and surrounding it the epiblast and hypoblast become continuous with one another, forming the wall of the perforation. The opening is wider below than above, and owing, I believe, to the curved condition of this specimen, was not visible from the surface. The whole length of the area is not drawn in the figure, and the portion anterior to the spot at which the reference letters *ep.* are placed, was bent back, and underlay the hinder portion of the area.

The cells forming the wall of the passage give rise to the first mesoblast cells, which are thus derived from epiblast and hypoblast conjointly; they extend in front and laterally for a short distance only as a thin sheet lying free between the two primary layers, while posteriorly they form a thicker layer and are united with the epiblast in the middle line.

From this point the primitive streak extends backwards, the embryonic area itself enlarging in that direction.

Figs. 11 and 12 are surface views of two areas, in which the primitive streak represented by the dark shading is well defined. In the former, which is the younger of the two, the opaque band extends about half way across the oval area, spreading out behind into two short horns; and down the centre of the band a lighter streak may be seen, which is caused by a groove in the epiblast, and is the well-known primitive groove.

At the front end of the primitive groove there is distinct evidence in section of the involution of the epiblast, although no actual perforation of the blastoderm exists. This I consider

is the point where in the earlier specimen the blastoderm was perforated (fig. 31), the increased size of the area being due hitherto to a growth backwards.

Fig. 12 represents the most advanced condition of the primitive streak. The embryonic area is pyriform, and the primitive streak is considerably longer than in the former specimen (fig. 11), and extends relatively further along the area; it is more opaque, and ends behind in a dark rounded mass or knob.

I could distinguish no primitive groove by an examination of the surface, and am obliged, therefore, to rely chiefly upon sections to determine the relations of the growth. Near the front end of the streak there is here also distinct evidence of an involution of the epiblast, although there is no actual perforation; and I am inclined to believe this point is identical, both with the front end of the primitive streak in fig. 11, and with the point where the perforation exists in the younger embryo (vide fig. 31). It is a curious fact, however, that the extent of the area anterior to the front end of the primitive streak appears to be less in this area than in the younger one (fig. 11), while the length of the primitive streak in fig. 12 is greater than that in the older embryos (figs. 13—14).

The presence of the involuted point at the front end of the streak appears to me to favour the view that this structure has not grown forwards, while the addition of the pyriform hind end is an argument in favour of its backward growth.

The reduction in size of that portion of the area anterior to the primitive streak may possibly be due to curvature, but this I am unable definitely to decide.

The eventual reduction in length of the primitive streak is more easily comprehensible, and is doubtless due to the widening out of the end knob, this structure having disappeared in older embryos.

I have frequently observed in surface views a darkly-shaded spot at the front end of the primitive streak, which is spoken of as the node of Hensen, and find that it corresponds with the spot where the three layers unite. It may also be seen some-

times when there is no other superficial evidence of the existence of the primitive streak, but in these cases I have invariably found by sections that a primitive streak does exist, but that the mesoblast to which it has given rise is so uniformly distributed everywhere except at the front end, that it is only there apparent.

The structure of the primitive streak is different in different parts, to illustrate which I have figured sections (figs. 33—36) through various regions of the blastoderm drawn in fig. 12.

The first section (fig. 33) is taken through the anterior portion of the primitive streak. A plate of columnar epiblast cells extends across the area; it is thinner at each edge, but of uniform thickness elsewhere, except in the middle line, where a keel-like ridge is formed. The upper half of the keel is wide and joins the epiblast, with the cells of which it is continuous, and the lower portion projects into a mass of cells below, but has no connection with them. These underlying cells I will deal with later, and will in this place merely draw attention to the fact that the lower borders of the cells of the keel are sharply marked off from them, and that these somewhat oval cells lying below the keel of epiblast are entirely different, both in shape and character, from the cells above them.

The second section is taken close behind the first; it passes through the front end of the primitive groove, and is, I believe, in an analogous position to the point immediately behind the perforation existing in the embryo, of which fig. 31 is a longitudinal section.

The epiblast is curved in the middle line constituting the primitive groove, and from the cells of this portion of the epiblast, mesoblast is produced.

Immediately below the primitive groove there is no layer of hypoblast to be distinguished, and here mesoblast is produced from hypoblast cells. Laterally all three layers are distinct, but in the middle line they may be said to combine with one another, and in this region, therefore, the middle layer is formed from both epiblast and hypoblast. The former does not here extend beyond the boundary of the embryonic area.

Between the two sections described above, the cord of cells (fig. 33) joins the front end of the mass of cells formed by the union of the epiblast and hypoblast in the middle line; and where this junction occurs there is distinct evidence of an involution of the epiblast layer.

From the front end of the primitive streak a tongue-shaped cord of mesoblast cells is projected forwards into the mass of cells underlying the epiblast in that region, and gives rise to the lighter shaded prolongation of the primitive streak seen in fig. 12.

I have been unable to find any complete perforation of the blastoderm at this stage of growth, although in a somewhat younger embryo and in an older one in which the medullary groove is formed, there is no doubt that it exists. I have, therefore, either missed the section in which the perforation occurred in this embryo, or it has been closed up by the rapid production of mesoblast which at this stage takes place.

Although I have only seen a complete perforation of the blastoderm in one embryo during the primitive streak stage, I have invariably found at the front end of the primitive streak evidence of an involution of the epiblast; on this account, as well as for reasons which will appear in the sequel, I conclude the front end of the primitive groove is the spot where the perforation of the blastoderm seen in fig. 31 occurs at an earlier and later stage. The cord of cells described in the first section is the front wall of the perforation seen in fig. 31, and the tongue of mesoblast projecting forwards is homologous with the anterior growth of mesoblast also seen in the younger embryo.

This statement is supported by a study of sections of an area but slightly older than that drawn in fig. 11, and somewhat younger than the one we have been considering. In this area there was no layer of cells underlying the epiblast at the anterior end of the primitive streak, and the behaviour of the forward growth of mesoblast from the front end of that structure could be more definitely determined. At the front

end of the primitive streak, at a point relatively similar to that drawn in section in fig. 34, the epiblast was involuted in the middle line and a deep pit formed which opened below into mesoblast, which is budded off from the lips of the ingrowth. At this point the epiblast, mesoblast, and hypoblast were united in the middle line, but in front of it an axial rod of mesoblast projected forwards for a short distance distinct from both epiblast and hypoblast, but soon becoming attached to, and indistinguishable from, the hypoblast. In this condition it may be spoken of as a thickened axial rod of hypoblast, and as such it extends forwards for some sections, gradually becoming reduced in size and eventually giving place to the single row of rounded hypoblast which elsewhere existed below the epiblast in front of the primitive streak.

The third section (fig. 35) demonstrates the structure of the area throughout the remainder of the primitive streak in front of the end knob. It is similar to fig. 34 except that (1) the hypoblast forms a complete layer across the whole of the area, and is nowhere combined with the layer of mesoblast; (2) the primitive groove is not present, and the number of epiblast cells concerned in the formation of mesoblast is greater than before, and (3) the mesoblast extends laterally, lying freely between the epiblast and hypoblast, outside the limits of the area. This is a typical section through the middle of the primitive streak of all the specimens I have examined, and it may be generally stated that throughout this region the epiblast only gives rise to mesoblast.

In the knob at the hind end of the primitive streak (fig. 36) the three layers are again seen to be closely combined, the hypoblast being indistinguishable from the mesoblast, and the epiblast throughout nearly the whole breadth of the area giving rise to mesoblast cells; there is also a much greater mass of the latter layer extending some distance beyond the limits of the area, which in this region is very narrow.

The junction of hypoblast and mesoblast does not appear to occur in this region in all specimens, although the embryo

from which this section was taken is not singular in exhibiting such a relation between the two.

The Hypoblastic Mesoblast.

A single layer of rounded hypoblast cells similar to those represented in section in fig. 30 is present throughout the lighter shaded anterior portion of the area drawn in fig. 11. At a somewhat later stage, however, these rounded cells in the region on each side of the thickened axial hypoblast, in front of the primitive streak, give rise to cells from which they are themselves indistinguishable; gradually the hypoblast situated anteriorly follows suit, and eventually the whole of that portion of the area in front of the primitive streak consists of a plate of epiblast below which lies a mass of cells several layers deep. These cells are rounded and appear throughout as do the lateral masses of cells below the epiblast in fig. 33.

Fig. 32 represents a section through the anterior region of the area drawn in fig. 12, a glance at which will, I think, prove the origin of these cells from the hypoblast.

It appears to me that the continuity of the intermediate layer with either of the primary layers is a safe guide as to the origin of the former—by continuity, I mean such relations as are shown at the node of Henson (fig. 34), where the boundaries of the three layers cannot be distinguished;—and if this be true I imagine there can be little doubt as to the origin of the mass of cells above described.

At a later period of development these cells become split up laterally into two layers, a lower single layer of flattened hypoblast and an upper layer of mesoblast several rows deep. This differentiation takes place from behind forwards, as does the original formation of this layer. These relations are seen by comparing figs. 32 and 33; in the former there is no trace of a separation of the cells into hypoblast and mesoblast, while further back (fig. 33), several cells (*hy*) along the lower border of the mass are more flattened, their nuclei more elongated, and they stain more deeply with hæmatoxylin than do the remainder of the cells

these become hypoblast cells. In the axial line no such change occurs, and the mass of cells existing there is continuous behind (by means of the axial rod described on p. 430) with the front end of the primitive streak, and continuous laterally with both the hypoblast and the mesoblast. Fig. 42, although it is a section through a considerably older embryo, represents these relations fairly accurately.

The axial mass of cells eventually gives rise to the notochord. The lateral mesoblast may be called hypoblastic mesoblast in accordance with its origin, and to distinguish it from the mesoblast of the primitive streak. The lateral masses of hypoblastic mesoblast adjoin posteriorly the mesoblast of the primitive streak, and it does not appear to me to be possible, with the existing methods of discrimination, to determine the exact extent of either layer; roughly, however, we may say that the front end of the primitive streak is the boundary line.

At the stage of development now reached the embryo may be compared with that of *Amphioxus*, as far as its structure is concerned in front of the primitive streak; two masses of mesoblast are formed from the hypoblast laterally and the axial hypoblast thickens and gives rise to the notochord. The latter is similar to the median diverticulum of the enteric cavity of *Amphioxus*, and the lateral masses of mesoblast to the mesoblast of the united diverticula on each side in that animal; the lateral diverticula do not, however, appear, but the median one is, as we shall see, formed later.

With regard to the embryonic vesicle it is much larger than in the previous stage, and no longer projects into the mouths of the uterine glands, but is exceedingly closely applied to the uterine epithelium, so closely that some of the latter is generally pulled away from the uterus when the ovum is obtained whole. Fig. 53 is a section of a portion of the vesicle wall which is formed of flattened epiblast only, and of the uterine epithelium to which it is closely adherent.

Historical.—Various accounts have been given by different observers as to the origin of mesoblast in mammalian embryos.

Beneden (No. 5) describes that portion of the inner mass which remains after the hypoblast is separated from it, as mesoblast, and states that it retreats to the hinder end of the embryonic area, becomes secondarily united with the epiblast, and gives rise to the mesoblast of the embryo.

Rauber (No. 21), Kölliker (No. 16), Hensen (No. 12), and Lieberkühn (No. 19), argue that the mesoblast arises first in the primitive streak. Kölliker considers that the epiblast alone gives rise to it, and that after being formed in the primitive streak it spreads, eventually, over the whole embryonic area, and also supplies the mesoblast of the area opaca. From this author's statement I gather he considers the primitive streak arises first in the end knob (Endwuldst), and extends from thence forwards.

Lieberkühn differs from Kölliker, and agrees with Hensen, in that he derives the mesoblast of the primitive streak from both epiblast and hypoblast; while Hensen differs from the other observers mentioned, in considering a certain amount of the mesoblast of the area opaca to be formed in situ from hypoblast.

Summary.—My own observations lead me to differ entirely from Beneden as to the formation of mesoblast, and to agree with the other observers mentioned above in concluding that it is first formed in the primitive streak. I cannot, however, accept Kölliker's statement, that the epiblast is alone responsible for its production, and that it is first formed in the hind knob. I consider that the hind knob is formed some time after the first portion of the primitive streak, and that the formation of the latter takes place from before backwards instead of from behind forwards, as this observer states; my main reason for this being, the universal presence at the anterior end of the primitive streak of an indication of the involution of the epiblast.

Again, I agree most distinctly with Hensen and Lieberkühn in regarding the epiblast and hypoblast as the originators of mesoblast at the front end of the primitive streak, but I must differ from them, and from Kölliker (*loc. cit.*) and Schäfer

(No. 26), in believing that the primitive streak mesoblast supplies the whole of the embryonic area.

With regard to this point my results are in entire agreement with those of Balfour and Deighton, expressed in their account of the development of the chick (No. 2), who consider that the anterior portion of the mesoblast is derived as two lateral plates from the hypoblast, while the axial hypoblast gives rise to the notochord.

Further, the similarity of the origin of the epiblast, hypoblast, and mesoblast of the embryo and of the notochord in the mammal is so strikingly similar to the relations of the same organs in *Amphioxus* that Kölliker's (*loc. cit.*) statements as to the dissimilarity of the germinal layers of mammals with those of other animals appears to me to require some modification; and Repiachoff's recently expressed opinions (No. 24) that there is no homology between the germinal layers of higher *Vertebrata* and *Amphioxus*, receives no support from what is known of mammalian embryology.

Finally, it is very generally believed that mammals are descended from animals which possessed a large yolk sac, and it is stated that the blastodermic vesicle is a remnant of this yolk sac. If this be true (and as far as we know there seems to me to be no reason to doubt it), the primitive streak of mammals is homologous with the same structure in birds, and the existence of such an arrangement, together with the presence of a complete neurenteric canal (which I shall describe later) in the mammal, is another instance of the morphological facts which led Balfour (No. 1) to conclude that the primitive streak was homologous with the true vertebrate blastopore.

The views as to the relations of the layers at the front end of the primitive streak will be more advantageously noticed in the following section.

Stage D.

The Medullary Groove, Notochord, and Neurenteric Canal.

The main differences in the superficial appearance in an embryo of this stage of growth are :

1. The disappearance of the hind knob of the primitive streak and the widening out of that portion of the area.
2. The great enlargement of the area in front of the primitive streak, and
3. The appearance in the latter portion of the area of a broad, light-coloured band, the limits of which are at first vague, but which gradually become more emphasised.

This is the medullary groove which first arises near the anterior end of the primitive streak, and from there extends forwards.

Fig. 13 represents an embryonic area, in which a shallow medullary groove is formed ; the extent of the groove is faintly indicated in front ; at the sides it is more definitely marked off ; while behind it abuts upon the anterior end of the primitive streak, and terminates abruptly.

At the junction of the medullary groove and primitive streak a deep pit is visible ; this is the dorsal opening of the neurenteric passage, which, however, does not appear completely to perforate the blastoderm at this stage.

The primitive streak extends from the hind end of the medullary groove to the edge of the blastoderm, spreading out there into two horns.

In fig. 14 the medullary groove is more distinctly indicated ; a tongue-shaped band extends anteriorly from the front end of the groove towards the edge of the blastoderm in that direction, and is the anterior end of the thickened axial mass of cells underlying the epiblast.

The primitive streak meets the opposite end of the medullary groove, and sends a forward prolongation between its divergent walls.

There was no indication, as far as I could see from the surface, of a dorsal opening to a neurenteric canal in this embryo. The growth from the front end of the primitive streak is similar to what has already been noticed in younger embryos (fig. 31 and p. 48).

Fig. 15 is a drawing of the hind end of the medullary groove of a still older embryo. Here the walls of the hind end of the groove, hitherto widely separate, have joined each other, and have enclosed within the groove the front end of the primitive streak, and with it the hinder dorsal opening of the neurenteric passage. From the front end of the primitive streak a prolongation is sent forward similar to that seen in fig. 14.

The walls of the groove are now distinct.

The structure of the groove of such an embryo as that drawn in fig. 13 is represented in transverse section in fig. 43. The plate of epiblast is thin where it is grooved, on each side, however, becoming about double the thickness, and then gradually thinning off until it is only a single layer deep at the edge of the area; here it is curved upwards, and thus indicates the commencement of the amnion.

The cells underlying the epiblast in this region are divided in the same manner as we have seen are those of a much younger embryo (stage c, p. 51), into (1) lateral plates of mesoblast and hypoblast, and (2) an axial mass of cells, the commencing notochord showing no differentiation into those layers.

It will be noticed, however, that the axial cells are considerably more isolated from the lateral masses than heretofore, although still continuous with the latter. The lateral mesoblast is thick, and at the edge of the area becomes divided into two layers, which are the future somatic and splanchnic mesoblasts.

These relations remain the same throughout the medullary groove, excepting that at the posterior end the axial notochordal cells become thicker and join a forward growth from the front end of the primitive streak, while at the anterior end the groove widens, and all the cells underlying the epiblast

come into closer relations with one another. Fig. 42 represents the latter condition in a somewhat similar embryo.

Anterior to the medullary groove the lateral hypoblast and mesoblast are not yet separated, and a continuous mass of undifferentiated cells underlies the epiblast plate.

Behind the groove the primitive streak occasions changes identical with those already described (p. 55).

The mesoblast throughout the embryo projects beyond the limits of the area, and is there split into somatic and splanchnic layers. The relations of the neurenteric canal I will describe in detail in another place (on p. 57); for this specimen I will only say that at the junction of the primitive streak and medullary groove a deep pit is formed by the involution of the epiblast in the middle line; the pit is widely open above, but enters a mass of mesoblast below, and is there, as far as I could see, entirely obliterated.

The groove now deepens, forcing the notochordal cells underlying it further downwards, and in this way the latter, while remaining connected with the hypoblast, becomes separated from the lateral masses of mesoblast. Such relations are shown in fig. 44, which is a transverse section through the medullary groove of an embryo slightly older than fig. 13, taken from the same relative position as the section in fig. 43.

This is, however, the deepest portion of the medullary groove, and only in this section and those immediately on each side of it do the relations hold which are here figured. Both anteriorly and posteriorly the groove is more shallow, and the axial hypoblast is continuous with both lateral mesoblast and hypoblast.

The structure of the remainder of the embryo is identical with that described above for fig. 13.

The amnion in this embryo is completely formed over the hind end of the primitive streak, although not so far advanced at the front end of the area.

In describing the next embryo I will give an account of the structure of the neurenteric canal.

The arrangement of the layers at the front end of the primi-

tive streak and the structure of the neurenteric canal at this stage of growth will readily be understood by a glance at the drawing of the surface view of an embryo (fig. 15), and comparing it with the diagrammatic longitudinal section in fig. 50 and the transverse sections in figs. 37 to 41. The latter are taken from an embryo of an age between that of fig. 13 and fig. 14, and the walls of the medullary canal do not yet enclose the front end of the primitive streak, although the latter is already placed between them.

The longitudinal section is taken from a younger embryo.

The dorsal hinder opening of the neurenteric canal (figs. 15 and 37) is formed by an involution of the epiblast in the middle line at the head end of the primitive streak, and the separation of the lips of the involuted layer.

The passage so formed enters the mass of mesoblast cells, budded off from epiblast and hypoblast in this region, as it does in the embryo of which fig. 13 is a drawing; but it does not end there, it travels forwards almost parallel to the plane of the layers, and is seen eight sections further forward (fig. 38) as a canal within the axial mass of mesoblast, which we have invariably seen to be projected anteriorly from the front end of the primitive streak. There is no doubt the cells surrounding the canal at this point are mesoblast cells; they are continuous with the epiblast in the middle line and with the lateral mesoblast, and there is a distinct layer of hypoblast below them (compare fig. 38, and fig. 50 immediately in front of *p. sk'*); gradually, however, the canal dips downwards, and as this prolonged cord of mesoblast joins anteriorly the axial hypoblast, the walls of the canal also there, some sixteen sections in front of fig. 37, become hypoblastic (vide fig. 39). Here the lateral mesoblast does not join the thickened axial hypoblast, which is continuous with the lateral hypoblast only.

Three sections further on the axial cells become continuous with both lateral mesoblast and hypoblast. The lower wall of the canal now shows signs of becoming thinner (fig. 40), and five sections beyond this, that is, twenty-four sections from the hind opening, it becomes divided in the median line, and the

neurenteric canal opens below to the cavity of the vesicle (fig. 41). The arrangement of the layers at the front end of the primitive streak may be shortly described, therefore, as follows:—The epiblast and hypoblast meet and form the hind wall of the dorsal opening of the neurenteric canal; from the front portion of this wall a tongue of mesoblast is projected forwards, separated from the underlying hypoblast, but united with the lateral mesoblast and with the epiblast in the middle line; it then joins the thickened axial hypoblast, and becomes freed from the lateral mesoblast (fig. 39), while anteriorly to this point the axial cells are continuous with both lateral hypoblast and mesoblast.

With regard to the structure of the remainder of the embryo, the medullary groove is shallow and wide, and throughout its length the axial hypoblast causes a swelling upwards of the bottom of the groove. The lateral hypoblast and mesoblast join the notochordal cells throughout the region where the latter exist.

The notochord is formed of cubical or columnar cells, and is alternately in the form of an arch and a tube throughout the whole length of the medullary groove (vide figs. 40 and 41). Beyond the groove it becomes more flattened out (fig. 42), and the arrangement there is similar to that described for fig. 13.

This tubular form of the notochord appears to be very transitory, as I have not met with it in any other embryo except in that drawn in fig. 14, in which it is not either so definite or so continuous.

The sections through the area represented in fig. 14 show a slightly different arrangement. Fig. 47 is taken through nearly the same region as are the sections from which figs. 43 and 44 were drawn. The medullary groove is much the same as is represented in fig. 43, but the notochord is less substantial, and the single row of somewhat cubical cells of which it is composed form an arch whose cavity opens into that of the vesicle below. It will be obvious that the thickness of the notochordal cells is much less than in fig. 41, and that the arch

is not so completely tubular. The lateral mesoblast is not continuous with the axial hypoblast (notochordal) cells in the middle and deeper portion of the groove, but such is the case both further forwards and backwards.

Immediately beyond the anterior end of the groove the flattened epiblast is thickened to form the medullary plate, and below it the arched notochordal cells form a complete tube, the structure of which is similar to that already described excepting that the lower wall of the tube is thicker.

Fig. 46 is a section through this region of fig. 14, the dark streak seen in surface view being accounted for by this thickened mass of notochordal cells; it is the only portion of the notochord in the anterior region which remains thickened at this stage of growth. The relations of the layers at the hind end of the area are the same as are described on p. 58.

At a stage slightly older than that represented in fig. 14 the medullary groove is still deeper. A section (fig. 45) taken through about the same region as are those drawn in figs. 43 and 44 demonstrates this. The epiblast now exhibits a further change, that portion of it forming the walls of the groove are for the most part but slightly thicker than heretofore, but immediately on each side it becomes suddenly considerably thicker, and then gradually becomes thinner again towards the boundary of the area, where it is turned up to form the commencing amnion.

The mesoblast is here completely separated from the axial cells, being rounded off at the sides bordering the medullary groove, and at the edges it is split to form splanchnic and somatic layers. The hypoblast is continuous across the area, the axial portion exhibiting no increase in thickness to that situated laterally; the former being forced by the deep medullary groove into a bow projecting into the vesicle below.

In front of the point from which the section is taken, the groove first becomes narrower, and then more shallow and wider, the notochordal cells becoming at the same time thicker and continuous with the lateral mesoblast. In front of the groove the relations are similar to what were described for fig.

14 (p. 59), excepting that the lateral cells below the epiblast are not in such numbers as before, and the axial cells, instead of being in the form of a tube, as in fig. 46, are only one row deep, and are arranged as an arc, the bay of which opens below (vide fig. 48).

Behind the section (fig. 45) the groove also becomes shallower and the notochordal cells thicker, terminating, as in former specimens, in the front end of the primitive streak; the latter is, however, now cut off from the remainder of the streak, and lies within the medullary groove, into which the dorsal pore of the neurenteric canal now opens.

The section of the groove in fig. 45 exhibits evidence in both epiblast and hypoblast of an advanced growth on those drawn in either figs. 43, 44, or 47, although the measurements of the area are almost exactly similar to the one represented in fig. 14, from which fig. 47 was taken.

I do not propose to trace the development beyond this point, but may briefly say, that after the stage just described the medullary groove becomes much deeper, the epiblast of its wall being thick, while the epiblast over the lateral portions of the embryo is composed of only one layer of cubical cells. The groove deepens first about the hinder portion of the anterior third of the groove, and from there extends backwards and forwards.

The further development of the notochord takes place in the same direction. The flattened notochordal cells seen in fig. 45 become slightly more rounded as the lateral hypoblast and mesoblast sink to the level of the bottom of the groove, and then the lateral hypoblast grows inwards, and a small bunch of cells are isolated in the middle line and lie between it and the now closed neural canal.

The amnion is first formed, as I have stated, over the hind end of the primitive streak, and from there grows forwards a considerable distance before the head is covered by the anterior fold.

To recapitulate, we may conclude it is probable that the region of the area in which the main portion of the

embryo is formed is derived by a forward growth. The hind knob of the primitive streak is lost, and the pyriform hind end of the area becomes shortened and widened. The medullary groove appears first as a wide shallow groove in the region adjoining the head of the primitive streak, from which point it extends forwards.

The changes undergone by the axial hypoblast are somewhat complicated. At first the cells of which it is composed are numerous, they then become fewer, and are arranged first as a flattened then as an arched plate, which may or may not be completely closed in to form a tube. Later on the arched plate becomes flattened out again by the deepening of the groove, and the notochord is represented by a thin layer of flattened cells which, as the lateral mesoblast and hypoblast sink down to a level with the bottom of the groove, become again more cubical in form. Eventually the lateral hypoblast grows in from the sides and the axial cells are separated off as a notochord.

The isolation of the axial cells from the lateral mesoblast takes place, as does the separation of the notochord from the hypoblast, from about the middle of the embryo backwards and forwards.

The same may be said for the medullary groove, which is first formed from behind forwards; its conversion into a canal takes place from about the middle towards the hind and front ends.

The neurenteric canal is complete, opening at first dorsally at the head end of the primitive streak, and between the latter and the medullary groove, but eventually becoming enclosed within the groove and opening at the bottom of its hinder end.

The canal travels forwards in an anterior growth of mesoblast from the head of the primitive streak, and enters a thickened axial mass of hypoblast, from which it opens downwards to the cavity of the vesicle.

The amnion is first formed over the hind end of the embryo, and only at a considerably later period envelops the front end by a separate formation.

Historical.—The arrangement of the layers at the front end of the primitive streak has been described by Hensen (No. 12), Schäfer (No. 26), and recently by Lieberkühn (No. 20).

According to the former, the axial cells below the medullary groove at its posterior end are thickened and join the primitive streak at the node of Hensen, this portion of the primitive streak being composed of epi-, hypo-, and mesoblast fused together.

Schäfer describes a similar arrangement in a somewhat different manner. According to him the axis of the embryo in this region is “occupied by a continuous column of cells, which inseparably connect the epiblast and hypoblast, and, traced from behind forwards, would appear to be chiefly epiblastic in origin.”

This author does not appear to believe that the hypoblast takes part in the formation of the primitive streak, and he therefore considers, I imagine, that the latter organ begins where the hypoblast lies free below the mesoblast.

Neither of these observers described any canal perforating the blastoderm at this point.

Balfour, however, in his ‘Comparative Embryology’ (No. 3), has expressed his belief that the axial cord of cells described by Schäfer is the rudiment of the neurenteric canal of Lacertilia and birds.

Lieberkühn agrees with Hensen as to the arrangement of the layers at the front end of the primitive streak, and further finds a canal present in the mesoblast, which grows forwards from the front end of the “node.” He states that the canal arises in the mesoblast, and does not open dorsally through the epiblast, but that it is prolonged forwards, and opens below through the hypoblast.

The notochord he believes to be formed from mesoblast, which secondarily becomes united with the hypoblast. This author also compares the neurenteric canal, such as he finds in mammals, with that of birds and lizards, and declares they are essentially different, inasmuch as in the latter the canal arises

as an inpushing of the epiblast, and connects the neural tube with the gastric cavity.

Kölliker (No. 17) agrees with Lieberkühn as to the mesoblastic origin of the notochord.

Summary.—My own work indicates that a complete neurenteric canal is formed similar to that in birds and lizards, first of all by an inpushing of the epiblast; secondly, the canal is conducted to the hypoblast within a tongue of mesoblast, which grows from the anterior end of the primitive streak; thirdly, the canal enters the axial hypoblast, and opens below to the cavity of the vesicle; and fourthly, the dorsal opening of the neurenteric canal is eventually enclosed within the walls of the neural tube.

With regard to the notochord, it appears to me evident that it is an hypoblastic structure, since it arises from an axial mass of cells, which are themselves derived from the primitive hypoblast.

My observations are at variance with Schäfer's, in that I find no continuous layer of mesoblast in front of the medullary groove, such as he describes, but a mass of undifferentiated cells, whose development shows that they are of hypoblastic origin, and that they split up laterally into sheets of hypoblast and mesoblast, while axially they remain undifferentiated, and give rise to the notochord.

Further, that the differentiation of the mass of cells which gives rise to the notochord takes place, as does the first formation of the medullary groove, from behind forwards, but that the separation of the notochord from the hypoblast takes place first of all somewhat anterior to the middle of the embryo, in the region where the medullary groove first deepens, and where the lateral mesoblast first forms protovertebræ, and that from that point the notochord is separated off both backwards and forwards.

Finally, that the derivation of the notochord from hypoblast is still further evidence of the incompatibility of Repiachoff's views (*loc. cit.*) with the facts of development.

Comparison between the Early Stages of Development of the Mole and Mouse, &c.

Until a few months ago there had been no satisfactory explanation of the manner in which the extraordinary phenomenon of the inversion of the layers in the Guinea-pig had been brought about, although the fact that such an inversion really existed had been described many years ago by Bischoff (Nos. 8 and 9), Reichert (No. 23), and Hensen (No. 12).

Kupffer (No. 18), Selenka (No. 27), and Fraser (No. 10), have, however, recently worked at the development of the Field Mouse, House Mouse, and Rat, and have found that the position of the layers in these animals is also inverted. Further, they each discovered the method by which the inversion was accomplished, and at the same time Hensen (Nos. 13 and 14) arrived at somewhat similar results for the Guinea-pig.

From these papers and from that of Spee (No. 28) I gather it is probable that the fully-segmented ovum of these various animals is similar to that of the Mole.

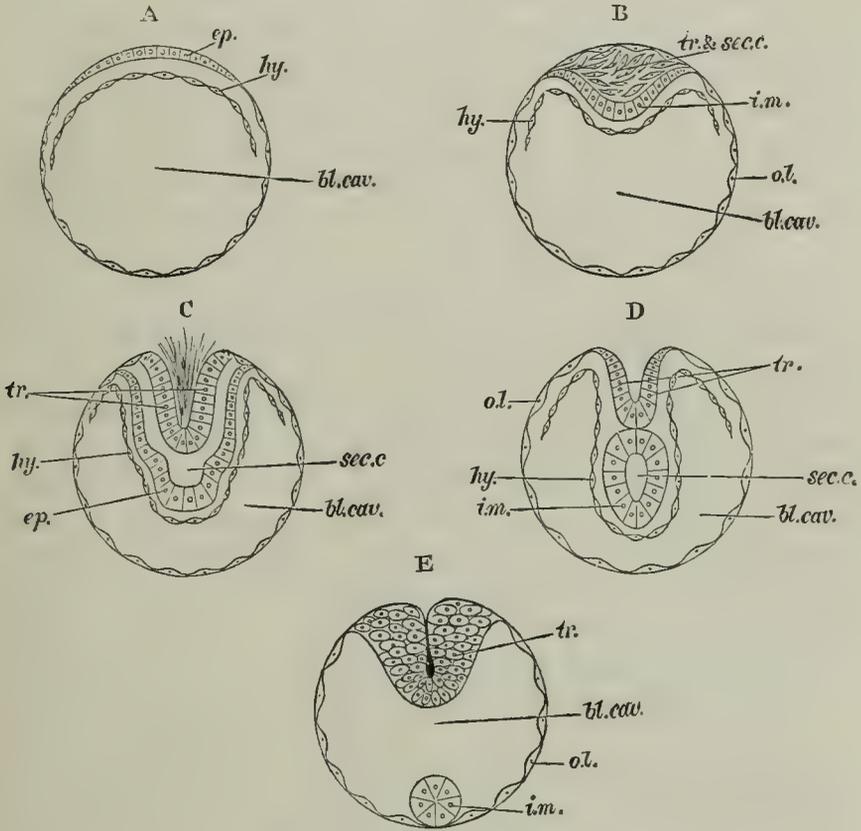
The changes which take place after segmentation are, however, somewhat different in each, and show a gradually increasing difference from the normal type to that one most specialised, viz. the Guinea-pig; while the phenomena exhibited during the development of the mole supply the connecting link between the two types.

These facts have not been, as far as I know, hitherto brought forward, and I venture to think merit some attention.

In the Field Mouse a blastodermic vesicle of flattened outer layer cells is formed, at one place on the circumference of which a solid inner mass is attached.

A layer of hypoblast is formed on the lower side of the inner mass, and the two shortly after flatten out; a thickening of the outer layer then takes place above the inner mass, and the flattened plate, with the hypoblast on its inner side, becomes involuted within the vesicle, and in this way an arched plate is formed the circumference of which rests upon the outer layer

cells. The cavity of the arch (the secondary cavity) is filled up more or less with cells derived from the outer layer, and thus a condition is arrived at remarkably like the stage of development in the Mole represented in the woodcut, Fig. B, and on Plate VII, fig. 24.



EXPLANATION OF WOODCUT.

Diagrammatic representation of :—A, ovum of Rabbit ; B, of Mole ; C, of Field Mouse (after Kupffer) ; D, of House mouse (after Selenka) ; E, of Guinea-pig. A, B, C, and D are at a similar stage of development. E is at a much earlier stage, before the formation of a secondary cavity.

bl. cav., blastodermic cavity ; *ep.*, epiblast ; *hy.*, hypoblast ; *i. m.*, inner mass ; *o. l.*, outer layer ; *sec. c.*, secondary cavity ; *tr.*, rudimentary träger.

There is, however, a difference in the future development.

The plate of cells in the Mole flattens out again, while in the Field Mouse it becomes further involuted within the vesicle, and the lower middle portion becomes the epiblast of the embryo, while the lateral portions form the amnion (Fig. c). In this manner the secondary cavity is surrounded by inner mass and outer layer cells, and into this cavity the embryo projects.

In the common House Mouse a layer of hypoblast is first formed below the rounded inner mass; next above the latter the outer layer cells become thickened and involuted within the vesicle, carrying with them the solid inner mass.

A cavity is subsequently formed in the latter, and it elongates until it nearly reaches the opposite pole of the vesicle, to which it was originally placed.

Thus the cells of the inner mass alone line the secondary cavity in this case, and into it the developing embryo projects (Fig. d).

The Rat develops similarly to the House Mouse, a secondary cavity forming in the inner mass after it is involuted.

In the Guinea-pig, however (Fig. e), the solid inner mass appears to become attached to the opposite pole of the ovum at a very early stage in the development of the blastodermic vesicle, and the outer layer does not become involuted, if observations made by Dr. Wilson and myself be trustworthy, until a considerably later period. A secondary cavity is eventually formed within the inner mass, and into it the embryo projects.

Thus a complete series of conditions may be traced in these various animals between the inverted and normal types. In the Rabbit the solid inner mass flattens out and remains on the surface of the vesicle; in the Mole it is first formed into a curved plate, which subsequently becomes flattened out and lies on the circumference of the vesicle; in the Field Mouse it flattens out first on the surface and then becomes and remains involuted; in the House Mouse it becomes involuted before becoming flattened; and in the Guinea-pig the inner mass remains attached at the opposite pole of the ovum before it becomes a vesicle, and an involution of the outer layer secondarily takes place.

A consideration of these facts, together with an examination of the conditions attending the later stages of development in some of these animals, leads me to believe that the difference in the development of normal animals and those in which the so-called inversion of the layers takes place is one of secondary importance, and, in fact, that no such fundamental differences exist as was supposed by the older observers; the temporary inversion of the layers which occurs in the Mole connecting the two types very closely.

I do not propose here to enter into a more detailed discussion of the points noticed above, or to attempt to compare the later stages of development; the only points which have immediate bearing upon my present work are—

(1) The explanation of the existence of the secondary cavity and the cells situated within it in the Mole; and (2) the fact that in the inverted types the epiblast of the embryo is formed entirely of inner mass cells.

The former may be considered as inversion phenomena of a temporary nature; while with regard to the second point the conditions of development appear to me to be sufficient explanation of the difference.

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DESCRIPTION OF PLATES VI, VII, VIII & IX,

Illustrating Mr. Walter Heape's Memoir on the "Development of the Mole (*Talpa Europea*). The Formation of the Germinal Layers, and Early Development of the Medullary Groove and Notochord."

List of Reference Letters.

a. a. Cut edges of uterus. *am.* Amnion. *bl. cav.* Cavity of blastodermic vesicle. *bl. of B.* Blastopore of van Beneden. *blp.* Blastopore. *c. m.* Circular muscles. *c. t.* Connective tissue. *ep.* Epiblast. *ep. k.* Epiblast keel. *hy.* Hypoblast. *hy. m.* Hypoblastic mesoblast. *i. m.* Inner mass. *l. m.* Longitudinal muscles. *m.* Mesoblast. *m. c.* Mucous envelope. *mes.* Mesoblast. *m. gr.* Medullary groove. *m. pl.* Medullary plate. *n. c.* Neurenteric canal. *nch.* Notochord. *nch. c.* Notochordal canal. *o. l.* Outer layer. *p. gr.* Primitive groove. *pl.* Papilla. *p. sk.* Primitive streak. *p. sk'.* Anterior end of primitive streak. *sec. c.* Secondary cavity between inner mass and outer layer. *sp.* Artificial space between zona and outer layer. *spt.* Septa of connective tissue. *t. c.* Transition cells. *tr.* Rudimentary trager. *ut. ep.* Uterine epithelium. *ut. gls.* Uterine glands. *ut. gls'.* Mouths of uterine glands. *z.* Zona.

Mr. H. A. Chapman, of the Fitzwilliam Museum, Cambridge, has drawn for me figure 51 and all the figures on Plate VI, with the exception of figure 4.

PLATE VI.

FIG. 1.—An optical section of a fully-segmented ovum showing the outer layer (*o. l.*) and the inner mass (*i. m.*) of segments, the blastopore of van Beneden (*bl. of B.*) perforating the outer layer, the radially striated zona (*z*), and the outer gelatinous mucous covering (*m. c.*). The whole ovum inside the membranes is .15 mm. by .17 mm. in diameter, diameter of inner mass is .1 by .12 mm., of outer layer .05 mm.; zona measures .01 mm., and mucous envelope .014 mm. in thickness. ($\times 177$ times.)

FIG. 2.—An optical section of an ovum soon after the appearance of the blastodermic cavity (*bl. cav.*). Beneden's blastopore is closed. The whole ovum inside the zona is .12 mm., and the inner mass .06 mm. in diameter. ($\times 177$ times.)

Figures 1 and 2 are silver nitrate preparations.

DESCRIPTION OF PLATES VI—IX—continued.

FIG. 3.—A surface view of a blastodermic vesicle at a stage before the formation of the secondary cavity between the inner mass and outer layer. The dark spot represents the inner mass attached to the wall of the now much enlarged vesicle. Diameter of whole vesicle $\cdot 31$ mm., and that of the inner mass $\cdot 04$ mm. ($\times 39$ times.)

FIG. 4.—A surface view of the inner mass of the ovum represented in figure 3 more highly magnified. Its nuclei appear smaller than those of the outer layer, but owing to its thickness can only be faintly seen. ($\times 265$ times.)

Fig. 18 is a section through this area.

FIG. 5.—A surface view of a blastodermic vesicle at a considerably later stage; the secondary cavity is present and is represented by the light space in the centre of the inner mass. Diameter of vesicle is $\cdot 9$ mm., that of inner mass $\cdot 15$ by $\cdot 16$ mm. ($\times 39$ times.)

Fig. 24 is a section through this area.

FIG. 6.—A blastodermic vesicle of the same stage as that represented in fig. 5; the wall of the vesicle (*o. l.*) has, however, become separated from the zona (*z.*) and, having collapsed, is more distinctly seen. (Silver preparation.) The diameter of the vesicle is $\cdot 9$ mm., and that of the inner mass $\cdot 16$ mm. (\times about 39 times.)

FIG. 7.—A surface view of an inner mass of the same stage; the secondary cavity is here seen to be placed asymmetrically, and to be in the form of a crescent. Compare the section of this embryo in fig. 25. The diameter of the inner mass is $\cdot 15$ by $\cdot 16$ mm. ($\times 146$ times.)

FIG. 8.—View of a blastodermic vesicle soon after the obliteration of the secondary cavity. The wall of the vesicle at this age is exceedingly thin and the zona so much attenuated, that upon opening the uterus and turning back its sides, the vesicle falls into the elongated shape here represented. The papillæ are processes of the wall of the vesicle, and project into the widely open mouths of the uterine glands at this stage. Compare figures 28 and 29. The embryonic area is drawn slightly too much elongated. Diameter of the vesicle $1\cdot 45$ by $\cdot 63$ mm., and that of the area $\cdot 25$ by $\cdot 18$ mm. ($\times 27\frac{1}{2}$ times.)

FIG. 9.—Another embryo from the same uterus, the area seen in profile. Diameter of vesicle $1\cdot 37$ by $\cdot 65$ mm. ($\times 27\frac{1}{2}$ times.)

FIG. 10.—A surface view of an embryonic area immediately prior to the formation of the primitive streak, formed of two layers throughout. In section, see fig. 30. Diameter of area $\cdot 43$ by $\cdot 35$ mm. ($\times 49$ times.)

FIG. 11.—Surface view of an embryonic area in which the primitive streak and groove are present. The former extends nearly half way along the area. The region in front of the primitive streak is shaded lighter than the remainder

DESCRIPTION OF PLATES VI—IX—continued.

of the area, and is formed of epiblast and hypoblast only. Posteriorly mesoblast is present. Diameter of area $\cdot 81$ by $\cdot 61$ mm. ($\times 49$ times.)

FIG. 12.—A somewhat later stage, in which the primitive streak reaches along $\frac{2}{3}$ rds of the length of the area, and ends behind in a knob or thickening. Mesoblast now extends over the whole area. Compare figs. 32—6. Diameter of area $\cdot 97$ by $\cdot 74$ mm. ($\times 49$ times.)

FIG. 13.—A still later stage. The medullary groove is now present in front of the primitive streak, and at the junction of the two the dorsal opening of the neurenteric canal is seen. Diameter of area $\cdot 97$ by $\cdot 79$ mm. ($\times 49$ times.)

Fig. 43 is a section through the medullary groove of this embryo.

FIG. 14.—Represents a blastoderm in which the medullary groove is considerably advanced. A dark tongue-shaped prolongation extends from the region of the anterior end of the primitive streak into the hind portion of the medullary groove, and another tongue-shaped dark ridge extends from the anterior end of the groove nearly to the edge of the blastoderm. The neurenteric canal is not seen on the surface in this preparation, but its position is indicated by a slightly lighter space at the hind end of the medullary groove. Diameter of area $1\cdot 15$ by $\cdot 69$ mm. ($\times 49$ times.)

FIG. 15.—The hind end of the medullary groove of an embryo slightly older than that drawn in fig. 14. The medullary folds now enclose the front end of the primitive streak, and the neurenteric canal perforates the floor of the medullary groove. In other respects the relations of the parts are the same as those in fig. 14. Compare figs. on Plates VIII and IX. Diameter of this area $1\cdot 79$ by $\cdot 76$ mm. ($\times 73$ times.)

PLATE VII.

All the figures on this plate are magnified 385 diameters, with the exception of fig. 18, which is magnified 585 times, and fig. 30, which is magnified 265 times.

FIG. 16.—A surface view of a portion of the wall of a blastodermic vesicle of the same size as that drawn in fig. 3. Several of the nuclei are seen to be undergoing division. (Silver-nitrate preparation).

FIGS. 17, 18, 19.—Are sections through the portion of the walls of blastodermic vesicles to which the inner mass is attached. They are all of the stage shown in fig. 3. The flattened outer layer cells lie above the inner mass, no hypoblast is developed, and there is no indication of the secondary cavity.

Fig. 18 is a section through the inner mass shown in fig. 4. The embryo from which fig. 17 is taken was preserved in picric acid, that of fig. 18 in corrosive sublimate, and that of fig. 19 in silver-nitrate.

DESCRIPTION OF PLATES VI—IX—continued.

FIGS. 20, 21, 22, and 23.—Are sections through the centre of the inner mass of four vesicles. They show the formation of the hypoblast from inner mass cells. In the two latter the outer layer cells above the inner mass have increased in number and size, and the cells of the latter are becoming columnar.

In fig. 23 the secondary cavity is shown.

Figs. 22 and 23 are Müller's fluid preparations.

FIG. 24.—Section through the centre of the inner mass drawn in fig. 5. The secondary cavity contains cells, which are homologous with the cells of the tråger of inverted types. The inner mass, in the form of an hemispherical plate, is continuous at the edges with the outer layer, and is composed of a double row of columnar cells. The hypoblast is now a distinct membrane, extending laterally beyond the inner mass. The zona is much thinner.

FIG. 25.—Section through the area drawn in fig. 7. The arched plate is becoming extended and flattened. In this specimen no cells are shown in the secondary cavity, but the outer layer above the cavity is much thickened.

FIG. 26.—Section through an inner mass still more flattened. Cells are present in the secondary cavity, and show signs of becoming incorporated with the inner mass. The cells of the outer layer at the edges give similar indications (*t. c.*).

FIG. 27.—Section a little to one side of the middle line through an inner mass, in which the secondary cavity is almost entirely obliterated. The inner mass is continuous at the edges with the outer layer, and several cells (*t. c.*) may be interpreted as outer layer cells, becoming incorporated with the inner mass. Compare fig. 49.

FIG. 28.—Transverse section through the embryonic area of the vesicle drawn in fig. 8. The cells of the inner mass have now united with the outer layer cells above them to form the epiblast plate of the embryonic area. The plate is not now uniformly two layers thick. The zona can no longer be distinguished. At the side one of the papillæ seen in fig. 8 is shown in section.

FIG. 29.—Section of another papilla of the vesicle drawn in fig. 8.

FIG. 30.—Transverse section through the embryonic area drawn in surface view in fig. 10. It is throughout composed of epiblast and hypoblast.

PLATE VIII.

The figures on this plate are all magnified 265 diameters, with the exception of fig. 32, which is magnified 170 times.

FIG. 31.—Longitudinal section through the middle line of part of an embryonic area in which the primitive streak has begun to form. In front of the

DESCRIPTION OF PLATES VI—IX—continued.

primitive streak the blastoderm is perforated; a few mesoblast cells are present anterior to the perforation.

FIG. 32.—Transverse section through the region, anterior to the primitive streak, of an embryonic area similar to that drawn in fig. 12. The single row of hypoblast seen in fig. 30 is here giving rise to hypoblastic mesoblast.

FIGS. 33, 34, and 35.—Transverse sections through the blastoderm drawn in fig. 12.

FIG. 33 is a section through the head of the primitive streak. The layer of cells below the epiblast is nowhere differentiated into hypoblast and mesoblast, though a few cells (*hy.*) exhibit a tendency to flatten out and become separated from the rest. A keel of epiblast (*ep. k.*) is forced into the underlying hypoblastic mesoblast in the middle line.

A few sections further back (fig. 34) the anterior region of the primitive groove is cut through. The hypoblast, epiblast, and mesoblast are united in the middle line, while laterally they are distinct from one another. The mesoblast does not extend beyond the embryonic area in this region.

FIG. 35 is a section through the middle of the primitive streak where the epiblast and mesoblast are united in the middle line, and the hypoblast is free throughout. The mesoblast here extends beyond the embryonic area.

FIG. 36.—A transverse section through the hind knob of the primitive streak of an embryonic area very similar to that seen in fig. 12. The epiblast, mesoblast, and hypoblast are throughout the entire breadth of the area indistinguishable from one another; the mesoblast, however, extends beyond the area, and they are there distinct.

FIGS. 37 to 41.—Transverse sections through the neurenteric canal of an embryo slightly older than the one represented in fig. 13. The dorsal opening of the canal is seen in fig. 37; it passes thence through the mesoblast (fig. 38) to the hypoblast, along which it extends for some distance (figs. 39, 40), and eventually opens ventrally in fig. 41. The three layers are united together at the front end of the primitive streak (fig. 37). Anteriorly the hypoblast is first separated as a thin layer (fig. 38), and then becoming much thickened contains the neurenteric canal (figs. 39—41). Where the neurenteric canal enters the mesoblast the latter is united with both epiblast and lateral mesoblast, but freed from hypoblast. When the canal enters the hypoblast the lateral mesoblast becomes at first distinct, but afterwards is continuous with the axial mass of cells. Compare Fig. 50.

FIG. 42.—Transverse section in the anterior region of the medullary groove of the same embryo, showing the thickened axial hypoblast to which the lateral mesoblast and hypoblast is joined.

DESCRIPTION OF PLATES VI—IX—continued.

PLATE IX.

FIGS. 43 to 48 are magnified 265 diameters, figs. 49 and 53, 385 diameters, fig. 51 magnified 18 diameters, and fig. 52, 15 diameters.

FIGS. 43, 44, and 45 transverse sections through about the centre of the medullary groove of embryos at different stages of growth. Fig. 43 is a section through the embryo drawn in fig. 13. They exhibit the gradual deepening of the medullary groove, the isolation of the axial hypoblast from the lateral mesoblast, and the reduction in bulk of the former.

FIGS. 46 and 47.—Transverse sections through the embryo drawn in fig. 14.

FIG. 46 is through the tongue-shaped dark line projecting forward from the anterior end of the medullary groove.

FIG. 47.—A section through the medullary groove in a region similar to that from which the sections drawn in figs. 43 to 45 were taken.

FIG. 48.—Transverse section through the region anterior to the medullary groove of the same embryo from which fig. 45 is taken.

FIG. 49.—Transverse section through the embryonic area of a rabbit's ovum six days four hours old. The sections throughout are similar to the one here figured, and show the cells of the outer layer (*t. c.*), Rauber's Decksellen, becoming incorporated with the cells of the inner mass, the two together forming the epiblast of the embryo.

FIG. 50.—Diagrammatic longitudinal section through the middle line of the hind end of an embryo similar to that drawn in fig. 14. The medullary groove has not as yet enclosed the neurenteric canal. The latter is continuous from the epiblast through the mesoblast to the hypoblast, and opens dorsally and ventrally through the former and latter layers. At *p. sk'* the three layers are united, behind and in front the hypoblast is free. The amnion is formed over the hind end of the area.

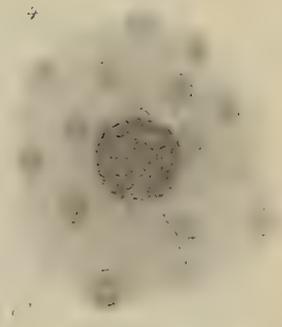
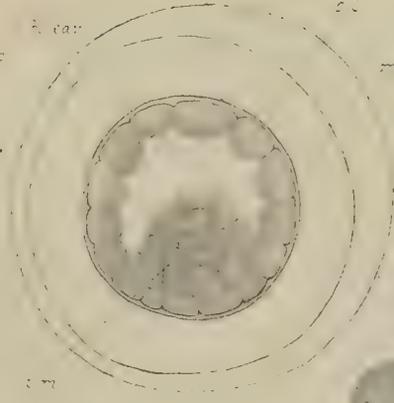
FIG. 51.—Transverse section through the uterus of the mole from which the vesicle drawn in fig. 8 was obtained. The section passes through the region in which the vesicle was placed. The widely opened mouths of the uterine glands into which the papillæ of the vesicle projected are seen in connection with the stems of the glands. The uterine epithelium is represented in the drawing slightly too thick, and the width of the mouth of the glands to which the reference lines *ut. gls'* point is somewhat exaggerated.

FIG. 52.—A piece of uterine epithelium torn away from the uterus. The papillæ are hollow, and are formed of the epithelium lining the mouths of the glands, which is continuous with the epithelium of the uterus. The uterus from which this was obtained is the same as that from which the section, fig. 51, was made.

FIG. 53.—Section of the wall of a blastodermic vesicle (at the primitive streak stage) at the non-embryonic pole, and of the uterine epithelium to which it was attached.

1. 1/2

2.

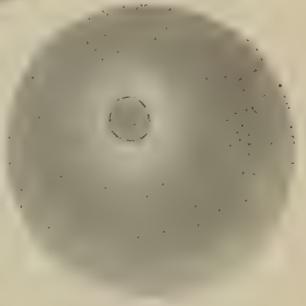
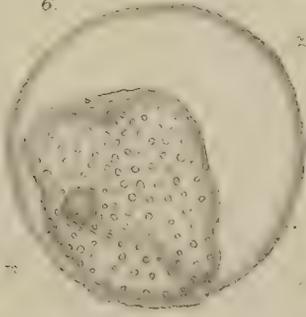


9.

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15.



Fig. 16.

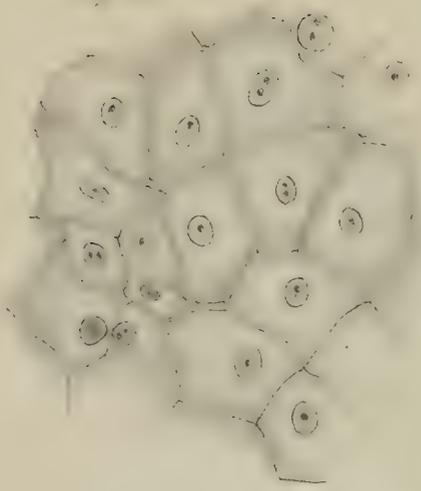


Fig. 20.



Fig. 21.

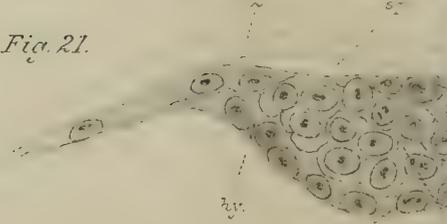


Fig. 22.

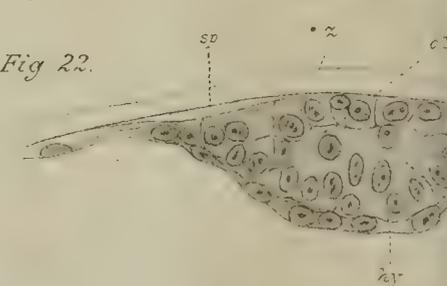


Fig. 17.

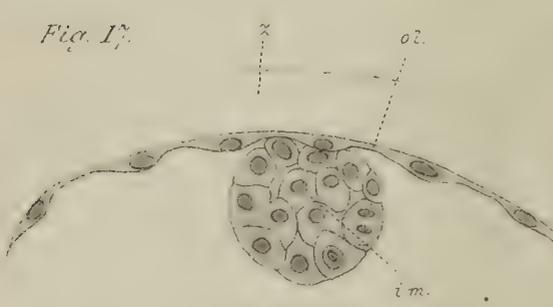


Fig. 23.

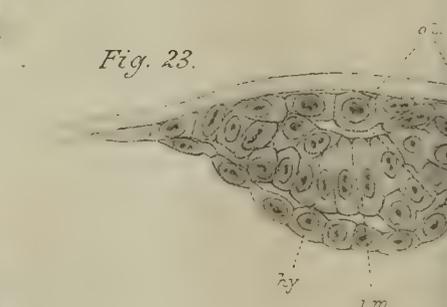


Fig. 18.

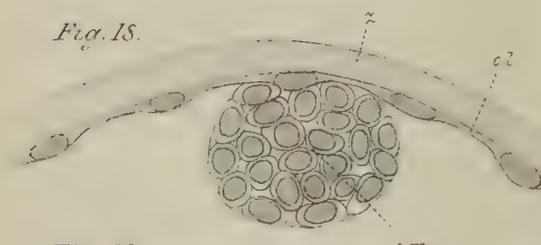


Fig. 19.



Fig. 30.

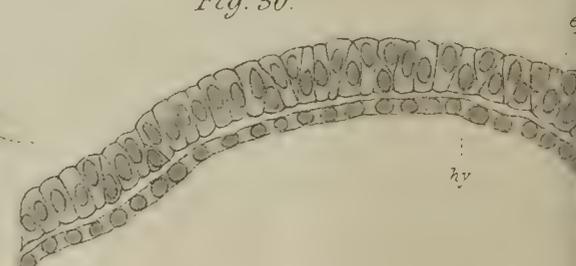


Fig. 29.

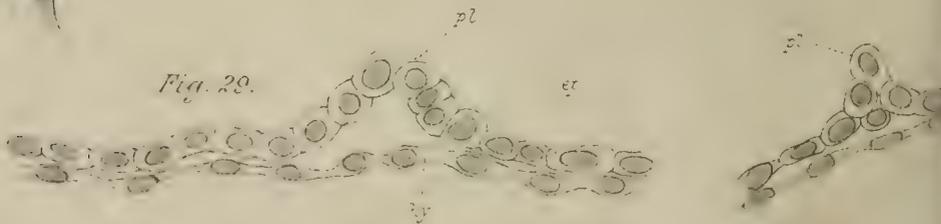




Fig. 25.



Fig. 26.

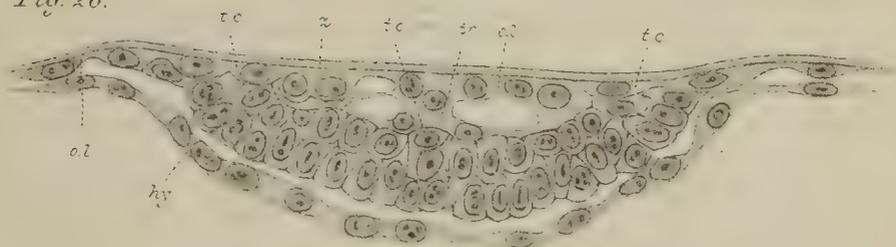


Fig. 27.

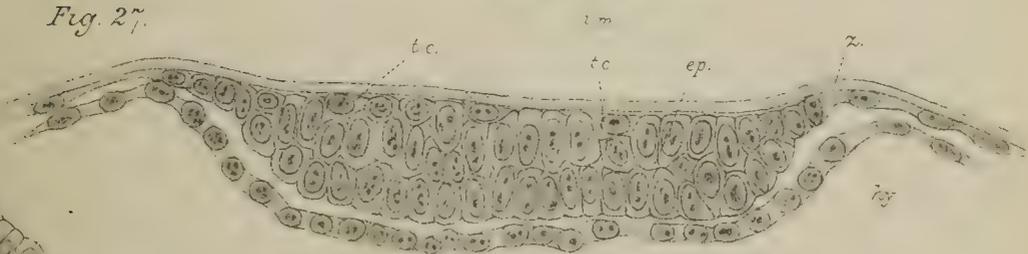


Fig. 28.

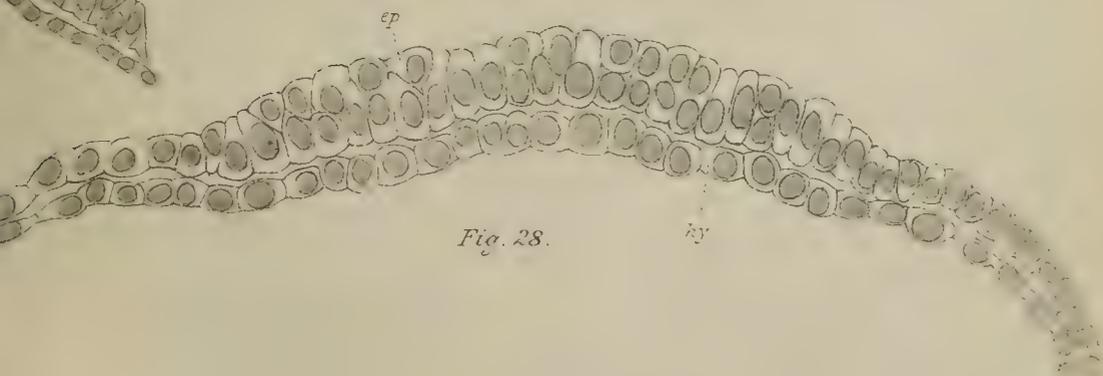


Fig. 31.

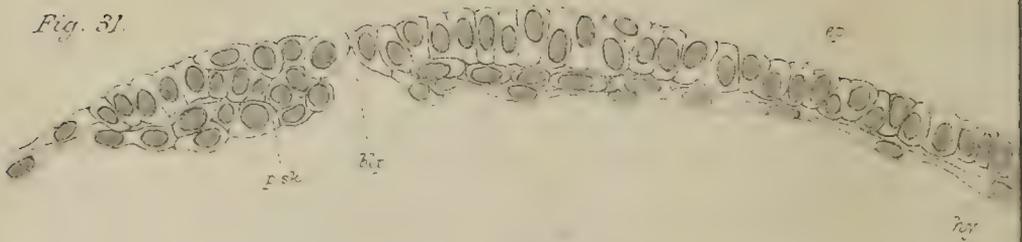


Fig. 32.



Fig. 33.

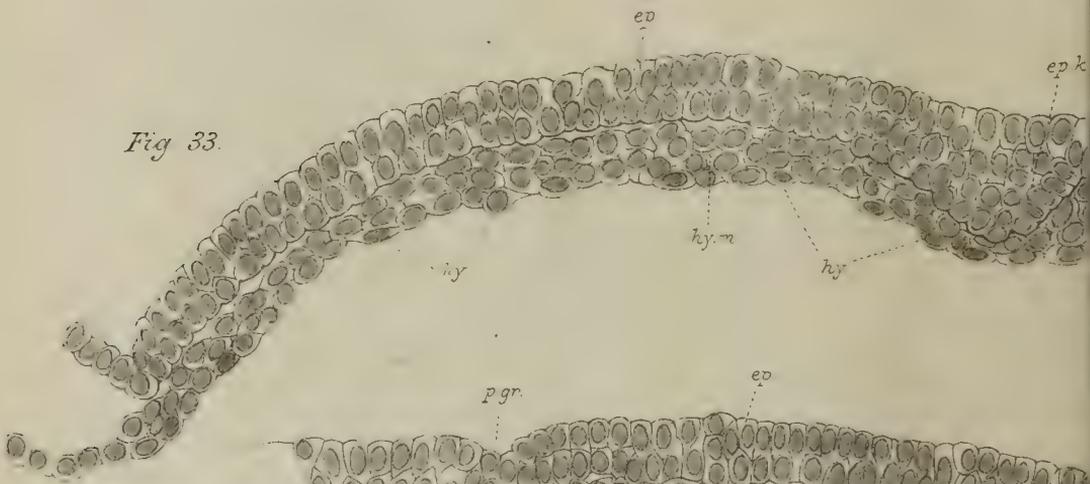


Fig. 34.

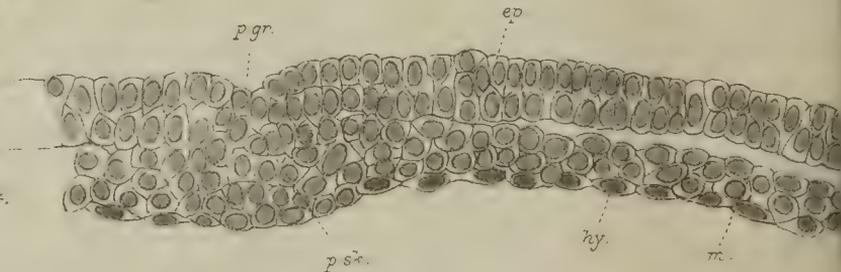


Fig. 35.

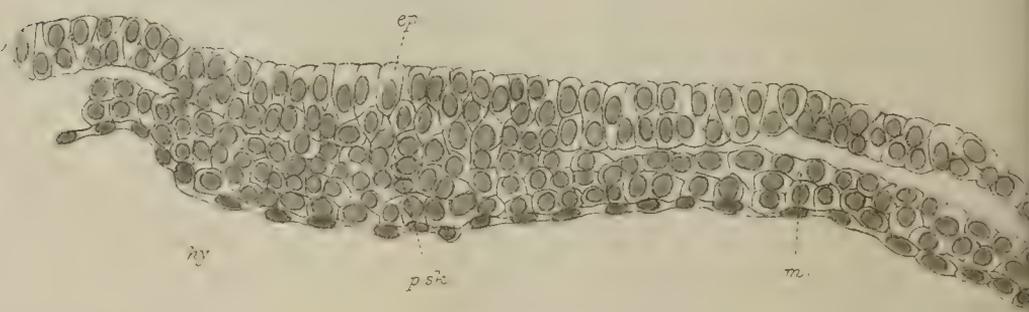


Fig. 36.

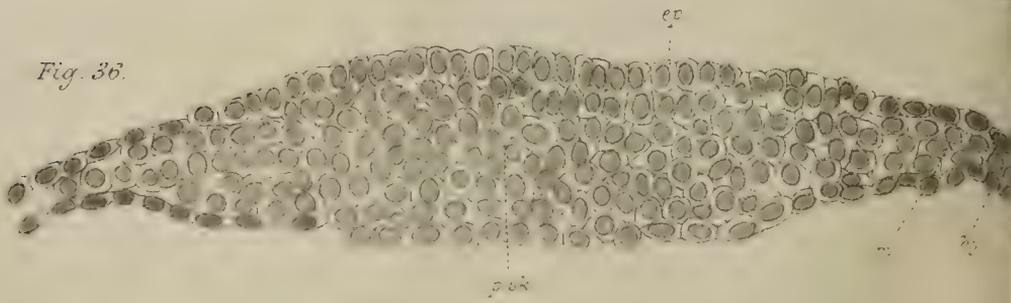


Fig. 37.

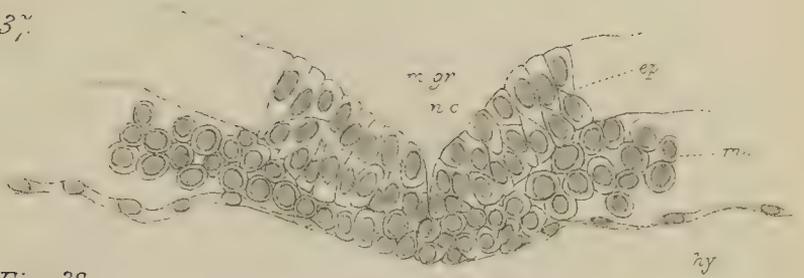


Fig. 38.

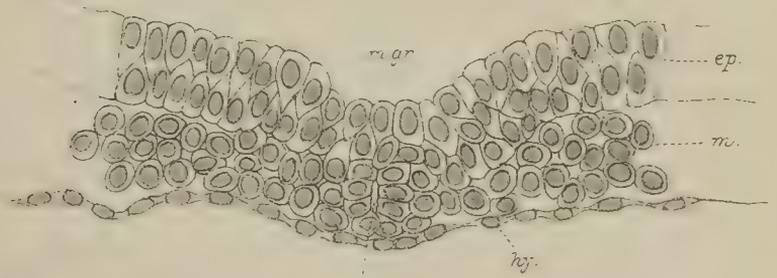


Fig. 39.

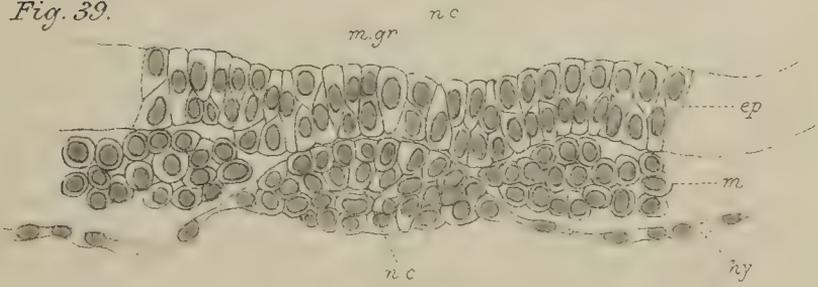


Fig. 40.

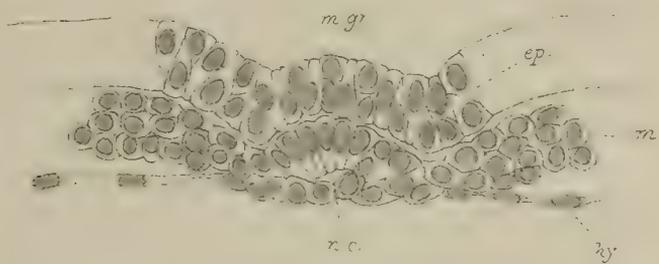


Fig. 41.

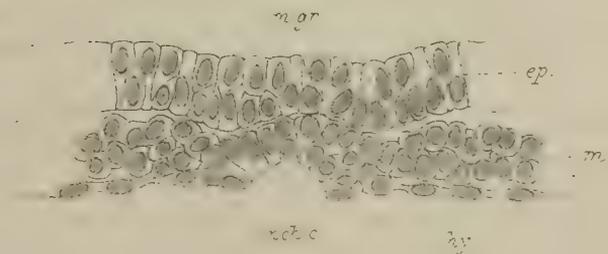


Fig. 42.

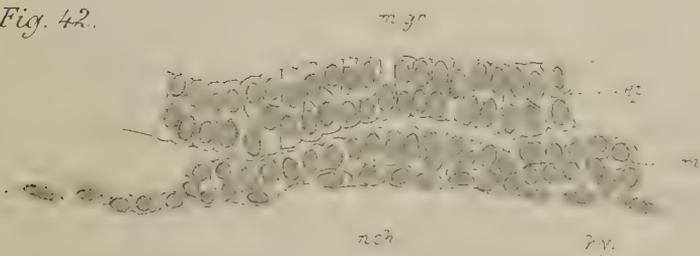


Fig. 43.



Fig. 44.

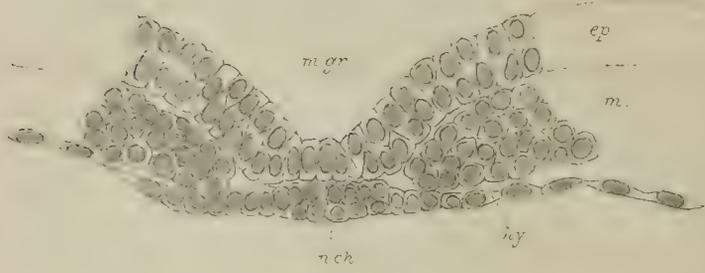


Fig. 45.

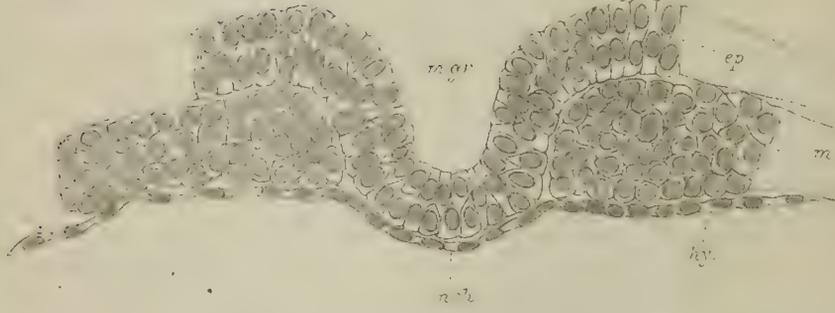


Fig. 50.

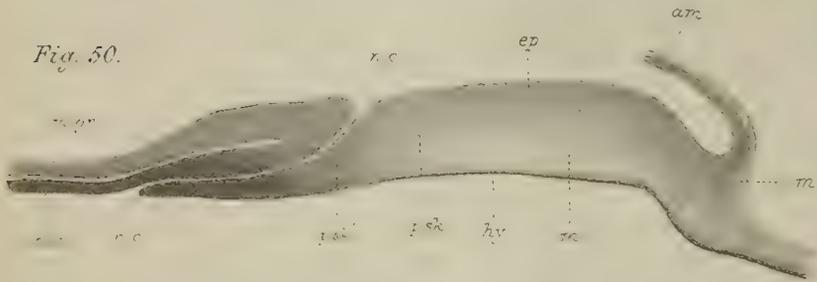


Fig. 52.



Fig. 53.

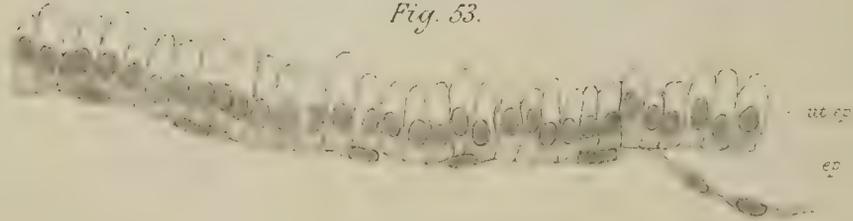


Fig. 46.



Fig. 47.

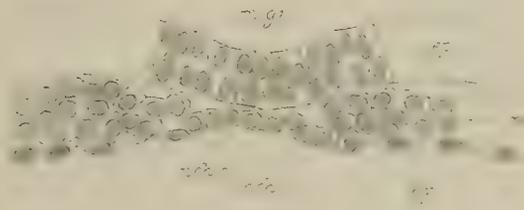


Fig. 48.

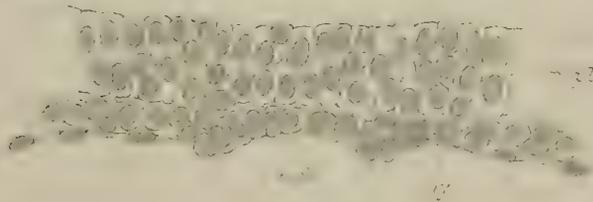


Fig. 49.

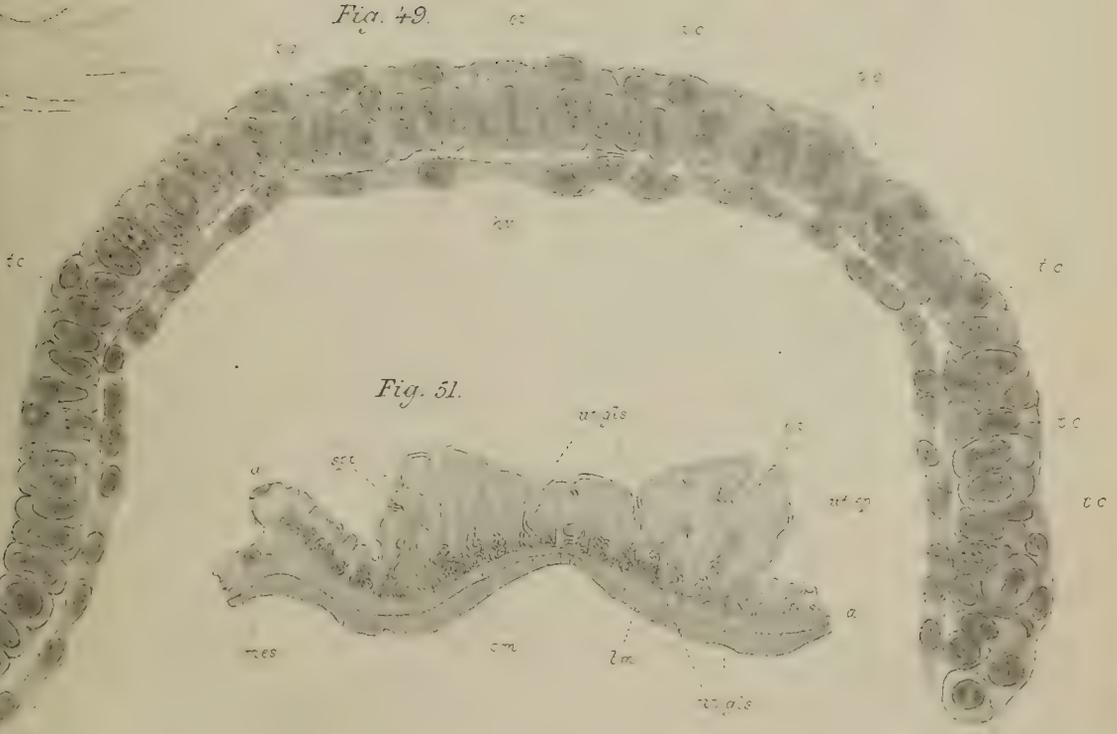


Fig. 51.

On the Origin of Metameric Segmentation and some other Morphological Questions.

By

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With Plates X and XI.

IN the following pages¹ certain hypotheses with regard to the evolution of segmented Triploblastica (Annelida, Arthropoda, Vertebrata), and some apparently unsegmented forms (Mollusca Brachiopoda, Sagitta, Balanoglossus) are suggested and discussed.

I have found it convenient to consider the Vertebrata specially in the latter part of the paper, because of the very pronounced views which are held at the present day with regard to their evolution.

The paper is divided into two parts. The first part deals with the evolution of certain organs; the second part with the evolution of the groups mentioned and especially with that of the Vertebrata.

My hypothesis concerning the origin of metameric segmentation has been in a sense anticipated by Lang. He regards the somites as derived from gut pouches such as are found at the present day in Turbellarians. It should be remembered that according to his view, the Turbellaria are specialised Cœlenterates. My view of the origin of Somites differs from

¹ A short account of the main points of this paper was communicated to the Cambridge Philosophical Society in November, 1883, and published in vol. v of the 'Proceedings' of that Society.

his in taking a simpler diploblastic form as the starting point for all the Triploblastica discussed.

Hubrecht in his recent paper on the "Ancestral form of the Chordata" has explained Lang's views and instituted some important comparisons between Vertebrates and Nemertines. I differ, however, from Hubrecht in taking a simpler form as my starting point.

I have purposely refrained from referring to the Turbellaria and other flat worms in this essay, because I cannot make up my mind as to whether they are degenerate Enterocœla or highly specialised Cœlenterata (without a separated cœlom). I am, however, very much inclined to the view that they are degenerate Enterocœla.

I have also avoided discussing the Echinoderms because, while their early development is easy to understand, the later stages and metamorphosis are not so intelligible.

My hypothesis with regard to the origin of the mouth and anus has, so far as I know, not been suggested before. I agree with Hæckel in regarding the blastopore as homologous with the primitive mouth of the gastræa.

I have attempted to explain the peculiar behaviour of the blastopore in a general way, in the first part of my paper. In the second part I again consider this question in connection with the Vertebrate blastopore. I dissent most strongly from the view that the Vertebrate mouth and anus are both secondary perforations, and not homologous with those of Invertebrates, e. g. Annelids. I regard them both as homologous with the corresponding structures in the other Triploblastica discussed.

But I have not been able to do justice to this part of my subject. I could only do so by reviewing critically the extensive literature on this subject, and by making a special investigation of the behaviour of the blastopore in animals with a prolonged larval life, and of the structures classed as primitive streaks, and this I have unfortunately been unable to do. I think that any such investigation would have valuable results.

I agree with Balfour in his view that the "conrescence"

theory of the growth of the Vertebrate embryo is untenable. It seems to me that the advocates of that theory have mixed up three distinct embryonic structures, the mesoblastic bands, the primitive streak, and the ridges of the medullary groove.

The primitive streak is in most forms at first a median structure. I agree with the current view as to its nature as a rudiment of the blastopore, and I suggest a reason for its persistence.

I ought particularly to mention that I regard the Annelid-origin of the Vertebrata and Arthropoda as untenable. This will be obvious to anyone reading the following pages.

I offer no suggestion as to the phylogeny of Mesoblast. I agree entirely with the current view that it has arisen from both of the primary layers.

Mesenchyme is obviously merely precociously developing mesoderm, and is particularly developed in free larvæ.

Finally, I may add that I do not put forward these hypotheses in a dogmatic spirit, and that I fully recognise that theories dealing with the complicated facts of morphology can only have in most cases a very temporary value. The main idea of the comparisons discussed below first occurred to me some years ago, when investigating the development of the Vertebrate excretory organs; but they have received such striking confirmation from Hatschek's work on *Amphioxus*, and more recently from a study of the embryo of *Peripatus capensis*, that I have at length decided to publish them, hoping that they may at least excite criticism and so lead to the increase of our knowledge, and to the greater definition of our ideas.

PART I.

In the discussion which followed the communication of the late Professor Balfour's notes and drawings of the early embryos of *Peripatus Capensis*, to the Royal Society (December, 1882), I drew attention to the great resemblance between the embryo of *P. Capensis* with its elongated blasto-

pore and somites, and an adult Actinozoid polyp. I pointed out that the comparison of these two structures suggested an explanation, which so far as I know has not before been suggested, of a great morphological difficulty, viz. the origin of metameric segmentation (vide 'Nature,' December 28th, 1882). At the same time I pointed out that by following up this comparison some other morphological difficulties received an explanation.

The hypotheses I suggested were shortly as follows :

1. The mouth and anus found in most of the higher groups (Vermes, Mollusca, Arthropoda and in all probability Vertebrata) have been derived from the mouth of an ancestor common to them and the Cœlenterata ; i.e. from an elongated opening such as is found at the present day in the Actinozoa.

2. That the somites of segmented animals are derived from a series of pouches of the primitive gut (archenteron) of a Cœlenterate-like ancestor, i.e. from pouches generally resembling those found at the present day in Actinozoid polyps and Medusæ.

That the excretory organs or nephridia (segmental organs) of the higher animals are derived from specialised parts of these pouches which were in the supposed ancestor, as indeed they now are in many living Medusæ and Actinozoid Polyps connected peripherally with each other by a longitudinal canal (circular canal of Medusæ, perforations in mesenteries of Actinozoa,) and with the exterior by a pore¹ one for each pouch ; further, that in the Invertebrata, e.g. Annelida, the longitudinal canal has been lost and the external pores retained, while in the Vertebrata the longitudinal canal (segmental or pronephric duct) has persisted and retained its posterior opening into the alimentary canal while the external pores have been lost.

I now add to these three propositions a fourth.

4. That the tracheæ are not developed from cutaneous glands

¹ Vide Hertwig, 'Organismus der Medusen,' p. 39 ; and "Actinien," 'Jena Zeitschrift,' Bd. xiii.

of a worm-like animal with well-differentiated mesodermal tissues (a view which on physiological grounds is hard to accept) but are rather to be traced back to simple ectodermal pits in the two-layered ancestor developed for purposes probably of aeration and represented at the present day in the Cœlenterata by the subgenital pits of the Scypho-medusæ, in the embryos of Arthropoda by the pits into the cephalic ganglia, and in the Vertebrata by the canal of the central nervous system.¹

The essence of all these propositions lies in the fact that the segmented animals are traced back not to a triploblastic unsegmented ancestor but to a two-layered Cœlenterate-like animal with a pouched gut, the pouching having arisen as a result of the necessity for an increase in the extent of the vegetative surfaces in a rapidly enlarging animal (for circulation and nutrition).

The hypotheses are based upon the embryonic development of the respective organs in the Triploblastica and the structure of the living Cœlenterata; in other words, upon facts precisely of the same nature as those which have been used in tracing the evolution of the nervous and muscular tissues.

Before proceeding to discuss the facts upon which the hypotheses rest, I may be permitted again to point out that it is no part of my view to derive segmented animals direct from the Cœlenterata, but to derive both Cœlenterata and segmented animals from a common Cœlenterate-like ancestor, whose structure can only be elucidated by studying the anatomy and the development of the living Cœlenterates, and of the higher segmented animals.

¹ Sedgwick, "On the Original Function of the Canal of the Central Nervous System of the Vertebrata," 'Proc. of Cambridge Phil. Soc.' vol. iv.

ON THE HOMOLGY OF THE MOUTH AND ANUS WITH THE MOUTH OF THE CŒLENTERATA.

It will be generally admitted that the mouth and anus of the Annelida, Arthropoda, and Mollusca are homologous structures—i. e. that the mouth of an Arthropod is homologous with that of an Annelid, and with that of a Mollusc, and that the anus in each of these groups is homologous with the anus of the other groups. It is well known that the blastopore in these groups presents considerable differences in its relation to the mouth and anus. In one form it is directly converted into the mouth, in another into the anus; while sometimes it entirely closes up and gives rise to neither (for summary of facts vide Balfour 'Comp. Embryology,' vol. ii, pp. 281, 282). This variability, in the fate of the blastopore was first pointed out by Lankester.¹ It is very puzzling, and has led some morphologists to regard it as a structure which is not homologous in the different animals, and of no particular phylogenetic significance. It seems to me, however, that a little consideration shows that this view of the blastopore must be given up, and that there are very strong grounds for regarding the blastopore as homologous in every case,² and also as homologous with the mouth of the Cœlenterata. Before proceeding to discuss the main point of this section of my paper, I must definitely examine this question about the blastopore.

On the Blastopore.—Either the blastopore has an ancestral meaning or it has not. It seems to me that we have no right to assume that this or any other embryonic structure or process is without a phylogenetic significance until all other views have been shown to be untenable.

It is often said when any peculiar embryonic process is discussed

¹ "On the Coincidence of the Blastopore and Anus in *Paludina*." 'Quarterly Journal of Mic. Sci., 1876.

² It must be distinctly understood that the only groups referred to in the following paper are the Vertebrata, Annelida, Arthropoda, Mollusca, Balanoglossus, Brachiopoda and Sagitta. For the present, I leave the Platyelminthes and Echinoderms out of consideration. The special case of the Vertebrata will be considered in Part II.

from a phylogenetic standpoint that it is only the way in which the animal develops, and that it is waste of time to attempt to explain it. I cannot agree to accept such a view of any embryonic fact. If there is anything in the theory of evolution, every change in the embryo must have had a counterpart in the history of the race, and it is our business as morphologists to find it out.

I wish to point out that I am not discussing how the gastrula arose. I take as my basis the undoubted fact that gastrulæ have existed, and I am trying to show that a two-layered, gastrula-like animal was the ancestor of most living Metazoa.

I must, therefore, reject the view that the blastopore has no ancestral meaning.

What, then, is its ancestral meaning?

It seems to me that there is very strong evidence for the view that it is homologous with the mouth of the Cœlenterata.

In the first place the Cœlenterate mouth either arises as a result of invagination, the blastopore remaining as the mouth (Cereanthus, Pelagia), or as the result of perforation. In the Triploblastica similarly the blastopore either arises as a result of invagination or as a perforation. The method of development, therefore, coincides, and we thus have a strong reason for regarding them as homologous.

The second important point to be examined in determining homologies is the relation to other important structures. The relation of the Cœlenterate mouth and the blastopore to the alimentary canal and the nervous system can in most cases be determined; and in all cases in which it can be so determined it is the same.

(1) The Cœlenterate mouth and the blastopore resemble each other in being the main communication by which the archenteric cavity or its rudiment communicates with the exterior.

(2) They resemble each other in always being perforations of the neural surface of the body.

With regard to the first of these agreements nothing need be said; it is a fact of little importance, as there are many other channels in the Cœlenterata through which the archenteron communicates with the outer world. The second agree-

ment is of great importance; but before it can be of any value to us, we must be able to decide whether the neural surfaces of the Cœlenterata, Annelida, &c., are homologous. It will be generally admitted that the nervous systems of the Annelida, Arthropoda and Mollusca are built upon the same type; and that the ventral surface of the body is homologous in each of these three groups. The late Prof. Balfour put forward the hypothesis that the nervous system of these types was homologous with that of Cœlenterata. He says:

“ In the first place it is to be noted that the above speculations render it probable that the type of nervous system from which that found in the adults of the Echinodermata, Platyelminthes, Chætopoda, Mollusca, &c., is derived, was a circumoral ring, like that of Medusæ, with which radially-arranged sense-organs may have been connected; Its anterior part may have given rise to supra-œsophageal ganglia and organs of vision; these being developed on the assumption of a bilaterally symmetrical form, and the consequent necessity arising for the sense-organs to be situated at the anterior end of the body. If this view is correct, the question presents itself as to how far the posterior part of the nervous system of the Bilateralia can be regarded as derived from the primitive radiate ring.

“ A circumoral nerve-ring, if longitudinally extended, might give rise to a pair of nerve-cords united in front and behind, — exactly such a nervous system, in fact, as is present in many Nemertines (the Enopla and Pelagonemertes), in Peripatus and in primitive molluscan types (Chiton, Fissurella, &c.). From the lateral parts of this ring it would be easy to derive the ventral cord of the Chætopoda and Arthropoda. It is especially deserving of notice, in connexion with the nervous system of the above-mentioned Nemertines and Peripatus, that the commissure connecting the two nerve-cords behind is placed on the dorsal side of the intestines. As is at once obvious, by referring to the diagram (fig. 231 B), this is the position this commissure ought, undoubtedly, to occupy if derived from part of a nerve-ring which originally followed more or less closely the

ciliated edge of the body of the supposed radiate ancestor." 'Comparative Embryology,' vol. ii, pp. 311, 312.

It seems to me that nothing can be added to make the case stronger. I only wish to make one addition to the hypothesis, and that is that the type of nervous system from which that of the above-mentioned groups has been derived was a broad ring round the mouth, in fact, more resembled the nervous system of *Actinia* in its general diffusion over the oral surface than the compact ring of the *Medusa*; the latter being a highly specialised part of this generalised nervous system, which has, however, in part persisted in the subumbrella plexus of ganglion cells described by Schafer and Claus. If this hypothesis is correct, i. e. if it be true that the oral surface of a *Cœlenterate* is homologous with the ventral surface of the mentioned groups; and if the nerve-ring of the *Medusa*, the nerve-ring of *Peripatus*, the nerve-ring and general ventral nervous plexus of *Chiton* and *Proneomenia*, the cerebral ganglia and ventral nerve-cords of other *Mollusca*¹ and *Annelida* and *Arthropoda* are all derived from a general peri-oral nervous system of a *Cœlenterate*-like ancestor, then the relation of the blastopore to the nervous system is the same in the *Annelida*, *Arthropoda* and *Mollusca* and the same as that of the mouth of *Cœlenterata*.

With these facts before us, viz. similarity in development and in relation to other important structures, I think we can hardly doubt the fact that the blastopore in the cases mentioned and the *Cœlenterate* mouth are homologous structures.

In the above discussion I have avoided referring to the ultimate history of the blastopore. The fate of the blastopore in the *Triploblastica* is extremely variable, and it is this variability only which has caused the homology ever to be doubted.

But I think we have here two distinct questions: one deals with the blastopore or mouth of the two-layered stage in

¹ The absence of the connection dorsal to the anus in some *Mollusca*, *Annelida*, and *Arthropoda*, will not I think be regarded as a fact of any importance if the hypothesis be accepted with regard to the nervous system of *Peripatus* and *Chiton*.

embryonic development and asks whether that stage has a counterpart in evolution; the other deals with the subsequent development of the blastopore and asks whether that subsequent development throws any light on the evolution of the mouth and anus.

But at the same time I must admit that the fate of the blastopore is so peculiar, that the doubts which on that account have been expressed as to its phylogenetic meaning are not unreasonable. The case stands thus. The blastopore in *Serpula* gives rise to the anus; in most other Chætopoda to the mouth; similarly in the Mollusc *Paludina* it becomes the anus, while the general rule among Mollusca is that it should become the mouth. It would seem to follow from these facts, as Lankester has already pointed out, that if the blastopore is in each case homologous, then the anterior end and mouth of *Serpula* must be homologous with the posterior end and anus of other closely allied Chætopods. This is manifestly absurd. There are two ways out of the difficulty; either the homology of the blastopore must be given up, or we must suppose that primitively it gave rise to both mouth and anus, and that its specialisation as a larval organ has caused the variability of its subsequent history. The latter view is obviously suggested by the elongated form the blastopore first assumes in many animals, extending as a slit along the whole of the ventral surface of the embryo.¹ The blastopore never retains for long this form, but soon becomes specialised to a round opening, the definite blastopore,² by the closure of the lip of the slit except at one point. The point at which it remains open must depend on the shape of the larva, &c., and will obviously be determined by the convenience of the larva.

This hypothesis that the mouth and anus of the Triploblastica is derived from a single opening, represented in living animals by the Cœlenterate mouth and, on the assumption

¹ This fact was first pointed out by Lankester, vide 'Quarterly Journal of Mic. Sci.,' vol. xvi, 1876, p. 326.

² A special name is wanted for this structure, to distinguish it from the blastopore of the gastrula stage.

(vide above p. 83) that the latter and the blastopore of higher types are homologous, by the early blastopore (before specialisation as the larval mouth) receives very strong support from the actual structure of the Actinozoid mouth, and from the newly discovered facts with regard to the history of the blastopore of *Peripatus capensis*; and has the merit of being on a priori physiological grounds easily conceivable.

Mouth of the Actinozoa.—In the Actinozoa the mouth-opening is elongated, and the animal is symmetrical on each side of the long axis of the mouth. At one end of the long axis the mouth is especially differentiated, and this differentiation extends down the stomodæum as a strongly ciliated groove called by Hickson¹ the Siphonoglyphe. The cilia of this groove produce a current from without inwards, while the cilia of the rest of the stomodæum work in the opposite direction. This differentiation of the stomodæum is particularly conspicuous in the Hexatinian *Peachia*, in which there is a deep strongly muscular groove along the whole length of one side (the so-called ventral side) of the stomodæum (fig. 6, *Si*); and the walls of the groove project at the mouth-opening beyond the rest of the wall of the stomodæum so as to form a semi-circular lip conspicuous from the exterior at one end of the long axis of the mouth.

The free edges of this groove are frequently united with each other, so that the groove is converted into a canal open into the general cavity of the body at the lower end of the stomodæum, and to the exterior at the mouth-opening. This junction of the lips of the groove seems to be simply a case of adhesion, as they may with very slight effort be separated without tearing the tissue. When the groove is thus converted into a canal there are obviously two openings into the body of the polyp, one through the general opening of the stomodæum, and the other through this highly differentiated siphonoglyphe. According to Hickson (*loc. cit.*) the cilia work in opposite directions in these two parts of the stomo-

¹ 'Proc. Royal Soc.,' 1883.

dæum, so that one may be regarded as a mouth and the other as an anus.

I have not been able to make out what causes the adhesion of the lips of the siphonoglyphe in *Peachia* (whether interlocking of cilia as in the Lamellibranch gill or what), but of the adhesion there can be no doubt whatever.

This differentiation of the mouth and stomodæum of Actinozoid polyps has been known for some time. The Hertwigs,¹ in their brilliant paper on the Actinozoa, summarise the facts and point out that the elongated mouth when closed has a dumb-bell shaped form, the median portion being closed, and the two ends remaining open.

“Wenn die Wandungen des Schlundrohrs an einander legt sind und der Mund geschlossen ist, bleiben sie (the ‘Schlundrinnen’) geöffnet und wird demnach ihre Bedeutung wohl darin bestehen, dass durch sie fortwährend ein Wasserstrom in das innere des Körpers hinein getrieben wird” (p. 513).

In view of the hypothesis under consideration, viz. that the mouth and anus of the higher animals is derived from an elongated slit-like opening such as is found in the Actinozoa, these anatomical facts are of the highest interest.

Blastopore of *Peripatus*.—The history of the blastopore of *Peripatus* has been given up to a certain point in the last volume (1883) of this Journal.² The youngest embryo found was a spherical or slightly oval gastrula with a slightly elongated blastopore (fig. 1). In the subsequent growth the embryo becomes elongated along the long axis of the blastopore and the mesoblastic somites appear (fig. 2). The middle portion of the lips of the blastopore then come together (fig. 3), and in the next stage (fig. 4) there are two openings into the mesenteron, an anterior and a posterior. Meanwhile, the primitive streak (connected with the formation of the meso-

¹ “Die Actinien,” ‘Jena Zeitschrift,’ vol. xiii, p. 513.

² The species of *Peripatus* which Dr. v. Kennel is working at is different from that described in Balfour’s memoir. Dr. v. Kennel does not mention this somewhat pertinent fact. Perhaps he was not aware of it; but if he was, I find it difficult to understand the positive nature of his criticism.

blast), which was present at the hind end of the blastopore in the earliest stage (fig. 1), has become marked with a groove (fig. 4). In the paper referred to, the question—Do these two openings become the mouth and anus of the adult?—was left open. I am now in a position to state that they do become the mouth and anus of an embryo of an age equal to the oldest stage described by Moseley¹ in his original paper, so that I think there can be no doubt that they do become the mouth and anus of the adult.

Thus, then, we have two undoubted facts :

1. That the mouth of the Actinozoa is differentiated into one portion for the exit and another for the entrance of matter, and that this differentiation is carried so far as to give rise to two separate openings (Peachia).

2. In the development of *Peripatus capensis* the single opening of the gastrula elongates, then divides into two parts, an anterior part which becomes the mouth, and a posterior which becomes the anus of the adult.

The argument may here be briefly summarised :

1. The blastopore of Annelida, Arthropoda, Mollusca, and the mouth of Cœlenterata are homologous because (*a*) of the development (*b*) of the anatomical relations in each case.

2. The structure of the mouth of *Actinia* and the position of the mouth and anus within the primitive nerve-ring, which is supposed to be homologous with the circumoral nervous diffusion of *Actinia*, obviously suggests the derivation of the mouth and anus from a single opening like the mouth of *Actinia* by the completion of the fusion which is there beginning.

3. The blastopore of *Peripatus*, which by hypothesis is homologous with the Cœlenterate mouth and with other blastopores, actually passes through the *Actinia* phase.

Is this development primitive? If it is primitive, then as the mouth and anus of *Peripatus* are homologous with those of Annelids, my point is gained and we shall have to take the second alternative (p. 86), and suppose that the peculiar

¹ 'Phil. Trans.,' 1874.

behaviour of the blastopore in other cases is due to larval specialisation.

The structure and distribution of *Peripatus* all point to its being an extremely primitive type. We should, therefore, a priori, expect to find that its development showed primitive features.

In the second part of this paper I shall attempt to show that the very variable behaviour of the blastopore is explicable.

It is hardly necessary to point out that the stomodæum and proctodæum are, on the above hypothesis, structures of purely secondary importance, and that I am in agreement with Balfour's suggestion that the stomodæum and proctodæum are not in all cases completely homologous. He says ('*Comp. Emb.*' vol. ii, pp. 285, 286), "As a rule an oral and anal section of the alimentary tract—the stomodæum and proctodæum—are derived from the epiblast; but the limits of both these sections are so variable, sometimes even in closely allied forms, that it is difficult to avoid the conclusion that there is a border land between the epiblast and hypoblast, which appears by its development to belong in some forms to the epiblast and in some to the hypoblast." In other words, the development of certain parts of the alimentary canal may be so much delayed that they appear to arise from the epiblast.

This view is of special interest in considering the structures classed together as primitive streaks. As is well known, these structures are generally regarded as rudimentary parts of the blastopore (Balfour, Rauber). I would go further and suggest that it is an attempted development of that portion of the alimentary canal of the original ancestor which gave off the cœlomic pouches; that the portion which is not wanted in the development of simple larvæ of living animals is delayed, and consequently modified. I shall discuss this question at greater length in the second part of this paper.

I may conclude this part of my paper by describing briefly the ideal ancestor of the Cœlenterata and Triploblastic groups now under consideration, so far as the nervous system and mouth are concerned.

The Triploblastica and the Actinozoa are descended from a common two-layered bilateral ancestor which possessed an enlarged oral surface, an elongated mouth opening which by the adhesion of its middle portion was functionally divided into two openings, one at each end of the long axis of the mouth. The nervous system was generally distributed on the ectoderm all over the body, but was probably, as in living Actinozoa, especially concentrated on the oral surface. This type has persisted with certain modifications in Actinozoa, but in *Peripatus* and the other triploblastic forms under discussion the primitive mouth has completely divided, the body has elongated, and the nervous system has become especially aggregated in a ring (as in *Medusæ*) round the mouth and anus.

ON THE ORIGIN OF METAMERIC SEGMENTATION.

It has for some time been recognised that the body cavity or cœlom of the Triploblastica has been derived from diverticula of the archenteron. Such diverticula have been known for some time in the Echinodermata, *Sagitta*, Brachiopoda, *Balanoglossus*, *Amphioxus*.

The development of the body cavity in Annelida, Arthropoda, Vertebrata, and other cœlomate forms without diverticula has been supposed to be an embryonic abbreviation of this primitive process. I may quote the following passages from Balfour on this head.

“The formation of hollow outgrowths of the archenteron, the cavities of which give rise to the body cavity, can only be explained on the supposition that the body cavity of the types in which such outgrowths occur is derived from diverticula cut off from the alimentary tract. The lining epithelium of the diverticula, the peritoneal epithelium, is clearly part of the primitive hypoblast, and this part of the mesoblast is clearly hypoblastic in origin. . . . There can be but little doubt that the mode of origin of the mesoblast in many Vertebrata, as two solid plates split off from the hypoblast, in which a cavity is secondarily developed, is an abbreviation of the process observable in *Amphioxus*; but this process approaches

in some forms of *Vertebrata* to the ingrowth of the mesoblast from the lips of the blastopore.

“It is therefore highly probable that the paired ingrowths of the mesoblast from the lips of the blastopore may have been in the first instance derived from a pair of archenteric diverticula. This process of formation of the mesoblast is (as may be seen by reference to the summary (pp. 291, 292), the most frequent, including as it does the *Chætopoda*, the *Mollusca*, the *Arthropoda*,” &c. (*Comp. Emb.*, vol. ii, pp. 293, 294).

It has been supposed until quite recently that only one pair of diverticula are developed (except in the *Echinoderms* and *Balanoglossus*). But Hatschek has shown that in *Amphioxus*, a very primitive and isolated animal, a series of diverticula are formed, each diverticulum giving rise to a mesoblastic somite, or, to put it in another way, that the lateral walls of the archenteron become folded before the region of the archenteron which they limit become separate from the central part of the archenteron. *Amphioxus* is the only segmented animal in which the body cavity is known to arise directly from archenteric pouches; development of the *cœlom* in other segmented animals being regarded as an abbreviation of a similar process. Now, however, that we know that the body cavity of *Amphioxus* is developed from a series of archenteric pouches, it seems to me that we are justified in concluding on similar grounds that the abbreviated development in other segmented forms is derived from a similar process.¹

So that the difference between a segmented and an unsegmented animal consists in this, that in the former the archenteric walls become more folded than in the latter and give rise to a greater number of pouches, each of which becomes a mesoblastic somite. This is exactly the difference between a *Hydra* and a *Medusa*.

The similarity between the diploblastic *Amphioxus* embryo with a pouched gut (pouches giving rise to the mesoblastic somites) and an *Actinozoid* polyp or medusa suggests

¹ This has been already pointed out by Hubrecht; see Hubrecht, “On the Ancestral Form of the *Chordata*,” this *Journal*, 1883.

very forcibly the hypothesis that the mesoblastic somites of segmented animals are derived from a diploblastic Cœlenterate-like ancestor with folded gut walls, the folding having arisen as a result of the necessity for an increase in the extent of the vegetative surfaces in a rapidly enlarging animal.

I would venture, therefore, to suggest that Medusæ, Actinozoa and segmented animals are all derived from a common diploblastic ancestor, the Gastræa; that as this Gastræa increased in size it became necessary that some arrangement should arise by which a proper circulation of the nutritive matter to all parts of the body should be effected. For this purpose the gut wall became folded in such a way as to give rise to the radial and circular canals of Medusæ; to the mesenterial chambers (communicating peripherally by mesenterial stomata) of Actinozoa, and to the pouched diploblastic form from which segmented animals have arisen (I do not mean to assert that the segmented animals are the only animals which have arisen from a diploblastic animal with a pouched gut; vide below p. 94).

In a segmented animal the mesoblast is the first part of the body to show segmentation. The rest of the segmentation is moulded on the segmentation of the mesoblast. That is to say, the segmented organs, primitively at any rate, correspond in their segmentation with the somites. For each somite there is the nephridium, nerve ganglion, &c.

Supposing there is anything in the hypothesis I am putting forward, viz. that the somites of segmented animals are derived from gut pouches, which are homologous with the alimentary pouches of Cœlenterata, then it ought to be possible to explain on the same hypothesis the similar repetition of other organs.

In a segmented animal the following organs usually show the same repetition as the mesoblastic somites; the external appendages, the nephridia, the muscular system and the nervous system.

In Cœlenterata, both in Medusæ and Actinozoa—

(1) The tentacles correspond as a rule to the radial canals or to the mesenterial pouches;

(2) In *Medusæ* there are a number of pores leading from the circular canal to the exterior, placed on the oral side of the insertion of a radial tentacle, i. e. opposite a radial canal; in *Actinozoa* there are a number of openings in the body wall, putting the pouches in communication with the exterior (for function and possible origin of these pores vide below, p. 96).

(3) In *Medusæ* the circular striated muscles of the sub-umbrella are interrupted by the radial canals (Hertwig) and so broken up into a number of segments.

(4) In *Medusæ* there are sense organs which may be in connection with special nervous aggregations (*Acraspeda*) at the periphery of each radius.

In segmented animals—

(1) When segmented appendages are present (*Arthropoda*, *Polychæta*) they are simply processes of the body wall containing prolongations of the body cavity (*Peripatus*, embryonic *Arthropoda*).

(2) The *nephridia* are essentially pores leading from the body cavity to the exterior on the neural side at the base of the appendage.

(3) The muscular system is sometimes broken up into bands corresponding to the segments.

(4) The nervous system sometimes presents swellings, one for each somite.

I further venture to suggest that the greater number of the *Triploblastica* have arisen from *diploblastic* animals with a pouched gut; that in some of these, in consequence of the form taken by the body (elongation) and the consequent necessity for jointing and the persistence and greater development of the paired appendages, the body has become moulded, so to speak on this primitive gut pouching, which has therefore left its trace in the "segmentation"; that in unsegmented *Triploblastica*, in consequence of the action of causes of an opposite nature to those just mentioned, the pouches, after becoming separated from the gut, have become completely continuous with one another and left no traces. As a known instance of the latter process I may mention *Echiurus*

(Hatschek); in this animal (in the adult) most of the nephridia have been lost, the three pairs which persist (two pairs of brown tubes and anal vesicles) being enlarged and modified; the gangliation of the ventral cord is lost and there are no traces of the somites.

To sum up in a few words:—The Cœlenterata differ from segmented animals only in the fact that the alimentary or archenteric pouches (mesoblastic somites) and the alimentary canal do not become separate; and connected with this absence of a distinct cœlom is the low state of differentiation of such cœlomic structures as the excretory organs and the absence of a separate vascular system.

ON THE ORIGIN OF THE EXCRETORY ORGANS.

This part of my subject is so closely connected with the preceding that it is difficult to separate the two.

I have already referred to the Hertwigs' observations¹ on the marginal pores of Medusæ and the cinclides of Actinozoa.

Metschnikoff² was, I believe, the first to observe these marginal pores in Medusæ, and he regarded them as excretory; in this view the Hertwigs concur.

There is, then, this common feature in the anatomy of the Medusæ and Actinozoa; they both possess peripheral pores, putting the alimentary pouches in communication with the exterior.

In the ACTINOZOA they seem to have an irregular distribution as tentacular pores and cinclides (vide Hertwig). In the Medusæ, however, they have a definite position, one pore for each radial canal.

It seems an obvious suggestion that in the less specialised ancestors of Medusæ and Actinozoa these pores were distributed more or less irregularly as in the Actinozoa: that their position was determined by the habits of life and form of the animal.

¹ Vide Hertwig 'Organismus der Medusen,' p. 39, and Hertwig, 'Die Actinien.'

² This is a mistake. I am indebted to Professor J. Reay Greene for knowledge of the fact that these pores were first observed by Milne-Edwards, and described in his Memoir on *Æquorea*, 'Ann. d. Sci. Nat.,' vol. xvi, 1841.

It is worth while trying to picture how such pores may have arisen. In the supposed ancestor the two layers of the body wall were in more or less close apposition. The animal had no vascular system, and only one more or less differentiated opening, the primitive mouth. It would obviously be convenient that the excretory products should pass out as near as possible to the point where they were formed, or that there should be some arrangement of ducts by which they could be carried to the mouth opening. The latter arrangement does not appear ever to have been developed in the Cœlenterata, while the former arrangement is present, if not in all, still in a great number of Medusæ and Actinozoa. My knowledge of the physiology of these low animals is not sufficient to enable me to offer any hypothesis of how the pores arose. But I may suggest that in the first instance the endoderm cells were of one kind only, whose function was to eat (in an amœboid manner) the food swept into the body cavity through the mouth opening, and to prepare soluble nutritive juices which passed to the ectoderm. The excretion of nitrogenous waste products must have been carried on by all the cells of the body, inasmuch as there is no circulatory system. The immediate undigestible remains or solid excreta from the endoderm cells would be cast into the alimentary cavity. Originally the latter must have been swept to the mouth and so got rid of. As the animal enlarged in size, and no well-developed canal apparatus appeared by which these solid waste products of the alimentary cavity would be directly carried to the mouth opening, some of the endoderm cells at the periphery of the animal became specially modified to eat these products, and pass them through or between the ectoderm cells to the exterior. So a close connection became established between the cells of the ectoderm and the endoderm, which eventually led to the establishment of pores, the excretory pores. For an example of this kind of excretion through the ectoderm, I may refer to Eisig's¹ observations on

¹ "Die Segmentalorgane d. Capitelliden," 'Mitth. a. d. Zool. Stat. z. Neapel,' vol. i.

the Capitellidæ, in which the excretory organs end blindly against the ectoderm; their products, therefore, must pass to the exterior in some such way as I have suggested. If my suggestion be correct, it follows that the excretory organs were in their origin not specially organs for the excretion of nitrogenous waste products (each cell of the body being in close relation to the exterior did this itself) but for the riddance of the undigested and solid excretory products; and also that the excretory process was in its origin an intra-cellular process, i. e. temporary passages (*amœba*) were formed in the cells, through which the solid products passed to the exterior. This latter deduction is supported by the fact that in the higher animals the first formed excretory organs of the larva (*Hatschek*, *Polygordius*; *Caldwell*, *Phoronis*) have the form of delicate ducts attached to and opening through the ectoderm and ending in the body cavity, each in a simple cell; i. e. they are blind internally, and the excretory products in the body cavity must pass through the cell to get to the exterior.

Whatever view may be held as to the origin of the pores, the fact of their existence in the *Diploblastica* is undisputable.

At first irregularly arranged (a condition retained in *Actinozoa*, but more markedly in *Sponges*), they soon acquired a regular arrangement (*Medusæ*), and on the differentiation of the alimentary cavity into a digestive part (gut proper), and a circulatory and excretory part (*cœlom*), they remained in connection with the *cœlom*, which latter became again differentiated into parts purely excretory and connected with the pores (*nephridia*), and into the general vascular space for the circulation of the nutritive fluids passed into it from the endoderm cells.

Turning to the development of the excretory organs of the higher animals, we find that in the *Vertebrata* they arise as special parts¹ (not mere outgrowths) of the *cœlom*, and I have no doubt that this will be soon shown to be the case for the development of the *Invertebrate* excretory organs.

¹ Sedgwick, "Development of Kidney, &c.," 'Quart. Journ. of Mic. Sci.,' vol. xx, 1880.

Here, however, an apparent difficulty presents itself. In the Vertebrata the excretory organs (which probably were primitively segmental¹) open not to the exterior direct, but into a longitudinal canal which opens behind into the alimentary canal; while in the Invertebrata each of them opens direct to the exterior.

As an explanation of this difficulty I suggest that in the Vertebrate ancestors the primitive alimentary cavity acquired a well arranged system of ducts, by which the peripheral excretory matters were carried to the part of the alimentary canal near the hind end of the primitive mouth (future anus), that in consequence the excretory pores were not wanted, and were either never developed or if developed lost. As confirmatory evidence I may refer (1) to the circular canal of the Medusæ, which might easily be conceived transformed into the Vertebrate segmental duct, the excretory organs themselves being developed from the outer part of the radial canals; (2) to the method of development of the anterior and least modified part of the Vertebrate excretory organ. In the osseous fishes and Amphibia the segmental or pronephric duct arises as a groove of the body cavity, and is therefore a direct product of the archenteric endoderm. In most Vertebrates the development of the segmental duct is much modified; but I pointed out some years ago that we can only get an intelligible explanation of the connection between the excretory tubules and the duct of the kidney by supposing that they originally developed in continuity, both as specialised parts of the body cavity, and that this method of development is repeated in the case of the anterior part of the kidney of Ichthyopsida, and in a more modified manner in the Amniota.

Turning to the Invertebrata, we find that the development is not direct from the cœlom, but from solid masses of cells²

¹ Elasmobranchs. For discussion of this question, vide Sedgwick, "Early Development of Wolffian Duct," 'Quart. Journ. of Mic. Sci.,' vol. xxi, 1881.

² Very various accounts are given of the origin of the Invertebrate excretory organs. I reserve a critical examination of these facts until I have worked out the development of the nephridia of Peripatus.

derived from its walls. This may reasonably be explained in the same way as I have attempted to explain in my paper quoted above, the development of the hinder part of the Amphibian kidney (modified larval development).

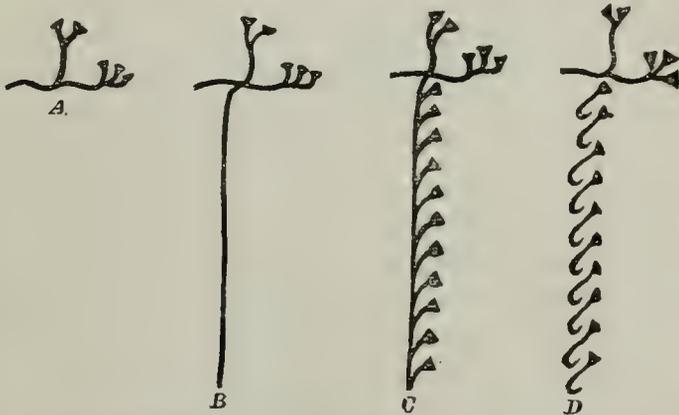


FIG. 1.—Diagram illustrating the development of the excretory system of *Polygordius* (from Balfour, after Hatschek).

The development of the excretory organs in *Polygordius* (woodcut, fig. 1) as described by Hatschek, is explicable on my hypothesis and so is confirmatory. The temporary longitudinal canal, which at first connects all the organs, is obviously a rudiment of the longitudinal duct found in the Vertebrata. The presence of this duct indicates that in the diploblastic ancestor of *Polygordius*, a system of canals was present in the cœlom together with the excretory pores.

ON THE ORIGIN OF TRACHEÆ AND GILL SLITS.

The view that tracheæ are derived from the cutaneous glands of a worm-like ancestor with a well developed middle layer is beset with so many physiological difficulties that I venture to suggest the following hypothesis, which agrees equally well with what we know of the development of tracheæ.

Tracheæ had their origin, like the organs so far discussed, in the diploblastic ancestor. In this ancestor they had the form

of simple ectodermic pits developed for the purpose of aerating those organs, whose position prevented their getting a sufficient supply of oxygen from the external medium or from the water circulating in the alimentary cavity. It must be remembered that there was no vascular system in this ancestor, and that therefore the living protoplasm of all parts of the body had to obtain its oxygen directly from the external medium. This method of aeration has persisted at the present day in certain *Medusæ* (sub-genital pits), in the *Tracheate Arthropoda*, and has left its trace in the *Vertebrata* in the canal of the central nervous system.

On this hypothesis the complicated distribution of tracheæ receives a physiologically satisfactory phylogenetic explanation.

The tracheæ were at first simple pits of ectoderm in a diploblastic animal, and they gradually became more complicated and branched as the other organs also became more complicated and folded.

The development of tracheæ fits in perfectly well with this view.

The tracheal respiration is then a primitive method of respiration, which has persisted in but few of the *Triploblastica*. It had its origin at a time before the vascular system was developed, and its essence consists in the fact that the living protoplasm takes its oxygen direct from the external medium. On this hypothesis the central canal of the central nervous system was a respiratory organ in a diploblastic *Vertebrate* ancestor without a well developed vascular system.

As soon as the vascular system became well developed, and the vascular fluid capable of carrying oxygen, the respiratory organs became localised. A special localisation of tracheæ is found in the pulmonary sacs of the *Scorpion*. In other animals external appendages have arisen. But in *Vertebrata*, *Balanoglossus*, and *Ascidians*, the circulation of water over the surface of the endoderm has been more developed. In the *Diploblastic* ancestor respiration was, as I have stated, partly effected by water circulating in the alimentary cavity. It entered by one end of the mouth and passed out

partly through the other end, and partly through the excretory pores of the alimentary pouches. Some of these alimentary pouches became, on the development of a vascular system, specialised as respiratory organs and retained their communication both with the exterior and with the alimentary cavity.

Thus gill slits are serially homologous with nephridia. This view of their origin is entirely supported by their development from pouches of the hypoblast of Vertebrate embryos, and by the fact that the kidney system in Vertebrata does not overlap them, but begins immediately behind them. A difficulty to this view lies in the fact that the cœlom does overlap the gill slits; but I think this difficulty is not a serious one when we remember that the cœlom being originally a vascular space had to extend in the region of gill slits as elsewhere, and that this extension might easily have proceeded either from the mesoblastic somite next behind the last gill pouch, or from a compression of the body in this region so that many somites (probably after separation from the archenteron) extended into the region of the gill slits.

SUMMARY.

The hypothesis suggested in the preceding pages are all based upon the gastræa theory, developed by Lankester and Hæckel. I take the gastræa as my starting point and do not inquire how the gastræa itself arose. I first (p. 82 to p. 91) by following the gastræa theory to its logical conclusion—and there seems recently to have been a disinclination on the part of some morphologists to do this—attempt to show that the gastræa mouth is not only homologous with the Cœlenterate mouth, but that the blastopore of the embryos of the Triploblastica is homologous with the gastræa mouth, and therefore homologous with the Cœlenterate mouth; and, finally, that if these necessary deductions from the gastræa theory are correct—and it should be noticed that the gastræa theory itself stands or falls with them—it necessarily follows (from the consideration of the *Peripatus* embryo) that the mouth and anus of the Triploblastica are derived from the gastræa mouth, i. e. Cœlenterate

mouth. I have pointed out that the blastopore in becoming the larval mouth must have become highly specialised and unable in most cases to repeat its ancestral history in the larval development, and that the behaviour of the blastopore becomes much more intelligible, though, I admit, not entirely so.¹ The remainder of my hypotheses are simply following the lines of the recent speculations on the origin of the nervous and muscular tissues. My speculations, like these, are based (1) on facts of Cœlenterate anatomy which have been mainly brought to light by the magnificent work of the Hertwigs. (2) On facts of embryonic development which have been for the most part long known, but have recently been added to in an important manner by Hatschek's work on *Amphioxus* and Balfour's discovery of the embryo of *Peripatus capensis*. The object of my speculation has been to extend Balfour's theory of the Triploblastic nervous system to the remaining systems of organs; in other words, I have attempted to show that the majority of the Triploblastica (I confine myself to the Annelida, Arthropoda Mollusca, Vertebrata and certain small groups, e.g. *Balanoglossus*, *Sagitta*, *Brachiopoda*) are built upon a common plan; and that that plan is revealed by a careful examination of the anatomy of Cœlenterata: that all the most important organ systems of these Triploblastica are found in a rudimentary condition in the Cœlenterata; and that all the Triploblastica referred to must be traced back to a common diploblastic ancestor common to them and the Cœlenterata.

¹ I shall return to a consideration of the behaviour of the blastopore in the second part of this paper.

PART II.

APPLICATION OF THE ABOVE HYPOTHESES TO THE VERTEBRATA, ANNELIDA, ARTHROPODA, MOLLUSCA, AND CERTAIN SMALLER GROUPS.

Fig. 7 represents a diagram of the ideal ancestor of all the above-mentioned Triploblastica. It closely resembles the common ancestor of the Cœlenterata but may be supposed a little more advanced in specialisation. For instance, the peripheral excretory pores (*o*) have a regular arrangement. This animal is supposed to have a bilateral symmetry shown in the gut pouches and in the excretory pores. It is supposed to have an elongated mouth partly differentiated into two parts, and the nervous system is generally diffused over the oral surface (which will henceforth be called the neural surface) with a tendency to specialisation into a narrow tract.

This ideal ancestor soon gave rise to two stocks, the first differences between which may be supposed to depend on the shape of the body.

In the one stock the mouth and anus (which soon became separated) remain on the neural surface, a præoral lobe was developed on the abneural surface of the body (fig. 12); this præoral lobe being carried first in movement became specially sensitive and the nervous system largely developed.

This stock is the Invertebrate stock. The præoral part of the nervous ring in consequence of the shape the body has taken becomes enlarged and sense organs largely developed in connection with it. The hinder præanal parts of the nervous ring have more or less approximated to each other, and are connected by commissures and become swollen at intervals where many nerves pass out to the locomotive organs (appendages). The postanal part of the ring becomes weak and often disappears, never having more than a commissural function (absence of nerve-cells in postanal connection of lateral nerve trunks of *Peripatus*, vide Balfour on *Peripatus capensis*).

With regard to the endodermal organs the alimentary pouches have lost not only their connection with the alimentary cavity and now constitute mesoblastic somites (fig. 8), but have also lost their peripheral connection with each other. The excretory pores persist and the part of the somites near the pore becomes developed into the nephridia.

In the other stock the body assumed a different shape, in consequence of which the mouth and anus became terminal (vide fig. 13, ideal). A projection overhanging the mouth then appeared on the neural surface and gave rise to a neural præoral lobe (fig. 14.) The præoral and postanal part (N^1 and N^2) of the nervous ring soon became inconspicuous and vanished. (It must be remembered that the nervous system of this stage of evolution was little, if at all, more developed than that of living Actinozoa.) This is the stock of the *Vertebrata* and *Balanoglossus*. The part of the primitive ring immediately behind the mouth is the most important in this stock; it is placed at the anterior end of the body, and therefore enlarges and develops sense organs. (Fig. 14.)

With regard to the endodermal organs the pouches have become differentiated into two kinds:

(1) Anteriorly a certain number retain their communication with the exterior and with the gut. (Fig. 10.)

(2) The majority, however, lose their connection with the gut and with the exterior, but remain connected by the peripheral canal, which behind retains (by means of a pouch?) its communication with the gut.

(3) A posterior pouch loses its connection with the gut and with the longitudinal canal, and gives rise to an abdominal pore.

The first group of pouches become the gill slits, the second become the cœlom, while part of each of them become differentiated into nephridia which opens into the longitudinal canal (pronephric or segmental duct). The last pair of pouches gives rise to a part of the cœlom and retains its connection with the exterior as an abdominal pore.

The further evolution of the *Invertebrate Stock*.—Paired

processes of the body wall (fig. 10), into which the cavities of the somites were continued are present (generally homologous with tentacles of Cœlenterata which correspond with the mesenterial chambers or radial canals). These become specially locomotive, and consequently muscular; hence the swellings (ganglia) on the nerve cords, each swelling corresponding to appendages, i. e. to a somite.

The septa between the pouches have more or less broken down, so that the cœlomic spaces become connected; the dorsal or ventral mesenteries, both or one of them, likewise break down.

Sometimes the appendages vanish (Gephyrea, Mollusca), the ganglionic swellings then disappear, and the only trace in the adult of the embryonic segmentation is seen in the nephridia. Many of these must, however, have vanished (according to Hatschek's account of development of Echiurus), and two or three or four pairs have become enlarged and alone persisted. It is interesting to notice the differentiation of the persisting nephridia in the Gephyrea into the brown tubes, which act as excretory organs and generative ducts, and the anal vesicles. This differentiation of the nephridia of different parts of the body is carried, as we shall see, much higher in the Vertebrata. In the Mollusca the disappearance of the somites has gone even further than in the Gephyrea, and the cœlom has become much modified. In Nautilus, however, a trace of the original segmentation persists in the nephridia and vascular system.

The development of *Sagitta* indicates that it is derived from an ancestor with three pairs of pouches, two of which retain their external pores (generative orifices). The Brachiopoda I at present leave out of special consideration.

Thus, the number of pouches (segments) in the Triploblastica varies in different cases, just as do the alimentary diverticula of the Actinozoa.

The further evolution of the Vertebrate Stock.—The central nervous system which is almost entirely derived from that part

of the primitive ring intervening between the mouth and anus, unites more or less completely across the middle line.

It and the superficial epiblast with which it is in connection become grooved; the groove becomes deepened and converted into a canal open close to the mouth in front and close to the anus behind (fig. 15).

The function¹ of this canal at this stage (the siphon stage) I have elsewhere discussed and ventured to suggest that it was in the main respiratory. (For the embryological counterpart of the siphon stage, see below, p. 75.)

It is important to notice that the nervous system of the Vertebrata becomes removed from the surface in quite a different way to that which obtains in the Invertebrata. In the latter it becomes removed from the surface by the ingrowth of mesoblastic tissue between it and the superficial layer in connection with which it arose. In the former, on the other hand, it never separates from the superficial epiblast from which it arises. The latter is involuted with the nervous mass and persists through life as the lining of the canal of the Vertebrate nervous system. This fact is of great importance in speculating on the origin of the Vertebrata, for it shows that the Vertebrate stock is a very primitive one, and must have separated from the Invertebrate stock before the nervous system of the latter separated from the epidermis.²

It will be observed that in consequence of the development of the præoral lobe (fig. 15 not marked enough), the mouth has become placed on the other side of the body, i. e. on the abneural side, and the neural canal has to bend towards this surface (the future ventral surface) in order to open into the mouth.

The water which was attracted by the ciliary movement

¹ For a discussion of the function of the canal at this stage, vide Sedgwick, "On the Original Function of the Canal of the Central Nervous System of Vertebrata," 'Proceedings of the Cambridge Philosophical Soc.,' vol. iv.

² This fact also holds for the cerebral ganglia of Peripatus; the invaginations of ectoderm become constricted off, and their cavities persist throughout life in the ventral protuberances of the brain.

divided at the anterior opening (fig. 15) into two streams, one of which passed through the mouth into the alimentary canal, while the other passed through the neural canal.

There was probably an olfactory sense organ developed from the epiblast close to the front end of the neural canal over which this water rushed.

The anterior convex wall of the neural canal now becomes bulged out forwards, and gives rise to a large anterior lobe, whose cavity opens behind into the neural canal, close to its opening into the mouth (fig. 16). This anterior lobe carries with it the olfactory epithelium, which, however, remains in connection with the mouth by grooves or canals. It becomes bi-lobed and transformed into the cerebral hemispheres of living Vertebrates.

The neural canal now closes both in front and behind, and assumes some other function than that of respiration. Behind the closing leaves no trace, while in front remains of the connection are seen at the present day in the infundibulum, and in the pituitary body.

It will be evident from the above hypothetical account of the origin of the Vertebrata, that I believe that the mouth and anus of the Vertebrata are homologous with the corresponding structures in the Invertebrate segmented animals. I have stated above, that I suppose that the blastopore of the Vertebrata is a specialised larval structure derived from the primitive mouth of a two-layered ancestor. It will be obvious also, that, according to my view, the position of this primitive mouth coincided with the middle line of the dorsal surface of the Vertebrate embryo, and that supposing it persisted in its primitive form in the embryo until the adult mouth and anus were formed, it would appear as a slit extending from the mouth anteriorly and ventrally round the front end of the head, along the whole surface of the medullary groove to the primitive streak round the hind end to the ventrally placed anus.

In the first part of this essay which deals with the blastopore, I have attempted to show that the mouth and anus of

segmented Triploblastica are in all cases derived from a primitive single mouth ; that this primitive mouth is represented in the embryo by the blastopore which should, if the phylogenetic development were repeated, give rise directly to the mouth and anus. I explained the fact that the blastopore so rarely does give rise to the mouth and anus by supposing that it became specialised as a larval¹ structure. My view is that in those animals in which it does not give rise to the mouth and anus, it functioned as the larval mouth while the animal was developing, and persisted until parts of the embryo were developed between it and the position of the mouth and anus of the adult, which parts had arisen in the phylogenetic history in the adult after the primitive mouth had completely divided into the mouth and anus. These parts never had been traversed by the original slit-like mouth, because they had appeared at a stage in evolution subsequent to the stage in which the mouth and anus were one. It cannot therefore be a matter of surprise if the blastopore does not elongate and bisect these later structures, which never had in the history of the animal been perforated by the blastopore. It is very difficult for me to express my meaning in clear language, and I am driven to take an instance to illustrate it. According to my view the cerebral hemispheres have appeared at a stage in the evolution of the Vertebrata long after the primitive mouth has become separated into the mouth and anus. The blastopore (primitive mouth), however, which has in some ancestral Vertebrate functioned for a considerable time in the larva as the only opening into the alimentary canal, persists and does not elongate to give rise to the mouth and anus which are not formed until after the cerebral hemispheres have appeared. It is now no longer possible, nor would it be advantageous if it could, that the specialised blastopore should elongate and give rise to the mouth and anus, the middle part closing up. The cerebral hemispheres have appeared, and they have never in the phylo-

¹ The larval stage, for which the mouth was specialised, has in the Vertebrata, as in many other animals, vanished ; it has probably been included in the embryonic period, and is rapidly hurried over.

genetic history been traversed by this slit. Consequently the only course open is that the mouth should be formed as a secondary perforation entirely independent of the blastopore.

From the nature of the case it is exceedingly difficult to bring forward any direct proofs derived from embryology in favour of this view. But I think it can be shown that there is reason to believe that the mouth and anus of the Vertebrata are placed in the line of the original blastopore. Amphioxus, so far as I understand its development, offers no support to my view, but the case is different with the Ascidians and the higher Vertebrata.

Weldon¹ has shown conclusively that the anus is formed within the area of the primitive streak, though after the disappearance of the latter structure. It is on all hands admitted that the primitive streak is a part of the original blastopore. I need, therefore, say nothing with regard to the anus.

The mouth, however, is a great difficulty. Dr. Dohrn has attempted to show that it is derived from a pair of gill slits. Now, without considering the embryological facts opposed to his view, which have been so ably pointed out by Balfour, I venture to suggest that it is exceedingly improbable that an animal should lose its mouth and develop a new one. It is surely, on a priori grounds, far more likely that it would change gradually the position of its mouth than that it should lose it and go through the labour of acquiring a new one, though that new one is supposed to be derived from pre-existing structures.

Turning to the actual development, I may mention here two facts which appear to me of importance.

(1) In Ascidians, Kowalewsky² has shown that the mouth at a certain stage is dorsal (neural), and that the neural canal opens into it (woodcut, fig. 2, V). The neural canal, also, at a slightly earlier, if not contemporaneous stage, opened behind into the gut. We thus find the hypothetical siphon stage of the evolution of the neural canal actually repeated in the

¹ 'Quart. Journ. of Mic. Sci.,' 1883.

² Kowalewsky, 'Arch. f. Mic. Anatomie,' vol. vii, 1871.

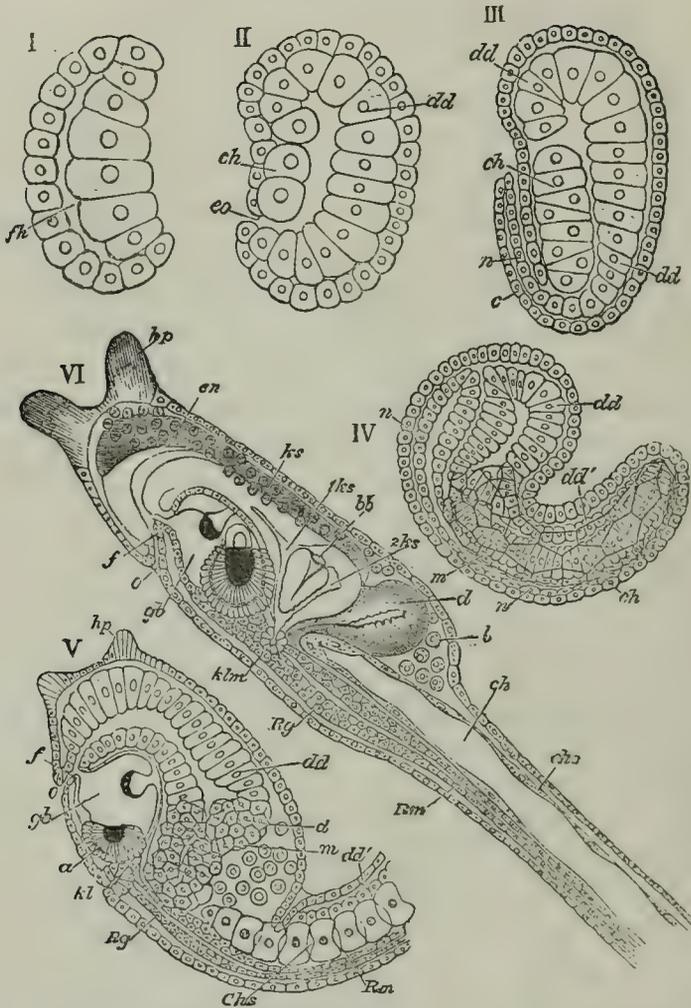


FIG. 2.—Various stages in the development of *Phallusia mammillata* (from Huxley; after Kowalewsky). I. Commencing gastrula. *fh*. Segmentation cavity. II. Late gastrula stage. *eo*. Blastopore. *ch*. Notochord. *dd*. Hypoblast. III. More advanced embryo. *n*. Neural tube. *e*. Epiblast. IV. Formation of neural tube completed. *dd'*. Hypoblast in tail. *m*. Muscles. V. Larva just hatched, the end of the tail is not represented. *a*. Eye. *gb*. Dilated extremity of neural tube, with otolith projecting into it. *Rg*. Anterior swelling of spinal division of neural tube. *f*. Anterior pore of neural tube. *Rm*. Posterior part of neural tube. *o*. Mouth. *chs*. Notochord. *kl*. Atrial invagination. *dd*. Branchial region of alimentary tract. *d*. Commencement of œsophagus and stomach. *dd'*. Hypoblast in tail. *m*. Muscles. *hp*. Papilla for attachment. VI. Body and anterior part of a two days' larva.

development of a living form. Salensky¹ long ago pointed this out. With a slight change in the shape of the anterior end of the body of the Ascidian larva in Kowalewsky's figure, the mouth would be removed from what we call the dorsal (neural) to what we call the ventral (abneural) surface. This would involve a flexure of the anterior end of the neural canal, and, I think, gives a clue to the phylogenetic meaning of the cranial flexure. The closure of the anterior pore of the neural canal is effected in such a way that it leaves a trace on the one hand as the infundibulum; on the other as the pituitary body. This homology has been often suggested. The persistence of the lower part of this pore, and its development from the epiblast of the buccal cavity, may be explained by supposing that the buccal end of the pore was glandular before the closure of the neural canal was effected. When this closure was effected, the buccal part remained in connection with the mouth as an excretory organ, a state of things persisting, according to Salensky, in Ascidians. It then acquired the new functions which it has at present, lost in the adult its connection with the mouth, and is known to us as the pituitary body. Meanwhile, some of the endoderm cells of the dorsal wall of the alimentary canal have become specially modified and separated from the rest as the notochord.

(2) In the Vertebrata the anterior end of the notochord is bent round, and becomes connected with the pituitary body at its extreme front end.² This condition of the anterior end of the notochord may be seen in the embryo before the pituitary involution is cut off from the ectoderm of the developing mouth—that is to say, the relation of the anterior end of the notochord to the ectoderm is similar to that of the hind end;

¹ Salensky, 'Zeitschrift f. Wiss. Zoologie,' vol. xxvii, p. 212; and 'Morphol. Jahrbuch.,' vol. iii, p. 600.

² The relation of the anterior end of the notochord to the pituitary body is somewhat complicated. For the knowledge of this fact I am indebted to Mr. Heape, who is at present engaged in investigating this very point. He informs me that the existence of the connection was known to the older embryologists (W. Müller).

behind it is closely connected with the front wall of the neurenteric canal; in front it is closely connected with the ectoderm of the developing buccal cavity.

At a still earlier stage, before the cranial flexure has appeared, the front end of the notochord is swollen, and runs into and is continuous with the front end of the medullary plate. This state of things I have myself observed at a stage before the medullary plate has begun to fold. Now, my view is that this connection of the notochord marks the site of the future mouth; that the site of the mouth—at first as in *Ascidians*—perforates the medullary plate, and is on the dorsal surface; that soon, however, this site bends round on to the ventral surface, and is eventually invaginated to form the buccal cavity and pituitary body. This hypothesis can easily be tested in the chick with the new Caldwell automatic microtome, but I regret that I have not hitherto found time to do so.

(Professor Hubrecht in his ingenious paper already quoted (this *Journal*, 1883), has instituted a comparison between the pituitary body and notochord of *Vertebrates* and the proboscis and proboscis sheath of *Nemertines*.)

The cerebral hemispheres appear relatively late in front of the notochord, and this fact fits in very well with the account of their origin which I have suggested. On this view *Amphioxus* has separated from the vertebrate stock before the appearances of the cerebral hemispheres.

The modification of the alimentary pouches, and the longitudinal canal connecting them, I have already alluded to. It only remains for me to point out that the cavities of the mesoblastic somites soon come to communicate ventrally both with each other and those of the opposite side; that the dorsal mesentery for the most part only persists, though the ventral mesentery remains in the region of the heart, liver, and behind in the region of the hind part of the body; that the nephridia become modified into groups, each with a special importance; the pronephros, or larval organ, is the first formed part of the kidney and atrophies in the adult; the hinder part differentiates

into meso- and meta-nephros; the meso-nephros becomes connected with the male generative organs, and loses its excretory function, while the metanephros persists as the functional kidney. I have, however, fully discussed the evolution of the Vertebrate excretory system in my papers already quoted on their development, and need not refer further to it here, except to point out that there is every reason to believe that the nephridia were originally segmental, one for each somite, that this segmental arrangement is, with the specialisation of the kidney, soon lost as it is in other organs.

ON THE STRUCTURES KNOWN AS PRIMITIVE STREAKS.

I may conclude this paper by a short review of these structures.

(1) They are always connected with the formation of the mesoblast.

(2) They are never, so far as I know, found in free larvæ. They are confined to the embryonic phase of development, and are only found in animals which undergo a considerable part of their development in the egg; in other words, only in eggs well-stocked with food yolk, or in eggs which have lost the food yolk. On the other hand, a primitive streak is not universally present in such cases, e.g. Cephalopoda, Elasmobranchii, Amphibia, Crustacea.

(3) They are always median and unpaired in their origin, but may in later development become grooved and present traces of a bilateral structure.

(4) They are always caused by rapid proliferation of cells, apparently from the epiblast.

(5) Their position seems to vary in different animals.

In Vertebrata, when present, the primitive streak is placed mainly behind the blastopore (according to Strahl¹ not entirely so in *Lacerta*, but this is not quite clear from his figures).

In *Peripatus* it is placed behind the blastopore, and, when the blastopore has divided, behind the hinder division (fig. 4).

¹ 'Arch. f. Anat. u. Phys.,' 1882.

In other Arthropoda in which a primitive streak is present, its position with regard to the blastopore cannot be determined; because the blastopore is not present in those cases in which there is a primitive streak.

With regard to the two first cases the blastopore of the Vertebrata closes, and the anus is subsequently (very late) formed within the area of the primitive streak.

In *Peripatus*, however, the hinder division of the blastopore does not close but travels slowly back over the area occupied by the primitive streak to its position at the hind end of the body.

I may here mention a fact which I observed last summer in the newt (*Triton cristatus*). In this animal the blastopore appears not to close but to persist as the anus. This statement is based on surface views of a large number of embryos from the stage when the egg is round until hatching. In all these stages I never saw an embryo without an opening at the hind end of its body. I very much regret that I have not had time to confirm this observation by means of sections.

If true it is most interesting as being the only known case in which the blastopore of Vertebrata actually persists as the anus.

In the case of larvæ which leave the egg at an early stage of development, no primitive streak is developed, but the mesoblast partly grows in from the lips of the blastopore, and partly arises as mesenchyme.

In *Amphioxus* fourteen pairs of somites are derived as hypoblastic pouches, the remainder are formed from hypoblastic tissue, the exact behaviour of which is not explained by Hatschek.

In those Vertebrata with a primitive streak, the anterior somites may be regarded as arising from hypoblastic mesoblast; but the greater part are formed from primitive streak mesoblast.

In *Peripatus*, the mesoblast arises behind the blastopore from the primitive streak, and grows forward as two bands, exactly as in worms; but it arises from a primitive streak.

I do not think any really satisfactory explanation can be offered at present of these facts. I venture, however, to suggest the following as an attempt at an explanation.

In many living Triploblastica the embryo leaves the egg at a very early stage as a larva; at a stage in which it is little more than a gastrula. Inasmuch as the parent of this ancestor has differentiated nephridia and muscles, &c., it is easily conceivable that the larva should precociously acquire as much of these organs as it requires. Hence mesenchyme. This larva is a small animal, and does not require a pouched gut; its hypoblast becomes specialised for digestion; now it would obviously hamper these exceedingly active larvæ if the gut repeated the phylogeny; at any rate, it is easily conceivable that it would be more advantageous if it were possible that the digestive cells should not have to undergo active developmental changes. Hence the mesoblast has to be formed in another way. The methods in which it is formed are, as is well known, various; it nearly always, however, originates at the lips of the blastopore, as the result of the proliferation of a cell, or cells which do nothing else but divide and give origin to the mesoblastic bands. This, as I have suggested above, may be looked upon as a modified development of that of the ancestral archenteron, which became pouched, and gave rise to somites (secondary invagination).

In those animals in which this larval phase has become merged in the embryonic development, this process is continued; but the area from which the major part of the mesoblast arises, i. e. from which the secondary invagination takes place, is larger. This may obviously be explained as being due to the fact that, the development being protected, it is not important that the amount of growing tissue present at any given moment should be as small as possible, in order not to hamper the larva.

On this view *Amphioxus* presents a most surprisingly primitive development, so far as its somites are concerned.

I need hardly point out that the prevailing order of develop-

ment, from before backwards, is just what would, a priori, be expected. The larva, being a free swimming animal, requires sense organs; it therefore develops its anterior part first and the organs belonging to this region of the body.

EXPLANATION OF PLATES X & XI,

Illustrating Mr. Sedgwick's Paper on the "Origin of Metamerie Segmentation."

Complete List of Reference Letters.

A. Anus. *a.* Anterior end of young embryo. *A. P.* Abdominal pore. *B.* Body-wall. *C. H.* Cerebral hemisphere. *C.* Longitudinal canal connecting pouches of archenteron. *E.* Ectoderm. *G.* Gill pouch. *H.* Heart. *K.* Nephridium. *K. D.* Longitudinal duct of Nephridia (segmental duct). *M.* Mouth. *M. A.* Coalesced part of primitive mouth. *ME.* Mesenteron. *me.* Edge of mesenteries. *M. S.* Mesoblastic Somites. *N.* Nervous system between mouth and anus. *N¹.* Præoral part of nervous system. *N².* Postanal part of nervous system. *N. C.* Neural canal. *Ne.* Posterior opening of neural canal. *O.* External openings of Nephridia. *P.* Pouches of archenteron. *P.* Præoral lobe. *Py.* Anterior opening of neural canal. *Si.* Siphonoglyphe. *Si'.* Upper end of siphonoglyphe projecting beyond general edges of lips. *St.* Wall of stomodæum.

Figs. 1—5.—Five young embryos of *Peripatus capensis*, ventral view. From drawings by Miss Balfour. *a.* Denotes the anterior extremity.

FIG. 1.—Youngest embryo, with slightly elongated blastopore.

FIG. 2.—Embryo, with three somites and elongated blastopore.

FIG. 3.—Embryo, with five somites. The blastopore is closing in its middle portion.

FIG. 4.—The blastopore has completely closed in its middle portion and given rise to two openings, the future mouth and anus. The primitive streak is deeply grooved.

FIG. 5.—Embryo, with about thirteen somites; flexure of hind part of body commenced. The remains of the original blastopore are present, as the mouth placed between the second pair of somites, and the anus placed on the concavity of the commencing flexure of the hind part of the body.

FIG. 6.—Stomodæum of *Peachia*, laid open so as to show the siphonoglyphe. This figure was very kindly drawn for me by Mr. W. F. R. Weldon. *T.* Tentacles. *St.* Wall of stomodæum. *Si.* Siphonoglyphe. *Si'.* Upper end of siphonoglyphe, projecting beyond the general edges of the lips. *B.* Body-wall. *me.* Edge of mesenteries.

EXPLANATION OF PLATES X & XI—continued.

FIG. 7.—Diagram of ideal ancestor of segmented animals, viewed as a transparent object from the ventral surface. *A*. Central part of archenteron. *P*. Pouches of archenteron (four represented on either side). *C*. Longitudinal canal connecting pouches. *O*. Excretory pores. *N*. Nervous ring. *M. A.* Dumb-bell shaped mouth. Ectoderm.

FIG. 8.—Diagram showing Invertebrate arrangement. Archenteric pouches separate from central part of archenteron (now called mesenteron). *E*. Ectoderm. *M. E.* Mesenteron. *M. S.* Mesoblastic somites. *K*. Nephridia. *O*. External openings of Nephridia. *M*. Mouth. *A*. Anus. *M. A.* Coalesced medium part of primitive mouth. *N*. Central nervous system; dumb-bell shaped like that of *Peripatus*. Wall of mesenteron, yellow. Mesoblastic somites, blue. Nephridia, red.

FIG. 9.—Diagram of Vertebrate arrangement from neural (dorsal, i. e. ventral of Invertebrata). Excretory pores are not developed, except behind, *A. P.*; and in front, *G*. Colours and letters as in Fig. 8, except *G*, gill pouch. *K. D.* Longitudinal duct of Nephridia, or segmental duct, opening behind into mesenteron. *A. P.* Pore retaining Invertebrate arrangement = abdominal pore. Mouth, anus, and nervous system not shown.

FIG. 10.—Diagrammatic transverse section through Invertebrate. Colours as before. *M. S.* Somite. *M. E.* Mesenteron. *N*. Nervous system. *K*. Nephridia.

FIG. 11.—Diagrammatic transverse section through Vertebrate. Colours as before. *N. C.* Neural canal. *M. E.* Mesenteron. *K. D.* Segmental duct. *K*. Segmental tube (nephridium). *M. S.* Somite.

FIG. 12.—Diagram of longitudinal vertical section of Invertebrate. Vascular system, red. *P*. Præoral lobe (hæmal). *H*. Heart. *N*. Nervous system. *N¹*. Præoral nervous system. *N²*. Postanal ditto. *M. E.* Mesenteron. *M*. Mouth. *A*. Anus.

FIG. 13.—Diagram of ideal intermediate type, with terminal mouth and anus. Letters and colours as in Fig. 12.

FIG. 14.—Diagram of arrangement of *Balanoglossus*, with neural præoral lobe and without præoral and postanal nervous system.

FIG. 15.—Diagram of arrangement of embryo *Ascidians* and *Vertebrata*. Nervous system folded in. (Siphon stage.) *P_γ*. Anterior opening of neural canal (site of the pituitary body). *N_α*. Posterior ditto. *N. C.* Neural canal.

FIG. 16.—Diagram of Vertebrate arrangement. *C. H.* Cerebral lobe.

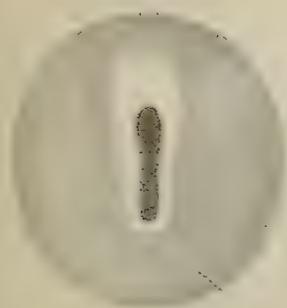


Fig. 1.

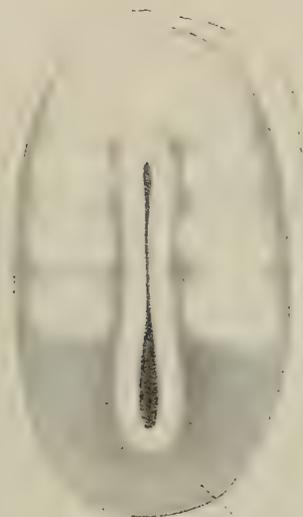


Fig. 2.

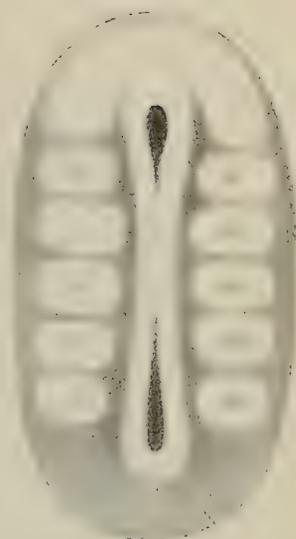


Fig. 3.

Fig. 4.



Fig. 5.



Fig. 6.



Fig. 7.

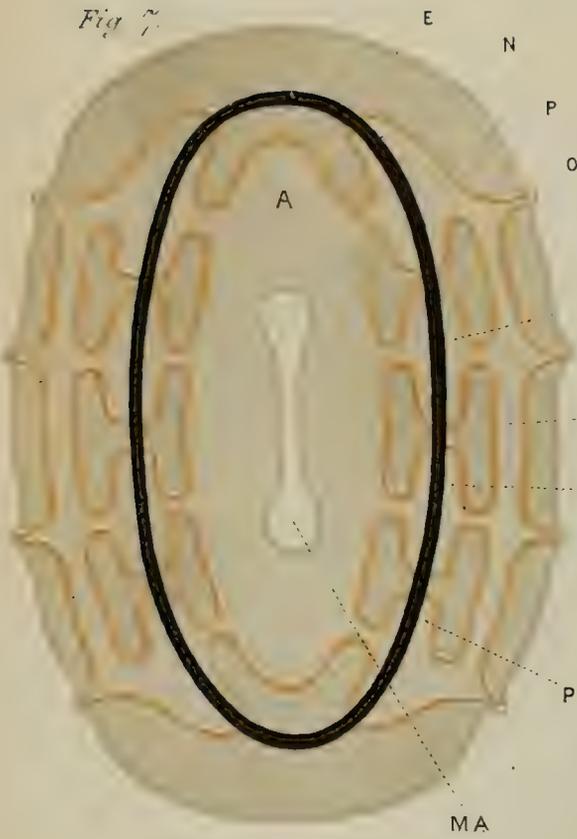


Fig. 8.

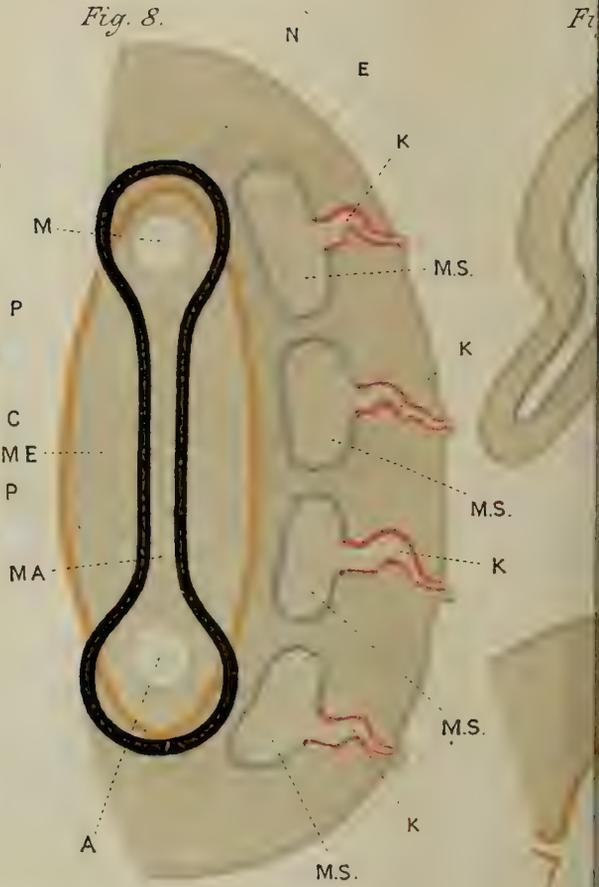


Fig. 15.

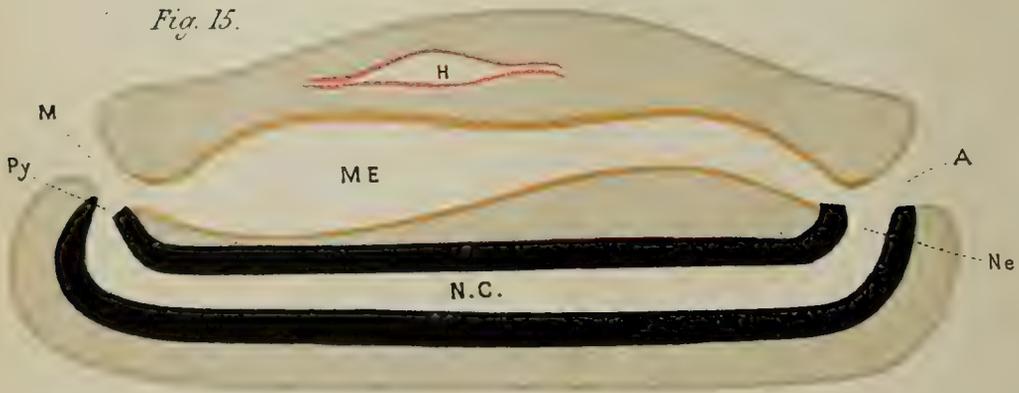
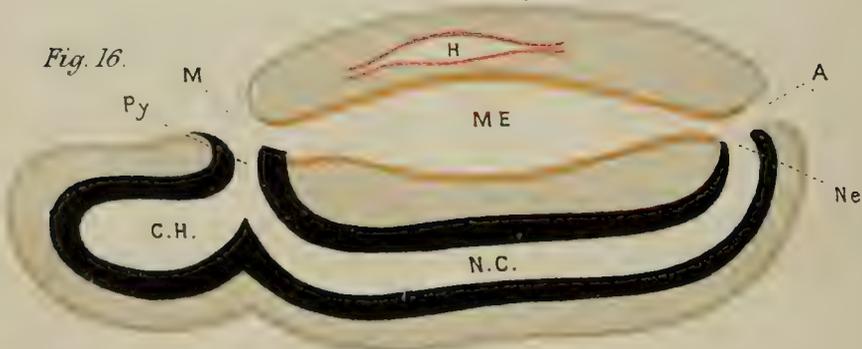


Fig. 16.



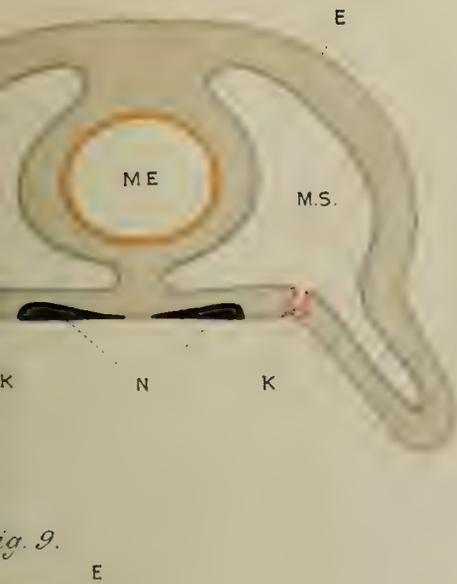


Fig. 11.

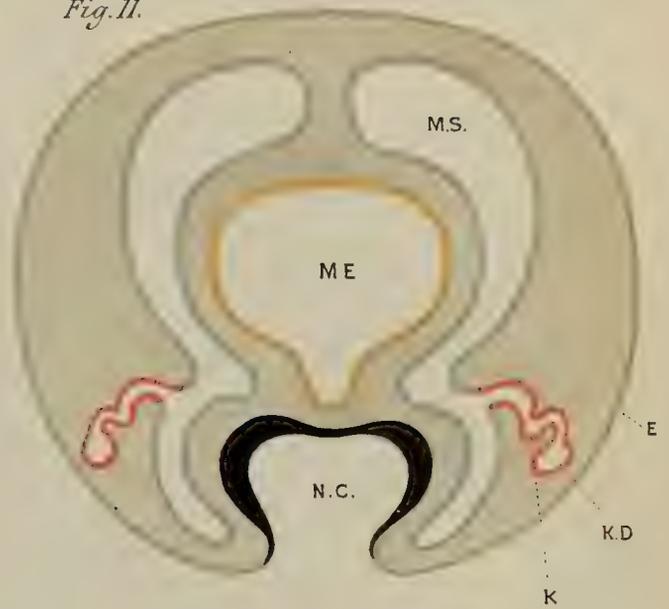


Fig. 9.

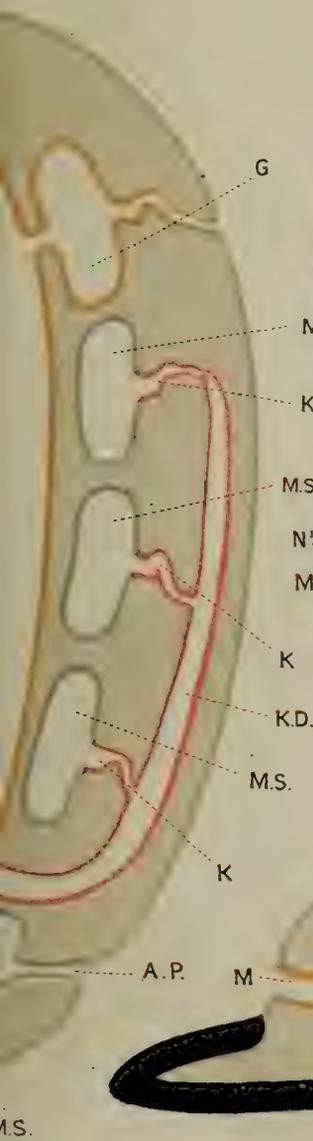


Fig. 12.

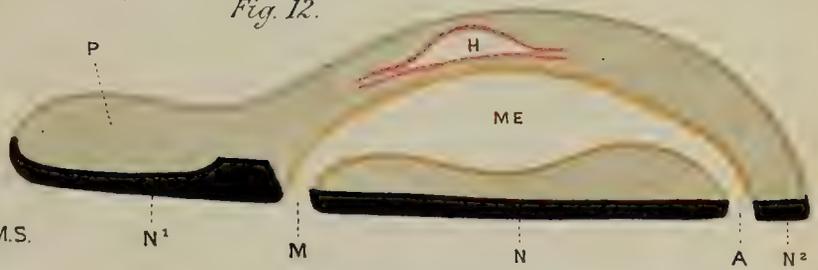
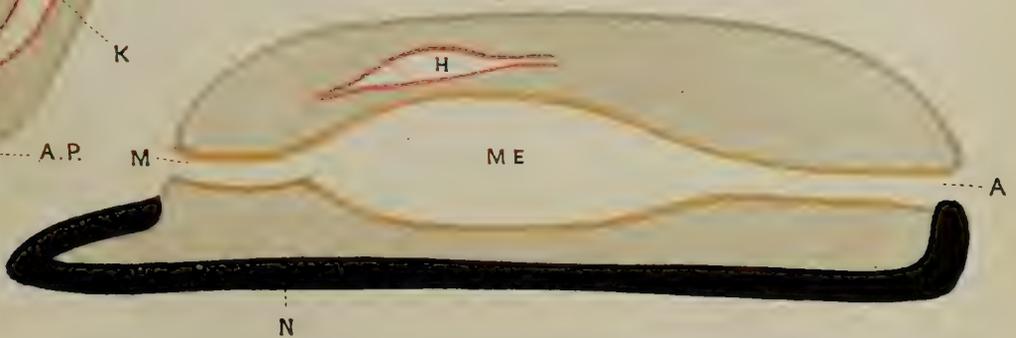


Fig. 13.



Fig. 14.



On the Head Kidney of *Bdellostoma*, with a
Suggestion as to the Origin of the Supra-
renal Bodies.

By

W. F. R. Weldon, B.A.,

Scholar of St. John's College, Cambridge; Demonstrator of Comparative
Anatomy in the University.

With Plate XII.

THE structure of the greater part of the kidney of *Bdellostoma* and the Myxinoids generally has been known since the time of Johannes Müller. It consists of a simple segmental duct on each side, which represents both the Wolffian and Müllerian ducts of the higher Vertebrates, giving off in its course a series of short tubules, each of which ends in a large glomerulus. These tubules are segmentally arranged, a single pair being present in every segment of the body in the anterior three fourths of the region lying between the anus and the hinder border of the pericardium. The relations of this system of simple segmental tubules, opening into a segmental duct, were described with perfect accuracy by Johannes Müller;¹ and no important additions have since been made to the account which he gave.

The whole system obviously represents the Wolffian body of the higher Vertebrates before the splitting of the segmental duct into Wolffian and Müllerian ducts.

In front of the kidney proper, however, there is on each

¹ 'Vergleichende Anatomie d. Myxinoiden,' Berlin, 1836—1845.

side a small, lobulated, apparently glandular body, whose structure is not so well known.

Each of these bodies is, in *Bdellostoma Forsteri*, from 20 to 25 millimètres long, and from 5 to 7 mm. broad; looked at with a simple lens, or with the naked eye, it is seen to consist of several small lobuli, which project into the cavity of the pericardium on the one hand, the whole gland being connected on the other with the connective-tissue adventitia of a great vein—the gland of the right side with the adventitia of the portal vein, that of the left with the anterior cardinal. In Pl. XII, fig. 1, an attempt has been made to represent the appearances seen on looking at the gland from the interior of the pericardium.

Johannes Müller¹ described these organs as consisting of “very small elongated lobuli, which are attached to blood-vessels, and are united with one another by loose connective-tissue. Each lobulus or cylinder consists . . . of a double row of cylindrical nucleated cells, like those of a columnar epithelium, the two rows of cells fusing with one another at the base of each lobulus. Between them run blood-vessels.”

No further statements on the subject were published till 1875, when Prof. Wilhelm Müller² gave an account of some observations made on *Myxine glutinosa*. In this animal Prof. Müller found that the bodies in question were connected each with the segmental duct of its own side, while the “double rows of cells” of Johannes Müller he found to be segmental tubules of a perfectly normal character, communicating by ciliated funnel-shaped openings with the pericardium, and provided with glomeruli.

The obvious inference was that these organs represented that anterior part of the kidney which is so well developed in many larval Ichthyopsida, and which is known as the “pronephros,” or “head kidney.”

Professor Müller, however, expressly states in his paper that his investigations were made upon very young animals.

¹ Loc. cit., part iii, pp. 7, 8.

² ‘Jenaische Zeitschrift,’ ix, 1875, pp. 111—113.

During the summer of last year, Mr. Sedgwick, while visiting the Cape of Good Hope, collected amongst other things a large number of very fine specimens of *Bdellostoma Forsteri*, var. *hexatrema*; and on his return to Cambridge he very kindly obtained permission from the Royal Society, for whom the specimens were collected, to allow me to examine their renal organs. On making a superficial examination of the so-called head kidneys, it was evident, as shown in fig. 1, that they were separated by a considerable distance from the anterior end of the segmental duct (*s. d.*), the only structures passing from one organ to the other being apparently blood-vessels. The subsequent preparation of a complete series of sections, the first of which passed through the anterior extremity of the "head kidney," the last through the beginning of the segmental duct, proved conclusively that with the exception of a rudiment to be spoken of presently, no trace of connection existed between the two organs.

Transverse sections showed the presence of a number of branched ducts, evidently the "pronephric tubules" of Wilhelm Müller, which opened on the one hand into the pericardium, and on the other into a central duct (figs. 3, 7).

These tubules (Pl. XII, fig. 7) had an average diameter of .06 mm.; there was no increase, but rather a diminution in diameter at the openings into the pericardium (figs. 2, 3, 7, *f.*). Each tubule was lined by cubical or columnar cells, the protoplasm of which was finely granular; each cell contained a large, elliptical, highly refracting nucleus, containing numerous coarse dark granules (fig. 7). At the mouth of each tube the columnar lining epithelium was continued into the flat pericardial epithelium (fig. 7, *p. c.*). No traces of cilia were found on the cells bounding the openings into the pericardium. Outside the lining epithelium was a well-marked basement membrane (fig. 7, *b. m.*); and outside this, in the spaces between the tubules, was a small quantity of connective tissue, and an exceedingly rich plexus of blood-capillaries (fig. 7, *b. c.*); so that during life a very considerable quantity of blood must be constantly passing between the tubules of the gland.

The tubules are for the most part aggregated into considerable lobuli; but here and there these lobuli become smaller, and in some sections tubules are seen which project separately into the pericardium; several such were cut transversely in the section from which fig. 3 was drawn, and they are seen to be separately invested by pericardium.

Passing inwards towards the centre of the gland, the tubules unite with one another, still maintaining the same characters, and not showing any appreciable change in diameter, till they finally open into a large central duct (fig. 3, c. D.).

The central duct is elliptical in cross section, its long diameter being about 0.5 mm., its short diameter 0.2 mm.; it may be single, as represented in the diagrammatic longitudinal section (fig. 2), or it may be divided into two or three anastomosing branches. It is lined by a single layer of very long and slender columnar cells, each about 0.07 mm. long by about 0.009 mm. broad, and having a large oval nucleus, with a dark outline and granular contents towards its outer end. The protoplasm of these cells is crowded with granules (fig. 4), and the free extremity of each is produced into a number of fine pseudopodia, round which are collected numerous granules (fig. 4). It is difficult to avoid the belief that the appearances described are due to the fact of the epithelium cells being actively amœboid during life, and of their pouring into the lumen of the central duct a quantity of secretion granules.

Outside this epithelium is a strong basement membrane (figs. 4, 6, *m*), which is connected with a tolerably compact coating of connective tissue, investing the whole duct.

The lumen of the duct was filled, in all my preparations, with a larger or smaller amount of material resembling a blood-clot, and consisting of a finely granular matrix (fig. 5), in which were contained oval nucleated cells, identical, so far as I was able to see, with the red blood-corpuscles found in blood-clots from the surrounding vessels. After a careful comparison, both with sections and with teased-out preparations of the blood-clots of the great veins, I have been unable to come to any other conclusion than that the central duct does actually con-

tain in preserved specimens a blood-clot, and, therefore, in the living animal blood.

The above description applies to the main body of the duct ; anteriorly it gives off a bunch of tubules, similar in all respects to those given off from its sides (see diagram, fig. 2), while posteriorly it ends in a mass of tissue (figs. 2 and 6 and 7), resembling the trabecular supporting tissue of a lymphatic gland. Fig 6 shows a portion of the periphery of a section through this tissue. It is seen to consist of a network of nucleated, branched connective-tissue cells, with elongated meshes, in which are several scattered blood-corpuscles (*b. c.*).

This lymphatic tissue is covered by a well-marked epithelium (B), forming the capsule of a large glomerulus (figs. 2 and 6, *gl.*), which lies close to it. From this glomerulus strands of blood-vessels pass off at frequent intervals into the lymphatic tissue. Such a strand is figured at *x* in fig. 6.

Owing to the impossibility of injecting a capillary plexus in an animal which has been preserved in chromic acid, I have not been able to obtain any very definite proof that blood can pass by these strands of vessels into the lymphatic tissue of the duct, and so into its lumen ; but I am strongly inclined to believe that this is the case.

I have occasionally seen capillaries leading directly from the lumen of the central duct, though I have been unable to follow them for any distance.

Until, however, further observations on fresh specimens can be made, I venture to think that I have shown tolerably good reason for assuming that the blood enters the lumen of the central duct of the "head kidney" through the glomerulus at its posterior extremity.

In some of my series of sections there is a considerable interval between the glomerulus just described and the segmental duct, which is occupied by nothing but connective tissue. In other, presumably younger specimens, I find traces of a continuation of the renal duct into the head kidney ; though in no case have I seen a continuous lumen in the connecting piece.

It therefore seems to me that the organ, whose anatomy I have just described, may very probably have resembled, in its earlier stages, the head kidney described by Wilhelm Müller, in which case it would have to be regarded as a part of the embryonic kidney, modified, in connection with the needs of the animal, to perform some unknown function in the elaboration or purification of the blood.

Such a modification of a part of the embryonic kidney is by no means unique amongst vertebrates. In the last paper which he wrote for this Journal, the late Professor Balfour¹ showed that, at all events, in a considerable number of Teleostei the head kidney becomes in the adult transformed into a mass of tissue resembling a lymphatic; and he subsequently discovered the same modification in the head kidney of *Lepidosteus*.²

This being the case, the question arises whether there may not exist, in all vertebrate animals, similarly modified portions of the primitive kidney. I believe that such structures are, as a matter of fact, to be found in the suprarenal bodies.

Though this view of the nature of the suprarenals is by no means in accordance with that generally held, none of the facts at present known concerning either their adult relations or their mode of development seem to me to disprove it.

First, as to their relations in the adult.

In Elasmobranchs there are, as Balfour has shown,³ two distinct sets of structures to which the name "suprarenal bodies" has been applied; first, a series of paired, apparently glandular bodies, arranged segmentally, and each connected with a sympathetic ganglion; these bodies, first accurately described by Leydig,⁴ are attached on each side to the dorsal wall

¹ Balfour, on the Structure of the Organ known as the Head Kidney in Teleostei, 'Quart. Journ. Micr. Sci.,' 1882.

² "On the Structure and Development of *Lepidosteus osseus*," by F. M. Balfour and W. N. Parker, 'Phil. Trans.,' 1882.

³ 'Elasmobranch Fishes.'

⁴ Rothen und Haie, Leipzig, 1852, 'Untersuchungen über Fische und Reptilien, 1853.

of the cardinal vein on each side, projecting into its lumen. They are best developed in the region of the mesonephros. In the region of the hind kidney, these bodies are replaced by a median, impaired structure, the lobuli composing it being closely connected on each side with the adjacent parts of the kidneys.

In Teleostei suprarenals are at all events frequently absent; or, as I would rather suggest, they are represented by the greatly metamorphosed head kidney described by Balfour.¹ In other cases, where suprarenals have been detected, they have always been attached to the surface of the kidney.²

In Amphibia, they are embedded in the substance of the kidney, either on its ventral surface (frog), or on its internal border (Triton); and they, like the kidneys, receive blood from the renal portal vein.³

In Reptiles⁴ the adult structure of the suprarenals strongly supports the view that they are modified portions of the mesonephros. In the male lizard, for example, they lie suspended in the mesorchium, between the testis and the seminiferous Wolffian tubules, with which latter they are closely connected.

In snakes the relations are very similar, while there is a remarkably well developed "adrenal portal" circulation.

In Birds and Mammals the highly specialised suprarenals retain, as might be expected, fewer traces of their mode of origin than is the case in lower forms.

It is evident, from the above sketch of their relations, that the only case among lower Vertebrates, in which any well-marked separation between kidneys and suprarenals occurs, is among Elasmobranchs, where the anterior paired portion of the suprarenal system is very distinctly separated from the mesonephros. This fact, however, is probably due simply to the extreme degree of specialisation undergone by the meso-

¹ Loc. cit.

² Ecker, 'Der feinere Bau der Nebennieren,' 1846.

³ Owen, 'Anatomy of Vertebrates,' vol. i, p. 543.

⁴ Braun, "Bau u. Entwick. d. Nebennieren d. Reptilien," 'Arb. Zool. Inst.,' Würzb., 1872.

nephros in the male, where it forms the complicated network of the epididymis, while in the female it by no means retains its primitive characters.

In Amphibians and Reptiles the intimate connection of the two sets of organs, and the great similarity between their means of blood supply—each receiving a portion of venous blood from the trunk or hind limbs, which passes through the organ (kidney or suprarenal as the case may be), to go to the vena cava—are surely most easily explained by supposing both organs to be parts of a single primitive structure, which are undergoing specialisation in different directions.

The very general absence of suprarenals, as separate structures, in Teleosteans, together with the existence of a peculiarly modified head kidney, has already been mentioned as leading to the same conclusion. The connection between the suprarenals and more or fewer of the sympathetic ganglia which exists in so many forms (Elasmobranchs, Reptiles, Birds, Mammals) can hardly be other than secondary.

The development of the bodies in question has been worked out in Elasmobranchs by Balfour,¹ in Reptiles by Braun,² and in Mammals by Mitsukuri³ and Janosik.⁴ In all these forms the first recognisable rudiment of a suprarenal is in the form of a compact mass of mesoblastic tissue, lying dorsal to the Wolffian body, between it and the aorta; and therefore just at the base of the ridge of the commencing generative epithelium. The cells composing this mass envelope a certain number of sympathetic ganglia; forming the cortical part of the adult suprarenal, while the cells of the ganglia form its central part. The question of the homologies of the cortical part of the suprarenals must, if it is to be settled by embryological evidence at all, be decided by observations on the mode of origin of the primitive cell-mass from which the cortical substance of the adult organ arises. On this point there is, however,

¹ 'Elasmobranch Fishes.'

² Loc. cit.

³ 'Quart. Journ. Micr. Sci.,' Jan., 1882.

⁴ 'Archiv für Mikroskopische Anatomie,' xxii Band, 1883.

very little evidence. Balfour and Mitsukuri give no definite account of the mode of origin of the cell mass, their observations beginning at a time when it is already formed. Braun considers that in Reptiles it commences by the formation of aggregations of cells round branches of the vena cava, while admitting¹ that "the rudiment . . . is often so close to the segmental tubules at their point of exit from the Malpighian capsules, that one is easily led to believe in the existence of a connection between the two." But the most striking observations on this point are those of Janosik, who finds that in Mammals there is, immediately in front of the Müllerian duct—that is, in the position of the head kidney—a number of solid cords of cells, connected at intervals with the peritoneal epithelium, and resembling exactly, so far as one can judge from the account given, a series of solid rudimentary segmental tubules. These strings of cells have no connection with the renal duct, but pass directly into the cortical substance of the suprarenals.²

Such fragmentary observations as I have hitherto been able to make lead me to hope that I may be able at no very distant date to show that, at all events in Reptiles and Mammals, the connection between the Wolffian body and the suprarenal is much more intimate than has generally been supposed. But should this hope prove unfounded, and should subsequent observations prove that the primitive mesoblastic rudiment arises simply as a mass of cells lying dorsal to the Wolffian body, this would by no means afford sufficient reason for asserting that the one structure had never been connected with the other, for we know that precisely the same kind of separation of two primitively continuous parts of the kidney has taken

¹ Loc. cit., p. 23.

² A short time before the appearance of Dr. Janosik's paper, Dr. Renson published in the 'Archiv für Mikroskopische Anatomie' (Bd. 22, p. 600), an account of some observations which tend to prove the presence, in earlier mammalian embryos, of functional segmental tubules in the position of the solid cords of Janosik. As neither of these authors figure the structures described, it is impossible to judge how far the one set may prove identical with the other.

place in the case of the metanephros, which, originally continuous with the hind end of the Wolffian body, now develops from a separate blastema which bears much the same relation to the hinder end of that structure as the suprarenal blastema does to its anterior end—both modes of origin being probably equally due to the delay which always takes place in the histological differentiation of an organ which is only functional comparatively late in life, and to the need of separating as soon as possible all such temporarily useless structures from the actively functional Wolffian body.

In the present state of our knowledge as to the function of the suprarenals, it may seem unjustifiable to assume that they have any essential connection with the blood system. At present, the only piece of direct evidence of their possessing any function at all is derived from the phenomena of Addison's disease, which seems, so far as I can learn, to be essentially due to alterations in the blood supply. The constant alterations in the behaviour of the red corpuscles, their refusal to form rouleaux, and the frequent difficulty in obtaining a good clot from the blood of patients suffering from this disease, are very suggestive.

An important indication of the probable need for some set of glandular structures in connection with the vascular system is found in the very general presence of such glands among the Invertebrates. It is not too much to say that in every group of Invertebrates in which the vascular system has been at all carefully investigated, glandular appendages to the vessels have been found, which can, from their anatomical relations, have no other function than that of elaborating some of the constituents of the blood. Thus, in Chætopods¹ there are very frequently present small cæcal diverticula of the great vascular trunks, which are coated with large, nucleated cells, loaded with granules; these cæca may simply lie loosely along the sides of the vessels, or may be collected into definite glandular masses lying on the floor of the body-cavity. In leeches,

¹ See Claparède, "Organisation des Annélides Sédentaires," and Cosmovici, "Les Annélides Polychètes," 'Arch. Zool. Exp.,' viii.

some glandular function may possibly be attributed to the large chains of "botryoidal connective tissue" in which many of the blood-vessels end.¹ In Echinoderms, the abundance of glandular cells in the cardiac plexus is probably a principal cause of the whole organ being regarded by many observers as an excretory apparatus. Among Molluscs, glandular structures, connected with the auricles, have long been known among Cephalopods, while the glands of the pericardium of Lamellibranchs, associated as they generally are with the auricles and afferent vessels, are probably of the same nature.² Among Arthropods, the "coxal glands," recently described by Professor Lankester,³ may perhaps prove to be connected with the vascular system, though the small blood supply at present recognised is certainly against such a view.

An investigation of the functions of these various structures in Invertebrates can hardly fail to afford an important clue to the real nature of the Vertebrate suprarenals.

EXPLANATION OF PLATE XII,

Illustrating Mr. W. F. R. Weldon's Paper "On the Head Kidney of *Bdellostoma*, with a Suggestion as to the Origin of the Suprarenal Bodies."

Complete List of Reference Letters.

B. Lining epithelium of a glomerulus. *b.-c.* Blood-corpuscles. *b. m.* Basement membrane. *c.* Blood capillaries. *cl.* Blood-clot in ventral duct of head kidney. *D. ep.* Epithelium of central duct. *gl.* Glomerulus at posterior extremity of head kidney. *g*¹. First glomerulus of functional kidney. *g*².

¹ Lankester, "On the Vasifactive and Connective Tissues of the Medicinal Leech," 'Quart. Journ. Micr. Sci.,' vol. xx, 1880.

² See Grobben, "Morphologische Studien über die Harnund Geschlechts apparat, &c., der Cephalopoden," 'Arb. Zool. Inst. Wien,' v Bd., 1883.

³ "On the Skeletotrophic Tissues and Coxal Glands of *Limulus*, *Scorpio*, and *Mygale*," 'Quart. Journ. Micr. Sci.,' Jan., 1884.

Granules attached to epithelium of central duct. *f.* Opening of head kidney tubules to pericardium. *Ly.* Lymphatic tissue at posterior end of central duct. *pc.* Pericardial epithelium. *S. D.* Segmental duct. *s. d.* Atrophied portion of segmental duct. *v. c.* Great vein. *x.* Strands of blood-vessels passing from glomerulus to lymphatic tissue of head kidney.

FIG. 1.—View of head kidney of *Bdellostoma*, from within the pericardium. The segmental duct is seen through the wall of the pericardium. $\times 4$ diam.

FIG. 2.—Diagrammatic longitudinal section through head kidney of *Bdellostoma*.

FIG. 3.—Transverse section through the middle of the head kidney. The tubules and the central duct are drawn, but the capillaries surrounding the tubules are omitted. Zeiss, obj. Δ , oc. 2.

FIG. 4.—Epithelium of central duct, showing granules thrown into its lumen. Zeiss, Obj. F , oc. 2.

FIG. 5.—Clot from central duct. Obj. F , oc. 2.

FIG. 6.—Transverse section through the lymphatic tissue at the posterior end of the central duct, showing strands of blood-vessels passing into it from the adjacent glomerulus. Obj. D , oc. 2.

FIG. 7.—Portion of periphery of a section similar to that shown in Fig. 3, showing the characters of the head kidney tubules and the surrounding blood-vessels. Obj. D , oc. 2.

Figs. 3 to 7 drawn with the camera lucida.



1. m.



2. x



Fig. 1.



Fig. 3.

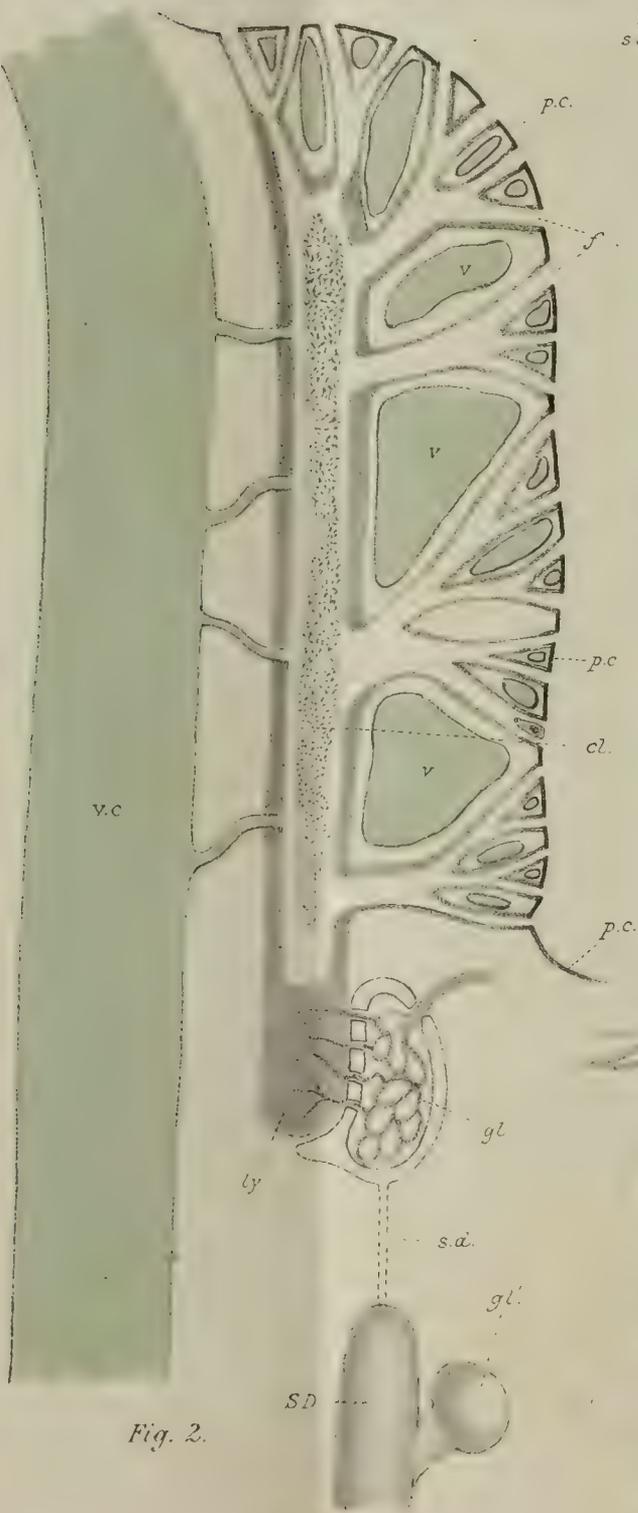
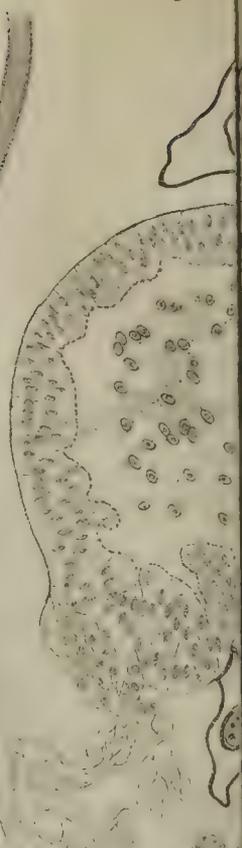


Fig. 5.

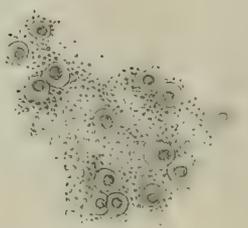


Fig. 4.

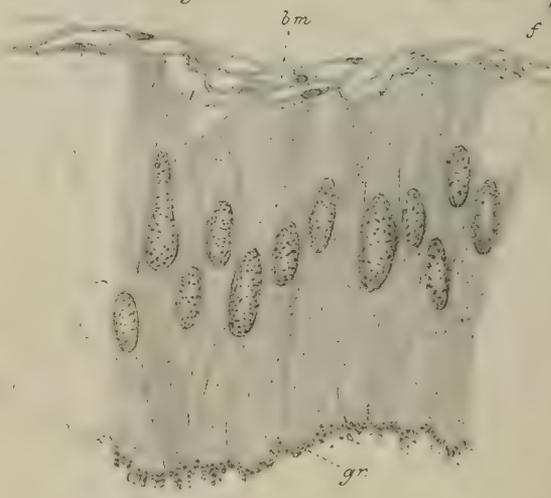


Fig. 2.

Fig. 7.

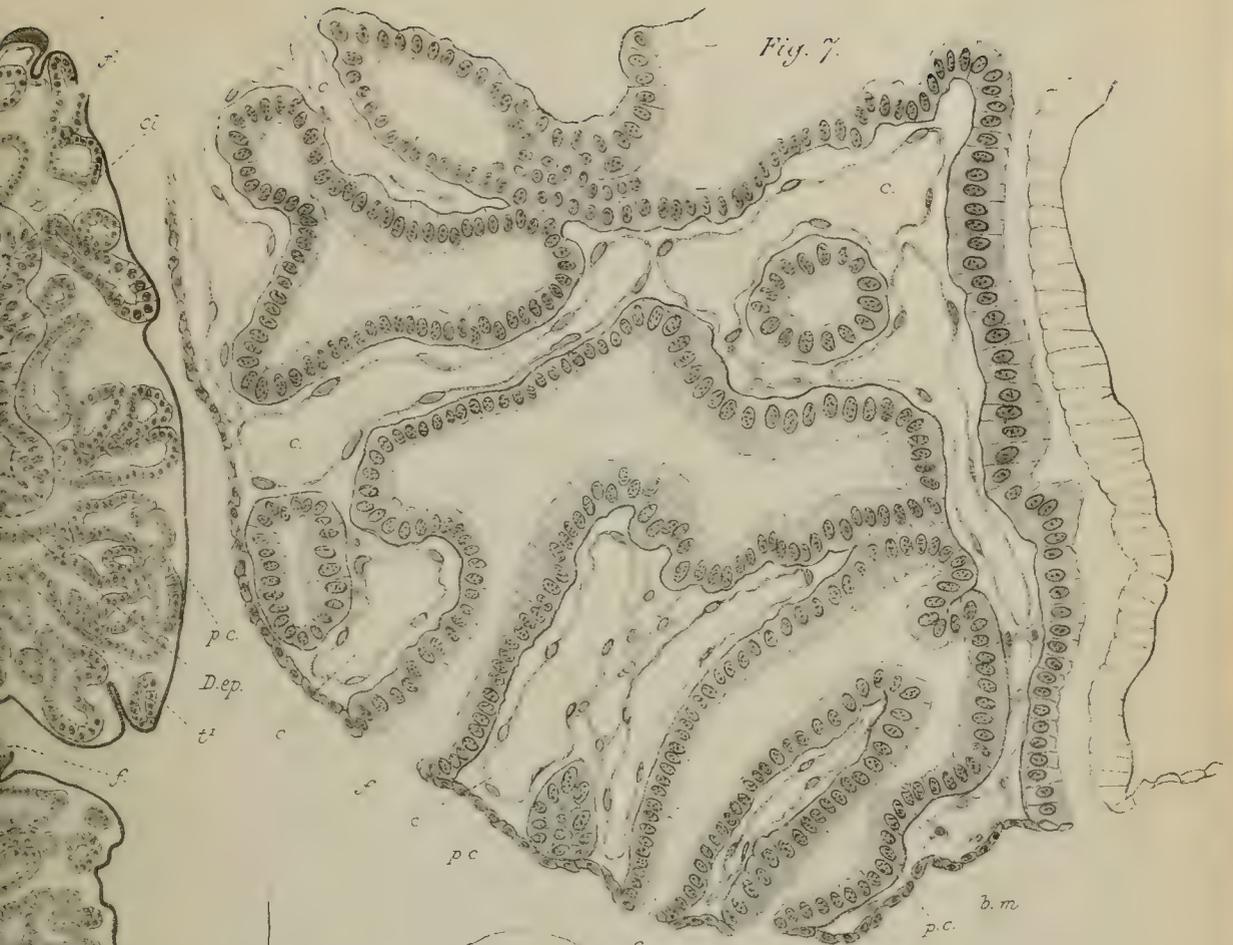
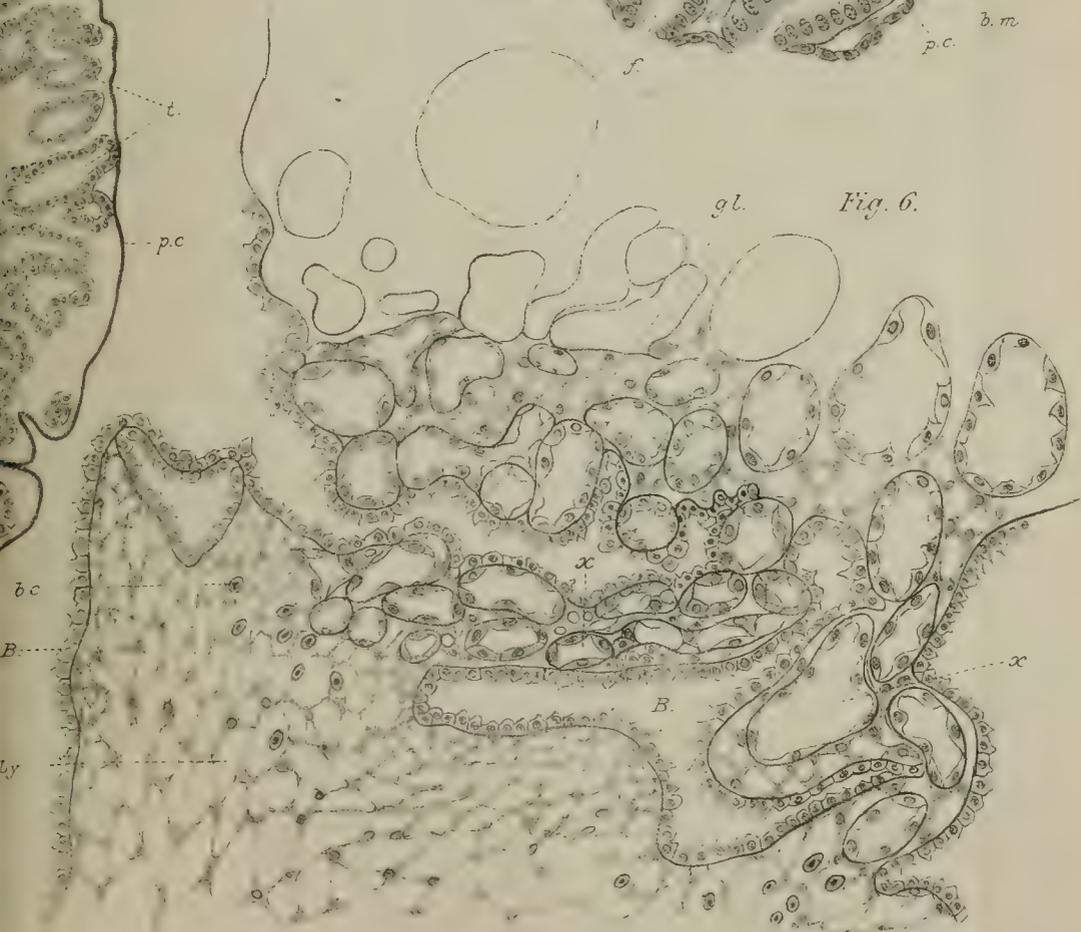


Fig. 6.



The Early Stages in the Development of
Balanoglossus (sp. incert.).

By

William Bateson, B.A.,
Scholar of St. John's College, Cambridge.

With Plates XIII, XIV, XV and XVI.

THROUGH the great kindness of Dr. W. K. Brooks and the Council of Johns Hopkins University, I was permitted to avail myself during the summer of 1883 of the facilities offered by the Chesapeake Zoological Laboratory, which was then situated at Hampton, Virginia. My best thanks are due to Dr. Brooks for the most valuable and manifold assistance which he gave me, both in my work and otherwise, during my whole stay at the Chesapeake Station.

While resident at Hampton I had an opportunity of obtaining a small species of *Balanoglossus*, which was to be found there in great numbers, buried in the sand at about half tide.

The characters of the adult animal agree very closely with the description given by Agassiz of the species named by him *B. Kowalevskii*; but as the species which I examined developed in a manner totally different from that which Agassiz has described for *B. Kowalevskii*, I am compelled to suppose that the two animals are not identical.

As will afterwards be shown, the points in which the anatomy of this animal differs from that described by Agassiz are of such a fundamental character that it is impossible to regard them as

specific variations. Most of these points are in accordance with the account given by Spengel for *B. minutus* and *B. clavigerus*.

The occurrence of another species of *Balanoglossus* (*B. aurantiacus*) on the North American coast has been mentioned by Professor Leidy (4) in a paper which I have not been able to see; this species may possibly be identical with the specimens I have examined. On the whole, therefore, I have thought it better to leave the name of the species an open question.

In this paper I propose to deal only with the history of the early stages in the development of the animal, up to the formation of the layers and the commencement of the nervous system, and to describe very briefly the most important external changes which occur in later larval life so far as I have been able to observe them. I hope shortly, however, to give an account of the subsequent development of the organs, and of some points in the anatomy of the adult.

The animals live at a depth of about eight inches below the surface of the sand, and are generally to be found with their bodies coiled in an even corkscrew-like spiral. The proboscis and anterior part of the branchial region are usually vertical, and the portion of the body posterior to the generative tract, which is about 6—9 inches long, is also, as a rule, not spirally disposed, but can be moved up and down a vertical shaft in the sand opening to the surface. By this shaft faecal matters, consisting mainly of sand and mucus, are extruded.

In this manner very characteristic conical coiled casts are thrown up, like that of the earthworm, the section of the coil being, however, elliptical. The whole body is from 8 inches to 1 foot in length.

Fertilisation and Segmentation.—The animals are dioecious. The ovaries lie in an irregular band along the dorso-lateral aspect of the animal, and the testes in the male occupy a similar position. The dehiscence appears to take place by a rupture of the body-wall in both sexes. When the eggs are laid they are small, ovoid, very opaque bodies, of a greyish

yellow colour, about $\frac{3}{8}$ mm. in length; enclosed in an elastic, close-fitting, transparent eggshell (fig. 1).

The spermatozoa dehisce in lobate spermatophoric masses; they have spherical heads, and short flagelliform tails, with which they swim actively.

No attempt at artificial fertilisation was successful, for though the spermatozoa attached themselves to the egg the result was an abnormal segmentation; the ovum dividing rapidly into a large number of isolated spherules, which subsequently died. It was, therefore, necessary to obtain naturally fertilised ova. These were to be found without much difficulty in considerable numbers deposited in the muddy sand which the adults inhabit.

After fertilisation the appearance of the egg alters; the ovum itself becomes spherical, and the eggshell increases in size, being separated from its protoplasmic contents by a considerable space (fig. 2). It is not at all clear by what processes this change is accomplished, since the shell of the ovarian ovum is a tough and apparently structureless membrane, which does not appear to alter in character after fertilisation.

The first furrow is formed in a median plane, dividing the ovum into two equal parts. It passes to a considerable depth (fig. 3). With regard to the subsequent segmentation I have no certain observations; for though some of the ova divided into four and eight nearly equal parts, these were obtained by artificial fertilisation, and the process of division was afterwards continued in an entirely abnormal manner as mentioned above.

Judging, however, from the characters of the blastosphere (figs. 4 and 18), and from the fact that yolk granules are uniformly distributed through the whole tissue, there can be little doubt that the segmentation is regular and complete.

In the next stage which was found a spherical blastosphere was formed (fig. 4). The walls of this were opaque, but the outlines of the cells composing them could be faintly distinguished in a surface view.

Subsequent External Changes.—Before proceeding to describe the internal structure of this and the following stages it will perhaps be best to describe the changes which

occur in the external appearance of the embryo from this point until the form shown in fig. 16 is reached.

For convenience of reference I shall allude to the Stage shown in fig. 7 as Stage A; in fig. 9 as Stage B; in fig. 10 as Stage C; in fig. 11 as Stage D; in fig. 13 as Stage E; in fig. 14 as Stage F; in fig. 15 as Stage G; in figs. 16 and 17 as Stage H.

The Gastrula.—The blastosphære is at first spherical. It next assumes an elliptical form and gradually becomes flattened on one side (figs. 18 and 19). The flattened side next becomes concave and is rapidly invaginated, forming a cup-shaped gastrula (figs. 5, 20, and 21). This gastrula is radially symmetrical and the blastopore is still circular. The edges of the blastopore then approximate, and during this process the embryo grows unequally, causing one of its axes to elongate slightly (figs. 6 and 7). As a result of these changes the blastoporic aperture forms a very short slit, placed in a depression lying rather towards the pole which afterwards forms the posterior end of the animal. As the closing of the blastopore proceeds the whole surface of the body becomes covered with very minute cilia and a ring of larger cilia develops round the blastopore which is placed in a slightly eccentric position within it (figs. 7 and 8).

Stage A is thus reached. When the cilia appear, the larva rotates in the eggshell on the blastoporic axis in the direction of the hands of a watch.

Stage B is attained merely by an elongation of the axis at right angles to the plane of the ring of cilia, whose position makes the determination of the approximate position of the blastopore possible throughout larval life (fig. 9). An examination of a series of sections through a larva of this stage shows that the blastopore has closed.

The changes from Stages B to F occupy about fifteen hours. In Stage C the long axis is still more marked.

In Stage D (figs. 11 and 12) the body is further elongated and slightly flattened, the flat surfaces being afterwards shown to be dorsal and ventral respectively. A transverse constriction

has appeared (fig. 12, g.) dividing the body into two nearly equal parts. Subsequent changes show that this groove marks off the region which is to form the proboscis from the rest of the body.

As this groove increases in depth a second groove is formed behind it (fig. 13, c. g.). The area between these two grooves forms the collar. The proportions of the body have also altered considerably; the length of the proboscis being now only about a third of the total length of the animal. At this stage arises also a tuft of long flagelliform cilia at the apex of the proboscis. No eye-spots are present, however, at this or at any subsequent stage.

The animal remains in this condition for some hours and is generally hatched without the occurrence of any further alteration. The time of hatching is, however, quite irregular. Larvæ may frequently be found swimming freely whose organisation is not much in advance of Stage C, and on the other hand, I have seen them in the condition of Stage G still enclosed in the eggshell.

While in the eggshell from Stage C onwards the larva swims about very rapidly, rubbing the membranous shell with its anterior end until it gives way, and the animal escapes. On leaving the egg it does not swim at the surface as pelagic larvæ do, but creeps about in the mud, burrowing with its proboscis, in the walls of which muscle fibres soon appear (*v. infra*), and also propelling itself by means of its ciliated band. If placed in a beaker of water it sinks to the bottom at once. Two specimens of Stage H were, however, taken on one occasion in the surface-net, but from the molluscs and other creatures present with them, it was clear that the net had been allowed to drag along the bottom. As the water was never more than four feet deep at high water this was easily possible, and occurred continually at Hampton.

Stage F.—The next feature of importance which generally occurred shortly after hatching, was the appearance of a longitudinal groove in the middle dorsal line of the collar (fig. 14, *n g*). This groove is a temporary structure, only lasting

two or three hours. As will be shown when the internal development is described, the appearance of this groove occurs at the time of the delamination of the dorsal nerve-cord in this region of the body.

Stages G and H.—Simultaneously with the disappearance of this neural groove, two pores are to be seen perforating the skin behind the collar, being placed in a dorso-lateral position, one on each side of the middle line. These pores in which long cilia may be seen working, constitute the first pair of gill slits (fig. 15, *g. s.*). In this stage the collar has become much shorter, and the cilia of the band are so densely crowded as to give an appearance of a thickened ring in preserved specimens. After the formation of this pair of gill slits no further external change of importance is to be noted in any of the oldest larvæ which I found. The proboscis becomes longer and more muscular, and the body increases somewhat in size; the region of the body behind the ring of cilia especially elongates. The ring of cilia may, moreover, in a profile view, be seen to lie no longer in a truly transverse direction, but rather obliquely with its dorsal limb anterior to the ventral portion (fig. 17). The body is usually somewhat bent upon the ventral surface while the animal swims. This feature is usually exaggerated after death, and in consequence from the dorsal side the whole length of the animal is not seen (fig. 16).

The mouth is formed in Stage F as a minute pore placed on the ventral surface in the groove which divides the proboscis from the collar; from its position and small size it is not visible when the animal is examined as a whole. The formation of the anus occurs rather later by a perforation of the skin at the posterior end; it also is best seen in sections. In position it is approximately coincident with the point at which the blastopore finally closed.

I have not hitherto been able to obtain any older larvæ whose external features differ essentially from Stage H. Judging from the fact that at this stage a considerable increase in size takes place, and from the commencement of several important internal structures at this time, it seems probable that the

animal passes a prolonged period in this condition without any external modification, but this, of course, is quite uncertain. The larvæ were all found between July 20th and September 6th, and I am in hopes that at some other season of the year it may be possible to find the remaining stages between H and the adult condition.

Very considerable structural modifications must, of course, occur before such a larva as Stage H can assume the external form of the parent. These changes must consist chiefly in a great increase in the length of the body, in the number of the gill slits, &c. ; but probably the point of greatest interest lies in the subsequent development of the collar, which in the adult presents an appearance considerably different from that of the same structure in this larva, both in position and extent.

Internal Structure.—Having dealt with the external appearances, I will now describe in detail the internal structure from the blastosphære to Stage E.

An account of the formation of the mouth and nerve-cord I also introduced into this paper, but I propose to leave the description of the other changes in Stages F, G, and H, together with an account of some points in the anatomy of the adult, to be given in a subsequent paper which I hope to prepare shortly.

The characters of the blastosphære are shown in fig. 8. The walls are formed of a layer of single cells enclosing a segmentative cavity. This individual has already become slightly flattened, having previously been spherical as shown in fig. 4. Simultaneously with this compression a differentiation has commenced between the cells which are to form the epiblast (*E*) and those which will be invaginated to constitute the hypoblast (*H*). The former are smaller and less granular than the latter, which are large and contain many yolk particles in their peripheral ends. The central ends of the hypoblastic cells have a characteristic amoeboid appearance ; between these two parts of the cell the large dotted nucleus is placed.

Between the epiblastic and hypoblastic portions the walls are composed of indifferent cells.

The segmentation cavity (*s. c.*) is a large empty space; and neither at this, nor at any subsequent period of development do any mesenchyme cells appear in it, the mesoblast arising entirely from the walls of the archenteron, as will be afterwards described.

By a progressive differentiation the plano-convex form shown in fig. 19 is reached. At this period the disparity in size between the cells of the two regions is still more marked, and no part of the wall is composed of indifferent cells.

Figs. 20 and 21 represent the appearances seen in transverse sections of rather older Gastrulæ. No histological change of importance has taken place. In fig. 22 the fusion of the layers which accompanies the closing of the blastopore is shown. This region of fusion is seen in a series of sections to be somewhat longer in one direction than in the other, conformably with the external characters of Stage A.

In Stage B the blastoporic fusion still persists, but the hypoblast is nearly separated from the epiblast, only remaining connected with it for a very small area, which marks the point at which the blastopore finally closed. When Stage E is reached this area is still further reduced, but can be traced until Stage D.

In a section through the anterior end of this stage, the epiblast is seen to be composed of large cuneiform cells containing granules, whose outer ends are covered with very minute cilia. Between their internal ends spaces are shown, which are no doubt due to contraction of the protoplasm caused by reagents; similar spaces are also observable between the cells of the hypoblast. At the front end of this larva these latter take a more amoeboid character than in the middle and posterior regions (fig. 25, *H'*).

The epiblast is closely applied to the hypoblast in the anterior half of the larva, causing the segmentation cavity to be obliterated. The hypoblast of the middle region is seen to be more columnar in character than that of the anterior region, while the other appearances are the same (figs. 23 and 25).

A section through the blastoporic tract of the same larva is given in fig. 24. The segmentation cavity is here still large, and the walls of the archenteron are found fused with the epiblast at a point at one side of the larva which is otherwise a radially symmetrical, double-walled cylinder. At this point the archenteric cavity also slopes to an angle which persists after the complete separation of the walls from the epiblast. By the persistence of this appearance the point at which the closure of the blastopore is completed can be identified as being nearer to the dorsal surface of the animal, being at the junction of the dorsal and posterior surfaces. The archenteric cavity contains a quantity of coagulum at this and subsequent stages.

The transverse constriction which now arises (fig. 11, *g*) lies at the junction of the columnar portion of the hypoblast with the more amœboid part, mentioned above as being found in the anterior end. It is, at first, merely a groove in the epiblast, which coincides with a similar depression in the hypoblast. As will presently be described, the walls of that part of the archenteron which lies in front of this groove become constricted off to form the unpaired anterior body cavity; and the commencement of the consequent histological differentiation may be already perceived in the more amœboid character of the hypoblast cells in this region (fig. 25, *H'*).

The internal relations of the parts between Stages C and D are shown in fig. 25, which is constructed from the same series of transverse sections from which figs. 23 and 24 were taken.

In Stage D the hypoblast separates completely from the epiblast, and the appearances to be described in Stage E begin to be present.

Stage E—Formation of the Mesoblast.—Leaving the larva in the form of the closed, two-walled cylinder described above, whose cavity has already begun to become constricted into two parts, the anterior being lined by somewhat amœboid, hypoblastic cells, and the posterior by regular, columnar hypoblast, a description of the changes to be seen in Stage E will be given in detail.

Figs. 26—34 are drawn from a series of sections through a larva at this stage, of which fig. 13 is an external view. It will be remembered that the body now consists of three regions. (1) An anterior lobe which on the formation of the mouth will be seen to be præoral; (2) a narrow area between two grooves, which will be afterwards spoken of as the collar; and (3) the rest of the body, which will be alluded to as the trunk; this trunk portion is secondarily divided into an anterior and posterior region by the transverse band of cilia.

The dorsal and ventral surfaces may now be distinguished in section, as a thickening of the epiblast is already to be found in the collar on one surface. This structure, which subsequently forms part of the central nervous system, marks the middle dorsal line. The body is now elliptical in section, the long axis of the ellipse being horizontal.

The epiblast now consists of small elongated cells, which are arranged from two to three deep, bearing minute cilia on their peripheral ends. This layer is of uniform thickness and constitution over the whole body, with the exception of the small linear area in the middle dorsal line of the collar, mentioned above. In this region it is somewhat thicker, but no differentiation has occurred among its cells. Moreover, the cells which carry the transverse band of cilia are slightly larger and more columnar than the rest.

The detailed structure of these epiblastic cells is shown in fig. 31, *a, E*. They are small, dense cells, containing a considerable quantity of granules, which stain deeply with reagents. The nucleus is proportionally large, and also contains granules.

The mesoblast arises at this period of development. It is formed directly by differentiations of cells belonging to the archenteron. These differentiations occur in five regions. The first comprises a median and primitively unpaired tract in the anterior end, which forms the lining of the body cavity of the præoral lobe. Behind this anterior body cavity a pair of mesoblastic differentiations occur in the region of the collar, constituting lateral outgrowths of the archenteric walls, each con-

taining a cavity which communicates directly with the cavity of the archenteron. Behind these, again, is another pair of regular archenteric diverticula, in the region of the trunk. This mode of origin of the mesoblast, which will be made clear by the diagram (fig. 40), will now be described in detail. It will, however, be perhaps simplest to describe all the remaining parts, beginning at the anterior end of the animal, and proceeding backwards.

1. The Anterior Body Cavity.

On reference to the longitudinal section of the larva between Stages C and D (fig. 25) it is seen that the cells lining the front of the archenteric cavity had a character different from that of the remaining hypoblast. When Stage E is reached this differentiation has greatly increased, and the cells which line this anterior region may now be seen to have an entirely peculiar appearance. They are still closely applied to the epiblast (fig. 26, *M'*) at one end.

This peripheral end is broad, and is continued into a narrower portion, which again dilates to form the round head of the cell, which projects into the cavity of the præoral lobe. These round central extremities, in which the nuclei are generally to be found, are continually budding off round cells into the cavity in which they lie. At this period, however, this process of proliferation is only commencing, and the cavity of the præoral lobe is therefore lined by a layer of cells, which is for the most part only one cell deep.

In the peripheral ends of these cells, which, as will be seen hereafter, are destined to form part of the mesoblast, an appearance may be noted which, together with the absence of granules in this part of the cell, gives it a look of semi-fluidity. This appearance is characteristic of a large part of the hypoblastic and mesoblastic tissue in a larva of this age, and disappears about the time at which the mouth is formed.

Whether it is actually due to the presence of fluid contents in the cells or not I cannot say; since, however, a considerable increase in the size of the body occurs before the animal leaves

the egg, and in the absence of a mouth, it is tolerably certain that this growth must be due to the taking in of water concomitantly with the using up of the yolk particles, deposited especially in the hypoblastic tissues.

An attempt is made to indicate this constitution of the cells where it occurs by a blurred shading in the figures.

The nuclei of these cells, and of all the cells of this layer, are irregular in shape.

The cavity, lined by these cells, which will be spoken of as the anterior body cavity (fig. 26, *bc*, 1), still communicates with the original cavity of the archenteron, and the layer of cells which forms its inner wall is still directly continuous with the hypoblast itself. This continuity is shown in fig. 27.

The gut here projects into the anterior body cavity as a tube, the end of which is obliquely truncated (fig. 35), so that the ventral lip projects further forwards than the dorsal. In a section taken behind that shown in fig. 27 the archenteron is therefore seen as an elliptical structure, lying inside the anterior body cavity, complete on its dorsal side.

The free edges of the hypoblastic tube are continuous with the body cavity epithelium, which is reflected backwards from it, owing to the backward prolongation of the cavity itself. This backward prolongation of the anterior body cavity is not of the same extent on all sides. Ventrally to the gut, it is very slight, and occurs in very few sections. It appears in fig. 27 as a small space below the ventral wall of the gut. On the dorsal, and especially on the lateral aspects of the body, the posterior parts of the anterior mesoblast are more conspicuous, and will be subsequently shown to be of considerable importance.

Now, since the anterior body cavity is continued behind the end of the gut on all sides excepting the ventral, it is crescentic in shape, the concavity being directed downwards. This appearance exists only for a short distance. Behind it the continuity across the dorsal surface ceases, and the mesoblast exists as a pair of small, hollow cavities at the dorso-lateral sides of the gut, which is here much more fully developed,

occupying most of the space enclosed by the epiblast. Still further backward the cavities in these two mesoblastic tracts close up, and their walls are continued for a short distance as two solid cords of cells, and then disappear.

The mesoblast of the anterior body cavity is, therefore, formed directly from the walls of the hypoblast, which occupied the same situation. It is separated off from it by a process of constriction in the region of the external groove, dividing the proboscis from the collar (fig. 13, *g*). While this process of constriction is being carried out, the pouch of mesoblast grows backwards, surrounding the gut except on the ventral surface, but especially forming the hollow horns (fig. 28, *r. M'*, *l. M'*), lying in a horizontal position, one on each side of the gut.

These relations are made clear by the diagram (fig. 40), and the continuity of the gut with this anterior mesoblastic wall is shown best in longitudinal, median, vertical section (fig. 35. This section is taken through a larva slightly younger than Stage E.)

The anterior body cavity is not completely constricted off from the gut until a later period.

In this larva the hypoblast itself is composed of large cells with rounded outlines, containing elongated nuclei of variable shape. Some of these cells are granular, while others have the fluid appearance described above. In the regions which are not concerned in the formation of the mesoblast, these cells lie in two to four irregular layers. A section through the region of the posterior horns of the walls of the anterior body cavity is shown in fig. 28. It is in about this position that the mouth is eventually formed on the ventral surface. The mesoblastic tissue of the anterior body cavity is seen as two lenticular masses of large cells, lying on each side of the archenteron in the dorso-lateral regions of the body, their cavities being closed.

The Nervous System.—In the dorsal middle line of this region a slight thickening of the epiblast is visible which is the rudiment of the central nervous system. As yet, however, no further development of it is present (fig. 28), and it merely

forms a slight inwardly projecting prominence in transverse section.

2. The Middle and Posterior Body Cavities.

A section through the tract behind the anterior mesoblast, and in front of the middle body cavities, is shown in fig. 29.

The form of the body is elliptical in section, and the epiblast does not differ from that previously described. No rudiment of the nervous system is as yet found as far back as this. The hypoblast is here closely applied to the epiblast throughout its circuit, no space being left between them. The cells of the hypoblast are here from two to three deep, and have the constitution described already. No other structures are present in this region; this part of the body consists, therefore, merely of a tube, with two walls placed in apposition. This simple, two-walled condition only extends for a very short distance, and transverse sections taken immediately behind that shown in fig. 29 exhibit a narrow split-like cavity in the wall of the archenteron on either side of the body. This pair of cavities is bounded on the inner sides by the cells forming the wall of the gut, and the external boundary is made up of a single layer of cells continuous dorsally and ventrally with the hypoblast. These two cavities are the middle pair of body cavities, and their walls constitute median mesoblastic tracts. Immediately behind the point at which they first appear, their cavities may be seen to be connected with that of the archenteron by means of two small pores rather below the middle horizontal line (fig 30, for. 2). This connection is only visible in very few of the larvæ, and may possibly be due to the action of reagents. Since, however, the middle mesoblastic tracts in *Tornaria* are said to be archenteric diverticula (Spengel (7), &c.), it seems more likely that the rarity of their occurrence is due to the shortness of the time for which they are present.

An examination of the succeeding sections shows that these foramina are placed almost at the anterior end of the second body cavities, and that their principal extension is therefore posterior to their openings. A section through these cavities

behind the openings is figured (fig. 31). They here extend along nearly the whole vertical depth of the archenteron. The cells forming their outer wall are very granular, and project in an irregular way into the cavity which they enclose. The inner wall is formed of cells still in connection with the hypoblast, whose contour is also rounded and irregular. As yet they only differ slightly from the rest of the hypoblast, being smaller and somewhat more granular. The minute structure of the layers in this region is shown in Fig. 31, *a*. The cavities extend from the anterior groove to the posterior one,—that is to say, throughout the length of the “collar” at this stage.

Behind them the archenteric wall is simple and the body is a two-walled elliptical cylinder, presenting nearly the same appearances which were described as occurring immediately in front of the collar. The archenteric walls are, however, somewhat thicker. This arrangement only occurs for a very short distance, and is continued as far backwards as the third mesoblastic region, which begins in front of the transverse band and cilia. In the anterior end of this area the hypoblast is split on each side, thus enclosing a pair of cavities similar in appearance to those of the second mesoblastic region, but differing from them in having a greater horizontal extent. The cavity of the archenteron is therefore reduced in this part of the body. These mesoblastic pouches open by large foramina (fig. 34) into the lumen of the gut; these openings occur in the posterior third of their extent. As will be seen in fig. 33 the outer walls of these two cavities do not continue the curve of the hypoblast, but spring from it, bending outwards, consequently forming a pair of archenteric diverticula.

In transverse section through the communicating foramina the ventral wall of the archenteron is thicker than the dorsal. Posteriorly this thickening increases, and, meeting the dorsal wall, closes up the lumen of the archenteron. As previously stated, the anus is not found until a later stage is reached. This completes the account of the anatomy of the larva at this period.

It may be useful to sum up the principal points of internal structure which are to be observed in Stage E. Of these the most important features are :—(1) The commencement of the nervous system as a median dorsal thickening of the epiblast in the region of the collar ; and (2) the formation of the mesoblast. Briefly to recapitulate the latter process, it consists of (*a*) the constricting off of the anterior portion of the archenteric cavity from the remainder to form a single median impaired mesoblastic pouch, which secondarily sends back a pair of hollow outgrowths placed one on either side of the gut ; (*β*) the formation of a pair of cavities in the archenteric wall in the region of the collar whose lumina open into the lumen of the gut ; (*γ*) the appearance of a pair of archenteric diverticula in the posterior region of the trunk.

It will therefore be observed that the process of formation of the first body cavity is constituted by a direct specialisation of the hypoblastic wall, and that while the posterior pair of mesoblastic somites are actual archenteric diverticula, the origin of the anterior pair partakes of the characters both of archenteric diverticula and of delamination from the hypoblast. These two modes of origin of the mesoblastic somites are, of course, essentially the same, differing from each other merely in degree. This case is perhaps interesting as affording an illustration of these two methods both occurring in the same animal, and the combination of the two processes in the case of the middle somites is especially noteworthy as representing the last stage of the phylogenetic transition from hollow archenteric outgrowths to mere plates delaminated from the hypoblast.

Fig. 35 represents a longitudinal vertical section through a larva slightly before Stage E is reached, in which the nervous system is not yet formed even in a rudimentary condition. As the plane of the section passes through the middle line the middle and posterior portions of the mesoblast are not shown. The relations of these to the regions of the body are illustrated by fig. 40, which is a diagram of a horizontal median section of such a larva.

The Separation of the Mesoblast.—The next changes of

importance consist in the closing off of these five mesoblastic pouches. The foramina opening from the archenteron to the middle pair are the first to become obliterated by the coalescence of the hypoblast surrounding them. Shortly after this has occurred the layers of cells forming their inner wall segregate themselves from the archenteric wall.

This process leads to the formation of a pair of closed sacs lying one on each side of the archenteron in the region of the collar.

While the middle pair are separating, the process of closure is also completed between the anterior body cavity and the archenteron by the fusion of the margins of the hypoblastic tube previously described as projecting into the anterior body cavity. The cells composing this tube then retreat backwards, and cease to project beyond the limit of the general anterior wall of the gut. The resulting condition is shown in fig. 36, which is drawn from a longitudinal section which does not pass quite through the middle dorsal line, and is nearly parallel to the longitudinal vertical plane of the body. (Owing to this fact the nervous system is not shown.)

In this section the cells forming the posterior wall of the anterior body cavity form a layer in apposition with the outer ends of the cells constituting the anterior wall of the gut in the region at which the communication between the two cavities previously existed (vide fig. 35). The anterior wall of the gut is here composed by a single layer of cells.

The posterior mesoblastic pouches are shut off from the archenteron in a manner similar to that of the middle pair.

On the completion of the separation of these various portions of mesoblast from the gut the cells of their walls change their primitive character.

Subdividing rapidly they become much smaller, assuming, except in the case of those of the anterior body cavity, the appearance of ordinary peritoneal cells. Those of the anterior body cavity proliferate rapidly in the manner mentioned above, especially in the dorsal and ventral middle lines, forming a pair of large masses of spherical cells in these situations.

Some of the cells lying on the external sides of these masses early become converted into muscular fibres before Stage F is reached (fig. 36, *m. f.*). A full account of these and the subsequent changes occurring in the anatomy of the præoral lobe will, I hope, be subsequently given, together with a description of the structure of the later stages of the larvæ.

Further Development, Stages F, &c.—With regard to the further development of the animal, I propose on this occasion to speak only of the formation of the mouth, and of the central nervous system.

The Mouth.—The mouth is found at the end of Stage F as a small perforation in the ventral middle line lying in the groove which separates the præoral lobe from the collar. At this point the wall of the hypoblast forms a short, downwardly directed diverticulum; this is partially shown in fig. 36. Its outer wall comes into close contact with the epiblast and then fuses with it, a perforation being formed through these coalesced tissues. There is therefore no regular stomodæal invagination. As mentioned above, when first formed the mouth is very minute and quite indistinguishable in a surface view.

Fig. 37 represents a transverse section through the mouth at a rather later stage. The body cavities occurring on either side of it are portions of the middle pair.

The Nervous System.—In Stage E a slight thickening of the epiblast could be distinguished in the dorsal middle line of the collar, having a short, longitudinal extent. It may be remembered that in Stage F a slight groove was visible in this position, which existed for a short time and then disappeared (fig. 14, *n. g.*).

A series of sections through a larva in which this groove is still visible exhibits the following arrangement in the epiblast of the collar. In the extreme front portion of the collar the epiblast is slightly thickened in the dorsal middle line, presenting an appearance very like that seen in the same region in Stage E. A depression is also visible on the dorsal surface at the point where the neural groove is cut across. In the middle

third of the collar this thickening is much more marked, and is formed by a cord of columnar cells whose characters differ from those of the rest of the epiblast (fig. 38, *n. s.*). These cells are rather larger and somewhat pyramidal in section, their bases forming the inner border of the epiblast in this region. Their apices converge towards the centre of the cord. By a continuation of this process of convergence this portion of the epiblast in the posterior third of the collar segregates itself from the skin, forming an apparently solid rod of cells immediately below the epiblast but detached from it in the dorsal middle line of the collar (fig. 39, *n. c.*) This separation from the skin is extended backwards and forwards along the whole length of the collar, but is never completed at either end of it, where the continuity persists throughout life.

As has been stated this nerve cord is at this stage apparently solid; but as may be seen upon examination of the same structure in the adult it eventually possesses a distinct lumen for a great part of its course.

[In fig. 39 it will be observed that a transverse section cuts both the middle and posterior body cavities. This is due to a forward growth of the posterior body cavities on the ventral side of the middle pair. As a result of this growth the septa dividing the two cavities come to lie obliquely, instead of being in a transverse place.]

As it is not proposed on the present occasion to proceed beyond this point in the account of the development I will now briefly recapitulate the chief facts in the history of the larva.

Recapitulation.—The eggs are elliptical and opaque, being fertilised outside the body. After impregnation they divide into two, the subsequent segmentation being probably regular and complete. Segmentation results in the formation of a hollow blastosphere, enclosing an empty segmentation cavity. One side of this blastosphere is next invaginated to form the hypoblast, thereby constituting a simple hemispherical gastrula. The blastopore closes completely; the point of closure being placed at the middle dorsal edge of the posterior surface. At

this period a posterior transverse ring of cilia is found. The body elongates, and becomes marked out into regions, by the appearance, first of an anterior groove situated nearly in the middle line, and secondly of a posterior groove shortly behind it. The area in front of these grooves is præoral, and is destined to form the proboscis, while the region between them constitutes the collar.

The invaginated hypoblast at first forms a simple lining to the cylindrical body. That portion of it which lies in front of the anterior groove then segments off from the rest, forming an anterior unpaired body cavity. This cavity sends back a horn for a short distance on either side of the gut.

In the region of the collar a pair of splits occur in the hypoblastic walls, whose cavities open into the archenteron. The cells forming their walls then separate themselves from the remaining hypoblast as a pair of closed pouches placed symmetrically, one on each side of the body.

A pair of archenteric diverticula are also formed in the region of the trunk, which, on losing their connection with the gut, persist as another similar pair of body cavities.

The nervous system is formed by a segregation of epiblastic cells in the dorsal middle line of the collar, forming a cord lying immediately beneath the skin, continuous with it at both ends of its course.

The mouth is a small pore in the ventral middle line placed in the anterior transverse groove.

The larva is always opaque, and on being hatched creeps about in the muddy sand which the parents inhabit, at no time leading a free life at the surface comparable with that of *Tornaria*.

I hope shortly to describe the remaining structures existing at Stage F, and to give some account of the older larvæ, and of certain points in the anatomy of the adult.

It may, perhaps, be convenient briefly to allude to some of the latter which are of value in interpreting the facts already given. Of these, three are of especial importance.

1. The nervous system (Spengel (7)) in the adult is made

up of four parts : a dorsal and ventral cord lying in the skin in the dorsal and middle lines respectively, connected with each other by a pericæsoophageal ring in the posterior fold of the collar ; a continuation of the dorsal nerve through the collar, first as a solid cord, and afterwards as a hollow tube running in the body, separated from the skin by mesoblastic tissues, this collar portion being continuous with the skin at both ends ; a continuation of the dorsal cord on to the proboscis, at the base of which it forms a considerable concentration, forming a ring in connection with the skin round the base of the proboscis stalk ; lastly, a plexus of nerve-fibres over the whole of the body in close connection with the skin, which is particularly well developed on the proboscis.

2. The Proboscis-pore is a small ciliated opening into the body cavity of the proboscis, situated in the middle dorsal line in the region of the proboscis stalk. It opens through the thickest part of that concentration of the nervous system which encircles this part of the body (Spengel (7), &c.).

As will be afterwards shown, when the anatomy of the older stages is treated of, this pore leads into a small chamber lined with ciliated columnar cells. This chamber is continuous with that horn of the anterior body cavity which lay originally on the left side of the gut. It thus communicates with the general tissue spaces of the proboscis. The right posterior horn never becomes connected with the exterior.

3. In the adult a forwardly-directed diverticulum opens into the alimentary canal in the dorsal middle line, a little behind the mouth. For the chief part of its course it lies in the proboscis (Spengel).

It consists of large vacuolated cells, whose structure is somewhat peculiar, and bears a strong resemblance to the notochordal tissue of a young Elasmobranch.

The lumen of this diverticulum is large posteriorly, and anteriorly is almost entirely obliterated. This rod of tissue is the supporting structure of the proboscis.

It arises as a forward growth of the hypoblast at Stage G, in a manner to be afterwards described.

Though a detailed comparison or general discussion of the significance of these developmental facts cannot, of course, be attempted until the later stages have been described, it may perhaps be advisable to point out the chief features of difference between the larvæ just described and *Tornaria*.

In the first place it will be seen that at no stage has this larva any superficial resemblance whatever to a *Tornaria* possessing a longitudinal posterior band of cilia. This fact is the more remarkable, as the adult appears closely to resemble *B. Kowalevskii*, which is described by Agassiz (1) as passing through a *Tornaria* stage.

At Stage H, however, the general contour of the body is very like that of the late stage of *Tornaria*, and especially that species described by Metschnikoff (5), in which a single pair of gill slits is present. (In the *Tornaria* figured by Agassiz, in this condition, four gill slits are already formed.)

Now *Tornaria* (Metschnikoff, &c.) is a transparent larva, possessing a præoral band of cilia; a longitudinal band of cilia, and one or more transverse posterior bands of cilia, also a nearly median water-vascular, archenteric diverticulum, opening to the exterior a little to the left of the dorsal middle line. It also has an apical epiblastic thickening, bearing a tuft of cilia and a pair of eye-spots, from which a contractile string passes to the inner end of the water-vessel.

On the other hand, this larva is opaque; it has no præoral or longitudinal postoral bands of cilia, water-vascular system, eye-spots, or contractile string, thereby differing markedly from *Tornaria*.

It resembles it in the possession of a transverse band of cilia and an apical tuft of cilia.

The opacity of this larva is however due to the presence of food yolk in its tissues, and most of these points of divergence are more or less such as might be expected to result from this fact, consisting, as they do, chiefly in the absence of such a complete apparatus for locomotion and of the sense organs; for it is clear that an animal which passes a large part of its larval life enclosed in an eggshell, and on emerging from it creeps in the

mud, has less need of bands of cilia and organs of sense than a larva which leads a free existence at the surface.

Less intelligible are the absence of a water-vessel and of a contractile string. With regard to the former, Spengel (7) states that in *Tornaria* it eventually forms the body cavity of the præoral lobe and its pore persists as the proboscis pore. It becomes, therefore, certain that the anterior body cavity of the opaque larva, which has a similar fate, is homologous with the water-vessel of *Tornaria*. Moreover, in *Tornaria* the water-vessel opens to the left of the middle line, while in this larva the external opening when it appears is connected with the left posterior horn of the cavity. The origin of the middle and posterior pair of mesoblastic sacs is similar in the two larvæ, and their eventual disposition in the opaque larva corresponds generally with the fate described by Spengel for the two pairs of archenteric diverticula in *Tornaria*.

Since Metschnikoff (6) has recently published a paper in which he proposes to class *Balanoglossus* together with the Echinodermata in a common class "Ambulacralia," and since this proceeding is mainly supported by arguments adduced from a comparison of *Tornaria* with Echinoderm larvæ in general, and with a typical Asteroid larva in particular, it will be necessary to see what new light the existence of a second type of *Balanoglossus* larvæ throws on this thesis. Until the later development of the opaque larva is known it is impossible to make any such comparison, but it is perhaps worth pointing out that while this larva differs from *Tornaria* in many points it happens that all of these points (if we except the presence of eyespots) are those in which *Tornaria* resembles an Asteroid larva. Nevertheless, most of these features, the præoral and longitudinal postoral bands of cilia, &c., are very possibly purely secondary structures whose presence is correlated with a pelagic habit of life. If this is true, however, they could not avail either to connect or to separate *Tornaria* from the Asteroid larvæ, and the existence of an opaque *Balanoglossus* larva which lacks them would have no significance in settling this question.

The absence of any proper water vessel and contractile

string is, however, of much more importance, and a full discussion of the meaning of it must be deferred. But in comparing the modified anterior mesoblastic sac in the opaque larva with the water vessel of the larvæ of Asteroids, &c., one point is obviously remarkable and may conceivably be of some morphological value, viz. that while in Asteroid larvæ the water vessel is developed always from the left primitive archenteric diverticulum so in *Tornaria* the original evagination from the gut to the exterior is on the left side of the body (Götte (2)). In the larva and adult of *Balanoglossus* (sp. incert.), and in the adult of *B. minutus* the permanent opening communicates with the left posterior horn of the anterior body cavity the cells of which become columnar, while those of the right horn have the structure of ordinary connective tissue. The origin of the water vessels from the left vesicle is also true generally speaking for Echinoidea and Ophiuroidea. It would therefore appear to be of some morphological importance. Upon these and other points in the relationship of *Balanoglossus* to other forms, the later development may be expected to afford some information.

There is one more comparison which may, I think, be shortly alluded to since it is suggested by even a superficial examination of the early stages of this opaque larva.

Since *Balanoglossus* possesses gill slits which are not comparable with any structures present in animals outside the Chordata, it appears *prima facie* as worthy of consideration whether the presence of these structures may not point to a common origin.

Now leaving this question aside for the present, I would suggest that a very striking similarity does exist between the general history of the early development of this larva and that described by Hatschek (3) for *Amphioxus*, this resemblance being more particularly strong in the situation and mode of origin of (1) the central nervous system and of (2) the mesoblastic somites.

For according to Hatschek's account of its origin, the nervous system in *Amphioxus* differs from the central nervous system in

this larva, mainly in its extent, and in the fact that in *Amphioxus* it encloses a neurenteric canal. Now, with regard to the second of these differences, from a consideration of the position of the blastopore in my larva (fig. 25), it is clearly only necessary to imagine the invagination of the dorsal nerve cord to have been extended along the back (instead of being confined to the region of the collar) in order to reproduce the condition which is found in *Amphioxus*; for the dorsal nerve cord in the adult *Balanoglossus* is as a matter of fact continued into this region, though in connection with the skin.

With regard to the origin of the mesoblast, the following is the arrangement in *Amphioxus* (Hatschek). It is formed anteriorly by a primitively unpaired pouch of hypoblast, which is continued into two posterior horns; this anterior pouch is followed by a great number of paired pouches lying on each side of the body which are constricted off from the gut. The anterior pouch is the last to close. As it does so, its cavity divides into a pair of pouches, lying one on the right hand, the other on the left. Of these the cells of the left become columnar and ciliated, and its cavity opens to the exterior, while the tissue of the right pouch becomes flattened epithelium, lining the body cavity of the head. On the other hand, in the larva now described the mesoblast is formed from an anterior archenteric pouch with two posterior horns, followed by only two pairs of pouches. Of the two incompletely separated divisions of the anterior cavity, that which lies on the left side becomes lined by ciliated columnar epithelium, and opens to the exterior, while the right hand one forms connective tissue. The origin of the mesoblast in *Amphioxus* differs, therefore, in being accomplished by a great number of paired posterior pouches instead of by two; and in the fact that the division between the right and left parts of the anterior pouch is completed instead of being partial. There appears, therefore, to be a general agreement in the early development of these two animals which holds good even in the remarkable asymmetry above described.

At first sight it seems likely that these points of resem-

blance are more than superficial, especially when it is remembered that the adults of both animals possess essentially similar branchial structures which, beyond the Chordata, are otherwise without parallel in the animal kingdom. There are, of course, many and great difficulties which preclude any assumption of relationship between them, notably the absence of any regular notochord in *Balanoglossus*.

On this occasion it is not profitable to discuss these questions at greater length. When, however, the later development of this form has been described, it may perhaps be possible to arrive at more definite conclusions.

In closing this paper I have to thank Mr. Sedgwick and Mr. Weldon for rendering me much valuable assistance and advice in connection with it.

LIST OF PAPERS REFERRED TO.

- 1.—AGASSIZ, ALEX., "The History of *Balanoglossus* and *Tornaria*," 'Mem. of the Amer. Acad.,' vol. ix.
 - 2.—GÖTTE, ALEX., "Vergl. Entw. d. *Comatula*," 'Archiv f. Mikr. Anat.,' xii, 1876.
 - 3.—HATSCHKE, B., "Stud. üb. Entw. d. *Amphioxus*," Claus's 'Arbeiten,' Wien, 1881.
 - 4.—LEIDY, J., "On *Balanoglossus*," 'Proc. Acad. Nat. Sc. Philadelphia,' 1881.
 - 5.—METSCHNIKOFF, "El. üb. d. Metam. einiger Seethiere," 'Z. f. w. Z.,' 20, 1870.
 - 6.—METSCHNIKOFF, "El. üb. d. Syst. Stell. v. *Balanoglossus*," 'Zool. Ausz.,' 1881.
 - 7.—SPENGLER, "Bau u. Entw. v. *Balanoglossus*," 'Tageblatt d. Naturf. Ver. München,' 1877.
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Fig. 1.

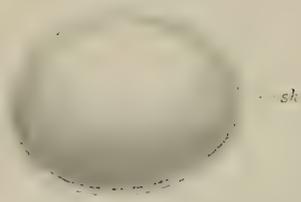


Fig. 2.

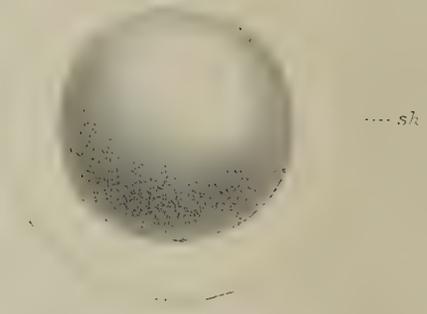


Fig.

Fig. 5.

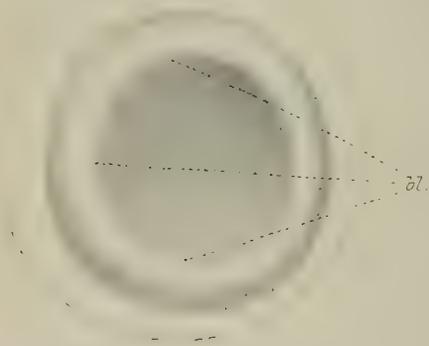


Fig. 6.

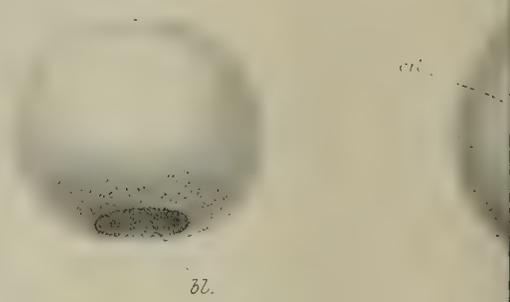


Fig. 10.



Fig. 11.

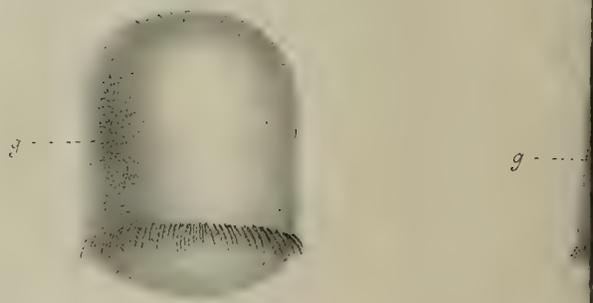


Fig. 13.

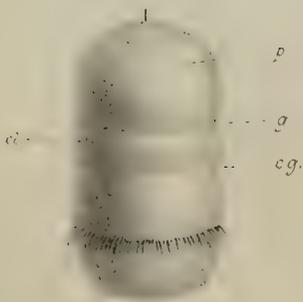


Fig. 14.

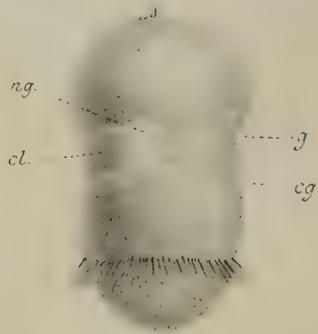


Fig. 15.



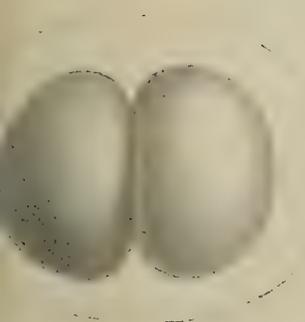


Fig. 4.

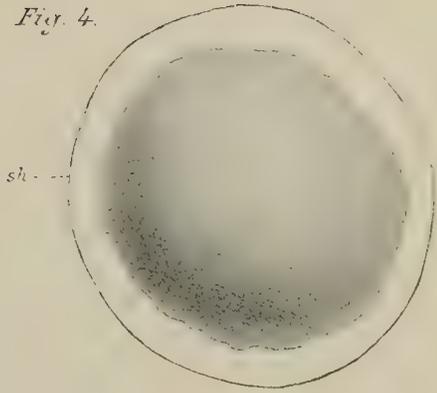


Fig. 8.

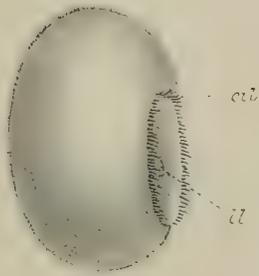


Fig. 9.

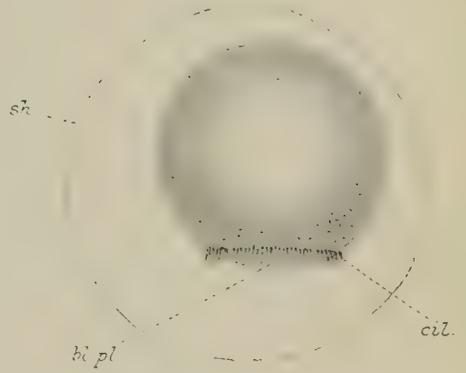


Fig. 16.



Fig. 17.



E

Fig. 18.

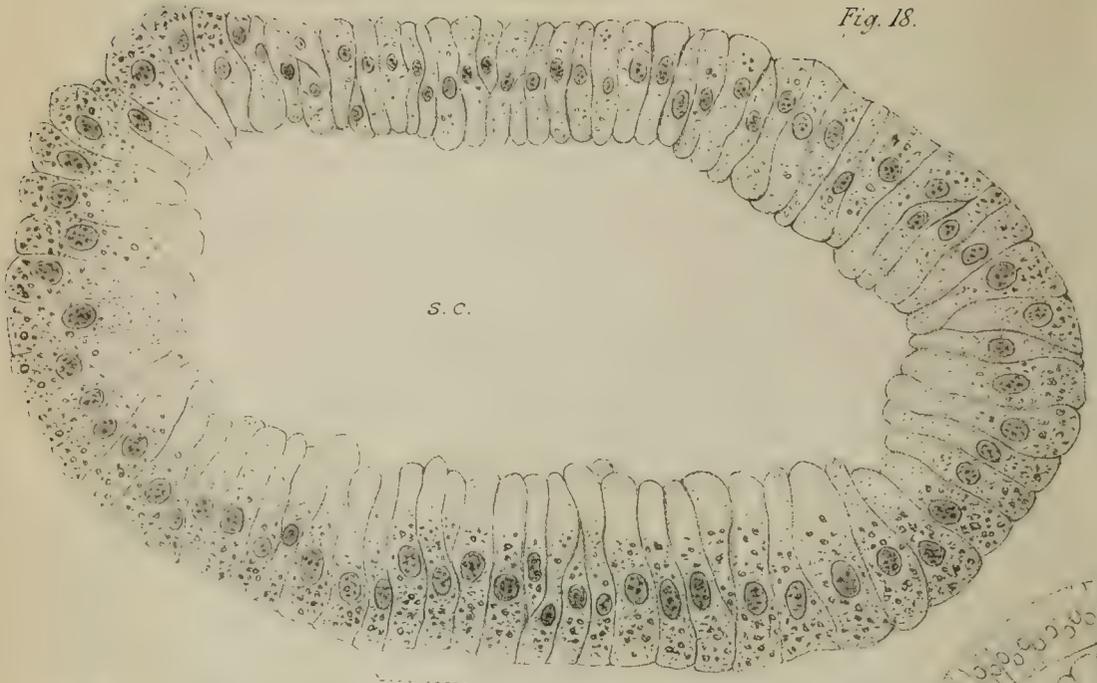


Fig. 20.

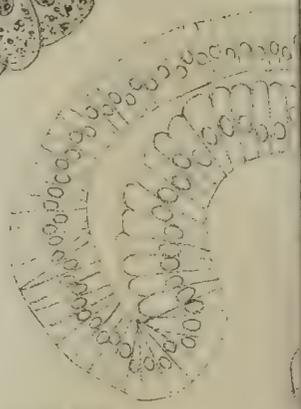


Fig. 24.

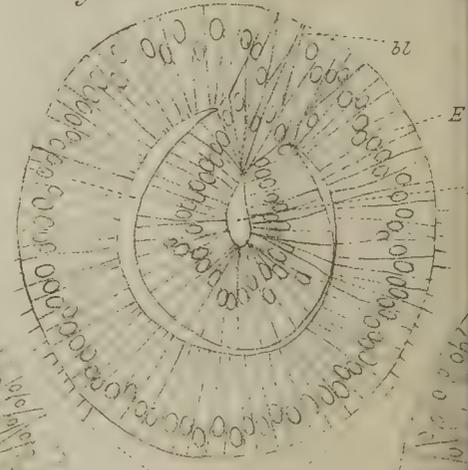
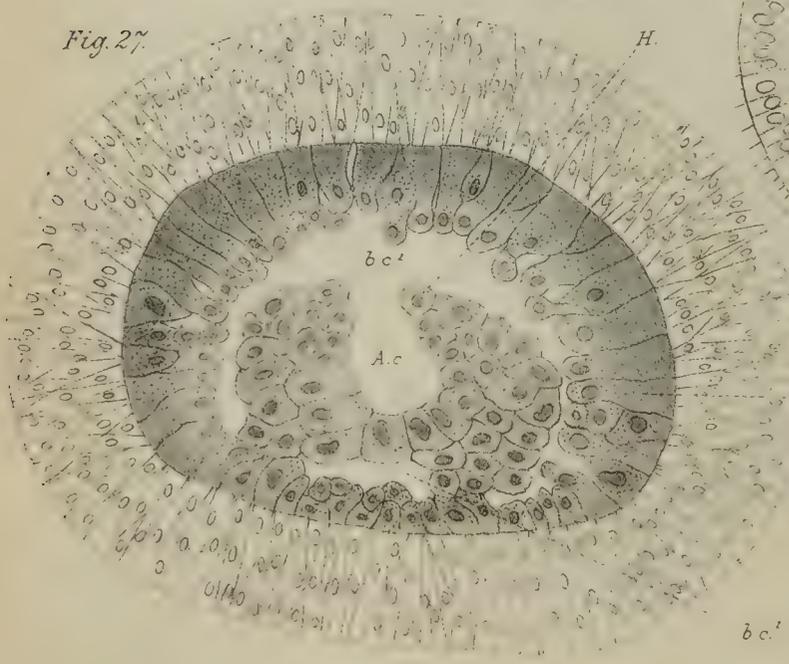


Fig. 27.



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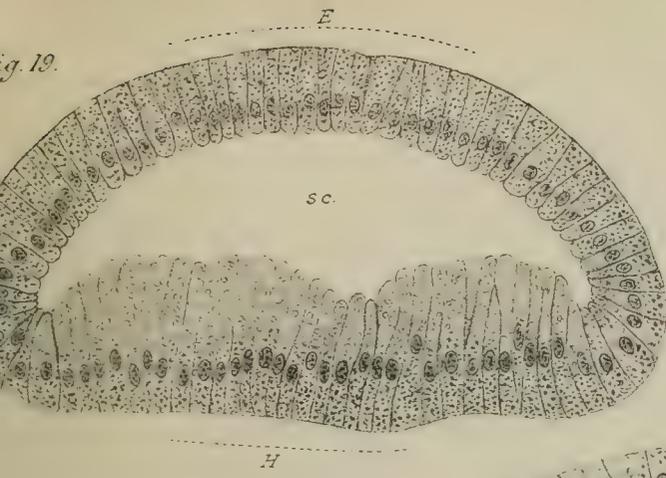


Fig. 22

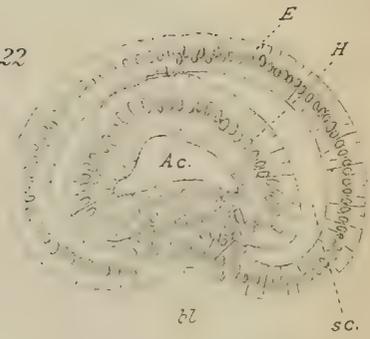


Fig. 23.

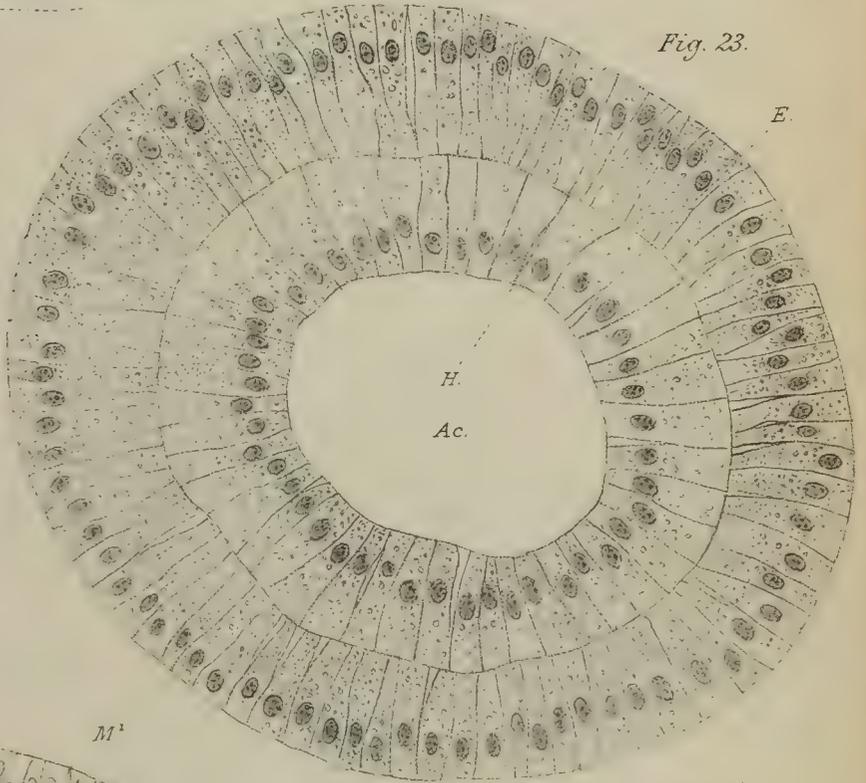
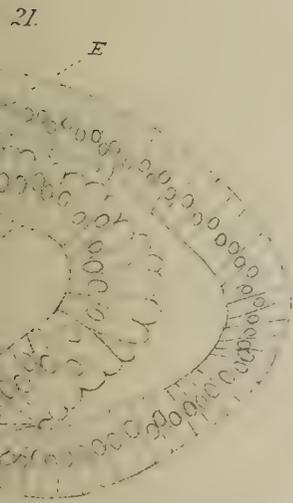


Fig. 26.

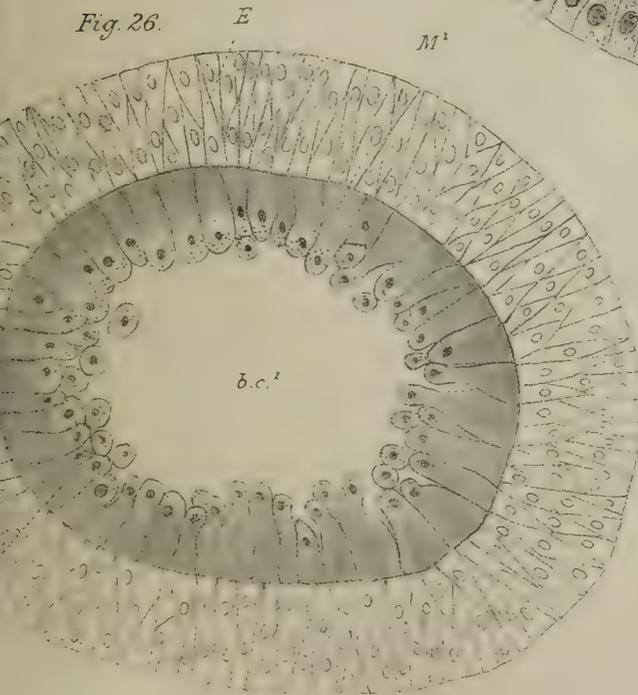


Fig. 25.

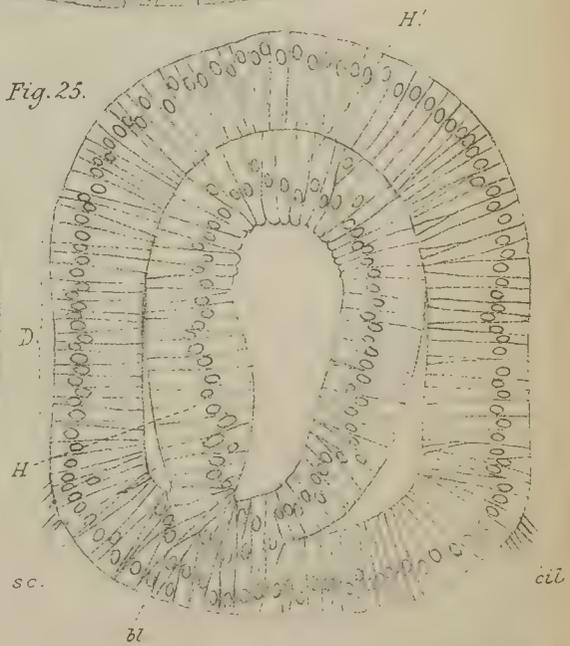


Fig. 28.

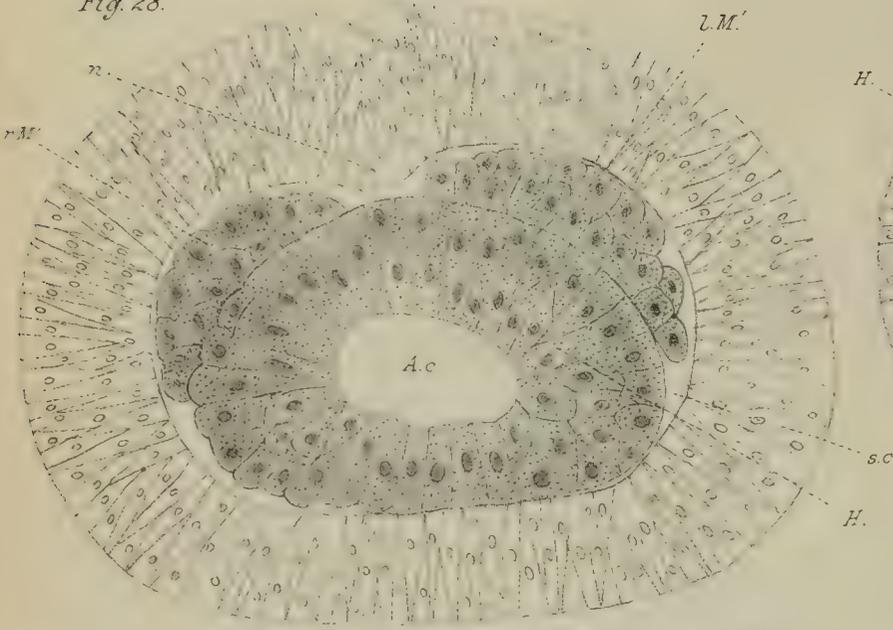


Fig. 29.



Fig. 30.

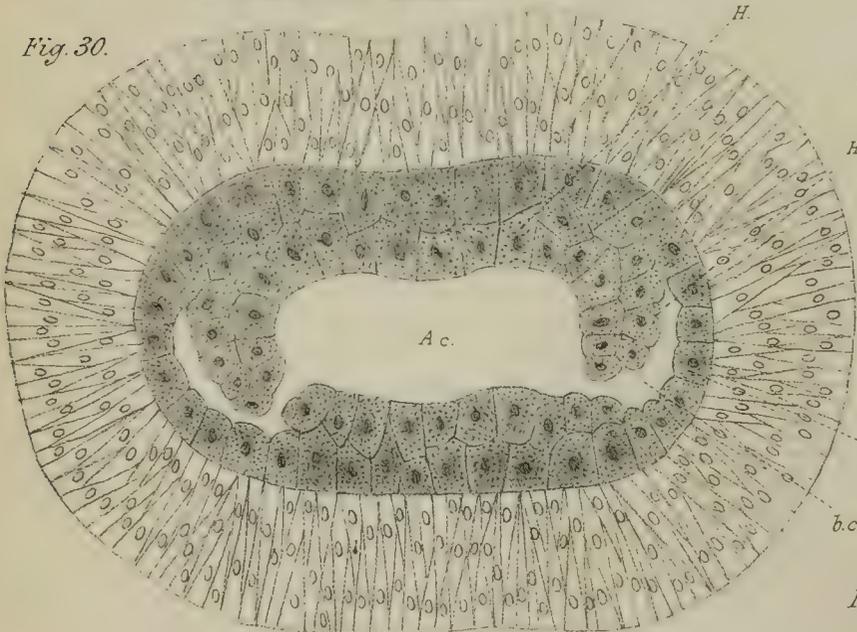


Fig. 34.

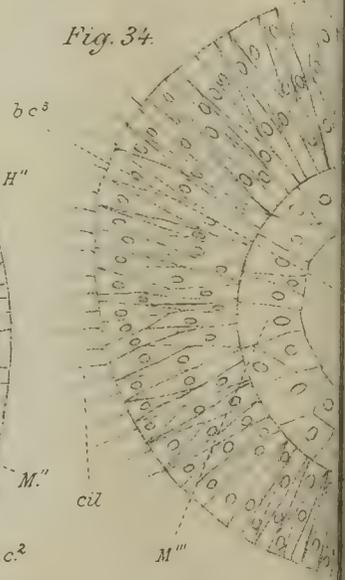


Fig. 33.

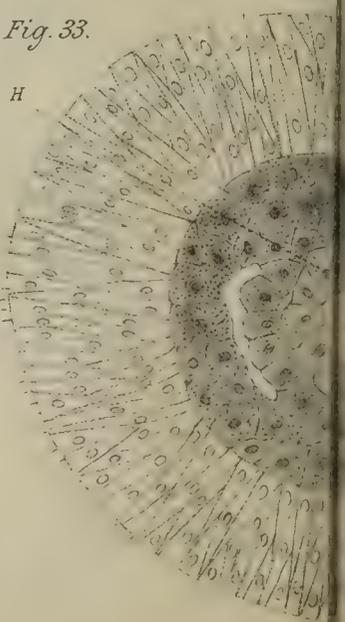


Fig. 31a.



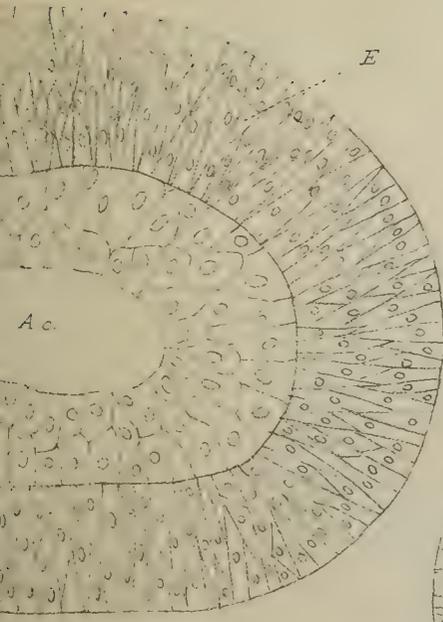


Fig. 31.

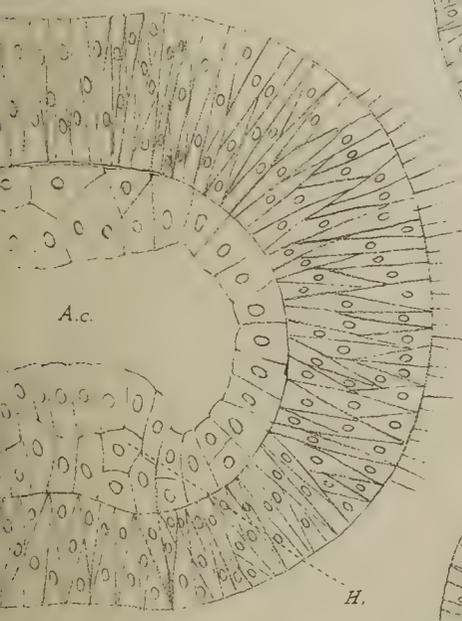
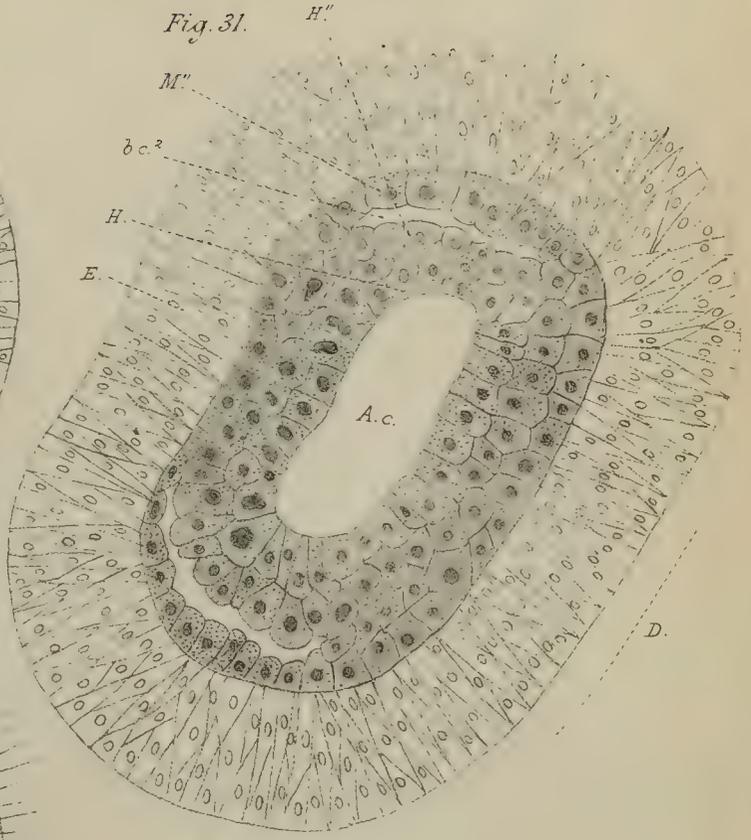


Fig. 32.

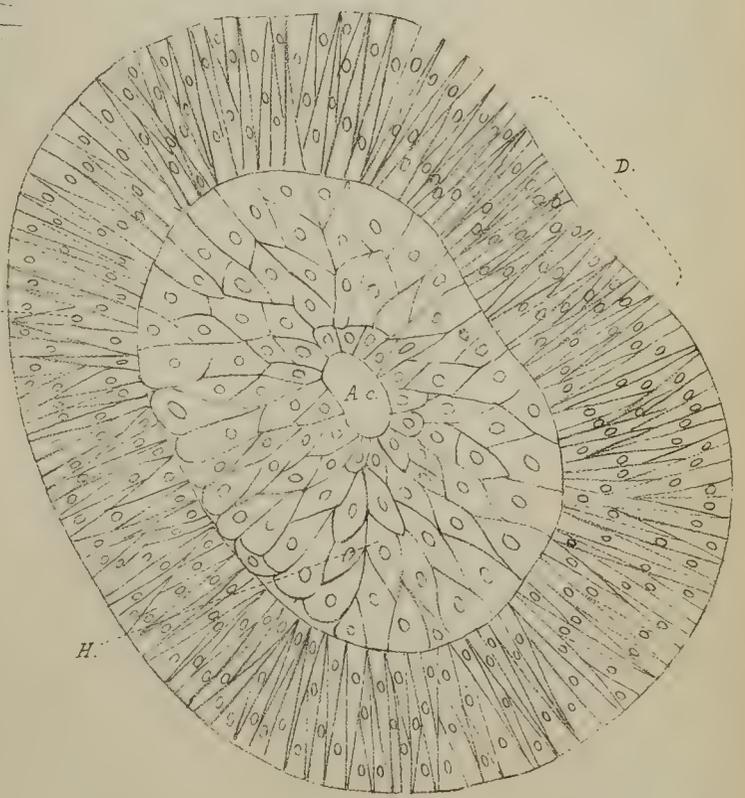
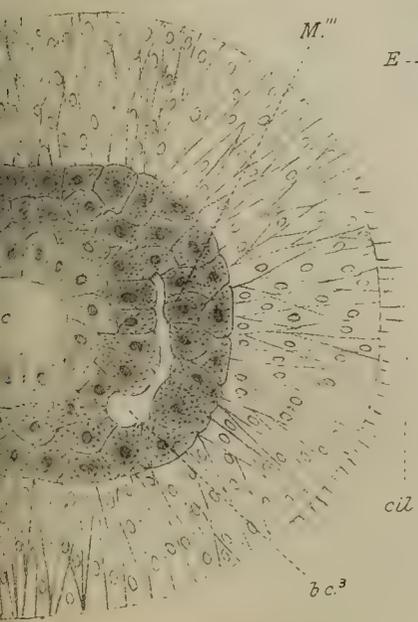


Fig. 35.

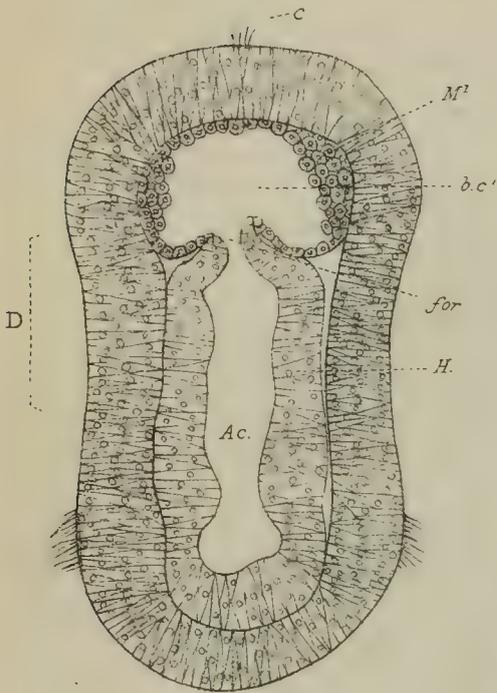


Fig. 36.

Fig. 38.

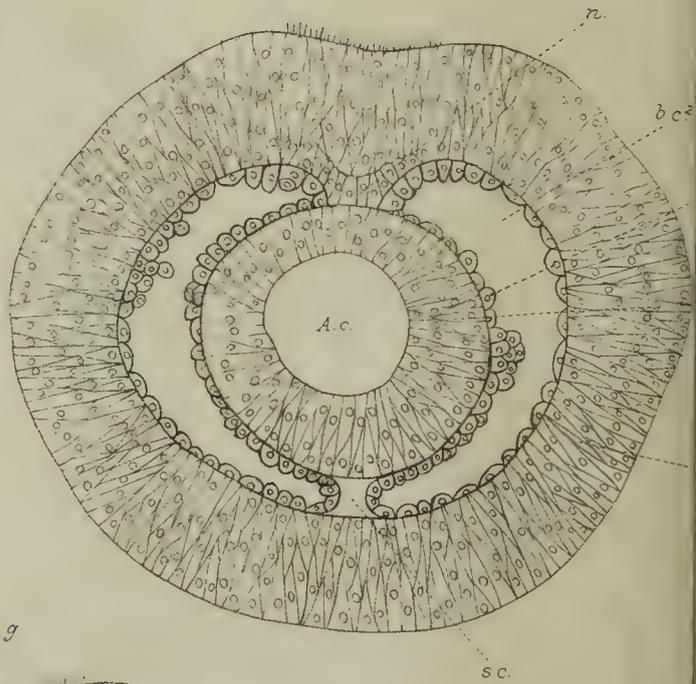
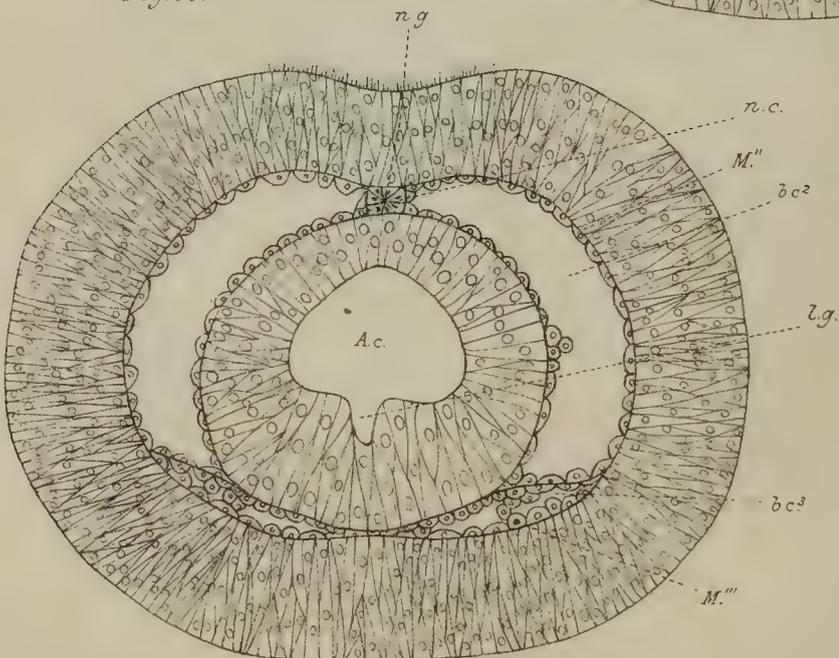


Fig. 39.



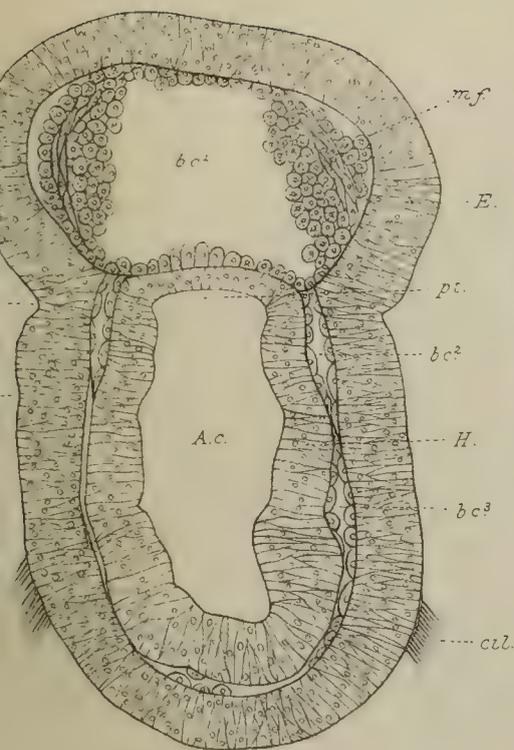


Fig. 37.

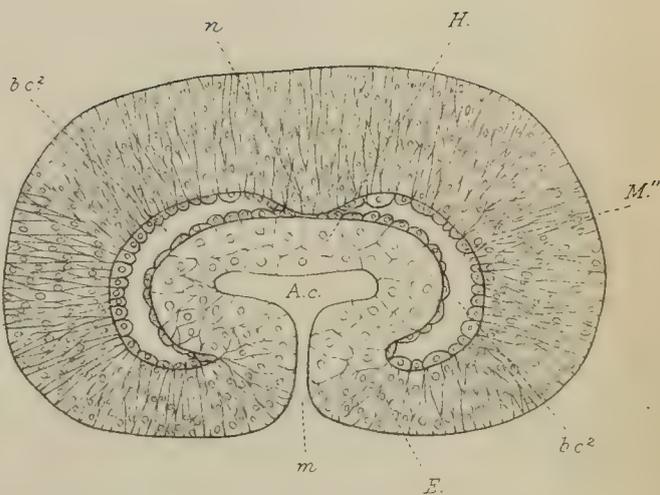


Fig. 41.

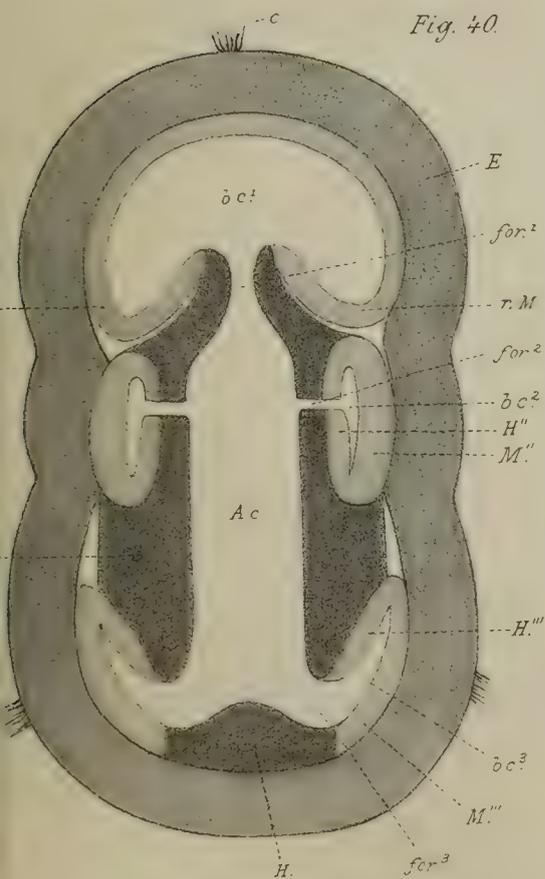
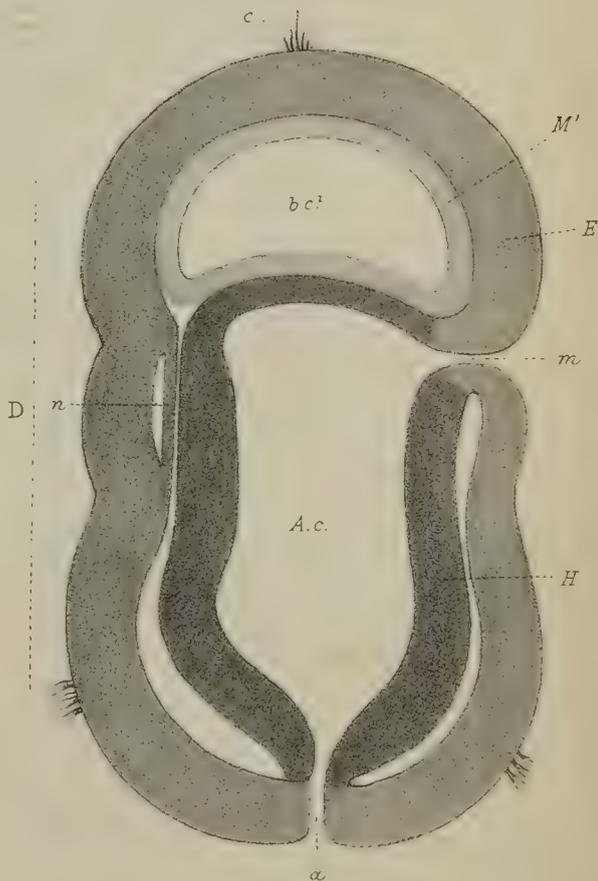


Fig. 40.



EXPLANATION OF PLATES XIII, XIV, XV & XVI,

Illustrating Mr. William Bateson's paper on "The Early Stages in the Development of Balanoglossus (Sp. incert.)."

Complete List of Reference Letters.

a. Anus. *A. c.* Archenteric cavity. *b. c.* 1, 2, and 3: first, second, and third body cavities respectively. *bl.* Blastopore. *bl. pl.* Blastoporic pole. *c.* Apical tuft of cilia. *c. g.* Groove separating the collar from the trunk. *cil.* Transverse band of cilia. *cl.* Collar. *D.* The dorsal side. *E.* Epiblast. *for.* 1, 2, 3: foramina, by which first, second, and third body cavities respectively communicate with the lumen of the gut. *g.* Groove separating proboscis from the collar. *g. s.* Gill slit. *H.* Hypoblast. *H'*. Hypoblast beginning to be differentiated to form the mesoblast of the præoral lobe. *H''*. *H'''*. Cells still forming part of wall of archenteron, but which are beginning to separate off as the inner walls of the second and third body cavities respectively. *l. M'*. Left posterior horn of anterior mesoblastic sac. (Vide note below.) *l. g.* Longitudinal ventral groove in gut. *m.* Mouth. *M'*. *M''*. *M'''*. Mesoblast of first, second, and third regions. *m. f.* Muscle fibres forming in the mesoblast of the præoral lobe. *n.* Commencing nervous system. *n. c.* Nerve cord. *n. g.* Neural groove. *p.* Proboscis. *pl.* Point at which anterior body cavity closes. *r. M'*. Right posterior horn of anterior body cavity. (Vide note below.) *s. c.* Segmentation cavity. *sh.* Shell.

I have to thank Mr. H. A. Chapman for assisting me with the figures of the surface views.

Fig. 31*a* was very kindly drawn for me by Mr. W. F. R. Weldon.

The outlines were drawn with Zeiss's camera lucida.

Figs. 1—17.—Surface views of eggs and larvæ of successive stages. (Zeiss's Obj. A, oc. 2.)

FIG. 1.—Ovum just laid, unfertilized.

FIG. 2.—Fertilized egg.

[Note.—In interpreting those figures in which the right and left sides of the animal are indicated, it must be remembered that as the figures are traced as seen through the microscope, the position of the real right and left sides is reversed.]

FIG. 3.—Egg segmented into two by a median furrow. The eggshell is not afterwards represented.

FIG. 4.—Blastosphære, cell outlines visible.

FIG. 5.—Gastrula seen from blastoporic surface.

FIG. 6.—Later gastrula; blastopore contracted, seen from the side.

FIG. 7.—Closing gastrula, seen from the mouth; ciliated. (Stage A.)

FIG. 8.—The same seen from the side.

FIG. 9.—Larva shortly after the blastopore has closed, from side. (Stage B.)

FIG. 10.—Older larva. (Stage C.)

FIG. 11.—Stage D. (Drawn from a preserved specimen.)

FIG. 12.—Larva between Stages D and E.

FIG. 13.—Stage E.

FIG. 14.—Stage F.

FIG. 15.—Stage G, from the side.

FIG. 16.—Stage H, from dorsal surface. (Drawn from a preserved specimen.) Appears shortened, owing to ventral flexure.

FIG. 17.—Stage H, from the side.

FIG. 18.—Vertical section through centre of a late blastosphære. (Zeiss's Obj. D, oc. 2.)

FIG. 19.—Section through plano-convex blastosphære. (Obj. C, oc. 2.)

FIGS. 20 and 21.—Sections through the blastopore of gastrulæ in two stages. (Obj. C, oc. 2.)

FIG. 22.—Transverse section through a closed gastrula, between Stages A and B. (Obj. B, oc. 2.)

FIG. 23.—Transverse section through the anterior end of a larva, between Stages C and D. The letter *H* is placed in the archenteric cavity. (Obj. D, oc. 2.)

FIG. 24.—Transverse section through posterior end of the same larva as the preceding. The transverse band of cilia and the point at which the blastopore has closed are cut through. (Obj. C, oc. 2.)

FIG. 25.—Longitudinal median vertical section through same larva as preceding. Constructed from the series of transverse sections, in which those represented in Figs. 23 and 24 occur.

FIGS. 26—34 represent some of a series of transverse sections taken through a larva in Stage E. The epiblast is represented diagrammatically. In each case the upper side is the dorsal one. (All, excepting Fig. 31*a*, were drawn with Obj. D, oc. 2.)

FIG. 26.—Section through præoral lobe, showing the amœboid cells forming the wall of the mesoblastic sac.

FIG. 27.—Section through the foramen, by which the anterior body cavity opens into the archenteron. The space lying ventral to the hypoblast is the slight ventral backward prolongation of the anterior body cavity, which would disappear in the next few sections.

FIG. 28.—Through the ends of the posterior horns of the anterior body cavity, whose cavities are not prolonged to this point. In this section the rudiment of the nervous system is shown.

FIG. 29.—Section between the anterior and middle mesoblastic regions.

FIG. 30.—Section through the junction of the second body cavities and the archenteric cavity.

FIG. 31.—Section through the middle of the second body cavities.

FIG. 31*a*.—Part of a section similar to the preceding. It represents the minute structure of the epiblast and hypoblast, and of the mesoblastic cells lining the second body cavity, which are here still connected with the hypoblast. (Obj. F, oc. 2.)

FIG. 32.—Section through the region between the second and third mesoblastic tracts.

FIG. 33.—Section through the anterior region of the third mesoblastic tracts.

FIG. 34.—Section taken rather behind the preceding, traversing the foramina by which the posterior body cavities open to the archenteron.

FIG. 35.—A longitudinal median section of a larva between Stages D and E, showing the opening of the anterior body cavity into the archenteron. (Obj. C, oc. 2.)

FIG. 36.—Longitudinal nearly median section, almost in the vertical plane, through a larva between Stages E and F. (Obj. C C, oc. 2.) The anterior body cavity is now seen to be closed.

FIG. 37.—Transverse section through the mouth of a larva between Stages F and G. (Obj. D, oc. 2.)

FIG. 38.—Transverse section through the anterior end of the collar of a larva between Stages F and G (slightly older than the preceding). (Obj. D, oc. 2.) The nervous system is shown still continuous with the skin in this region.

FIG. 39.—Transverse section through the same larva as Fig. 38, taken somewhat behind the section there represented. (Obj. D, oc. 2.) The nervous system is here detached from the skin, to become continuous with it again posteriorly.

FIG. 40.—A diagram representing the arrangement of the parts as seen in a median horizontal longitudinal section through a larva at Stage E. In this, as in Fig. 41, the epiblast is of the medium shade, the hypoblast dark, and the mesoblast light.

FIG. 41.—Diagram of a longitudinal vertical section through a larva between Stages F and G. The letter *D* indicates the dorsal side; shading as in Fig. 40. The anal perforation is here represented in order to show its eventual position. It would not, however, be found until Stage G is reached. The relations of the closed præoral body cavity and of the nervous system to other structures are here shown.

The Original Function of the Canal of the Central Nervous System of Vertebrata.

By

A. Sedgwick, M.A.

THE central nervous system of all known animals, with certain doubtful exceptions, arises from the epiblast. The region of the epiblast from which it arises may either persist in the adult as part of the superficial epidermis, or it may be pushed in so as to give rise to a tube, from the walls of which the central nervous system is developed. The last-mentioned method is characteristic of the Vertebrata. The walls of this tube become differentiated into a superficial epithelial layer lining it, and an external mass of nervous matter. The tube persists as the canal of the nervous system; the epithelium lining it becomes the ciliated epithelium of this canal, which therefore corresponds to the external epithelium of the body-wall.

I may here draw attention to the fact that the vertebrate stock must have separated from that of other animals before the nervous system was separated from the external epithelium of the body; that in fact the vertebrate nervous system never is separated by any ingrowth of mesoblast from the superficial epiblast from which it arose; as is the case in all but the most primitive of the Invertebrata. This superficial epiblast in Vertebrata is involuted and gives rise to the ciliated epithelium just mentioned of the central canal. Three stages may be distinguished in the development of this canal, and I suppose that all three have had a functional counterpart in the evolution of the organ.

In the first stage a groove extended along the whole length

of the middle dorsal (or ventral?) line of the body, the nervous system being placed in the deeper layers of the epidermis of this groove. This stage I propose to call the groove stage.

In the second stage, which may be called the siphon stage, the groove had become converted into a canal, open in front at or near the anterior end of the body, and open behind close to the anus.

In the third stage the canal has completely closed; this is the present stage.

There have been doubtless many other stages, each with its special functional importance in the evolution of the neural canal; but those which I have just mentioned are most obviously suggested by the ontogeny of this organ.

Of the existence of the groove stage there can, I think, be no doubt. It has left its mark in the medullary groove of Vertebrates; and indications of the occurrence of a similar structure are to be found in the embryos of Annelida and Arthropoda. The ventral groove of *Neomenia* and *Proneomenia* is probably the same structure persisting in the adult.

This groove was probably richly ciliated. Once established it soon became deeper.

The function of the groove was in my opinion partly respiratory and partly protective. As the nervous system increased in thickness, the deeper parts of it became so far removed from the surface that the supply of oxygen which reached them was inadequate. This would be remedied by an increase of the surface over which the nervous system was spread, and this increase might be produced by enlarging the superficial area over which the central nervous system extended. It is improbable that this should happen, because the tendency of evolution seems to have been to localise the central nervous system, probably for the sake of its greater security. The increase of surface required seems to have been obtained by the development of this groove, and the deeper the groove became the more completely would it serve its double function. Thus the way was prepared for the siphon stage which proceeded from the groove stage by the conversion of the groove

into a canal open at either end. The neural canal therefore owed its origin to the requirements of protection and respiration. When once formed it must have continued to discharge some function, otherwise it would have atrophied, as we know is the habit of useless structures. The function of the canal at this stage of its evolution is the subject of the present discussion.

The relations of the neural canal at the siphon stage, which is well marked in the development of Ascidians and Amphioxus, are well known to all students of embryology. It is open behind into the hind end of the alimentary canal and in front, in the cephalic region of the body.

How it acquired its opening into the alimentary canal is perhaps hard to understand. The discussion of this question involves the discussion of a still more difficult question, viz. the relation of the permanent anus to the blastopore; this I reserve for a subsequent occasion.¹ I may, however, point out here that development points to the fact that the blastopore was placed within the medullary plate, and that therefore on the conversion of the medullary groove into a canal, the alimentary canal would open into the hind end of that canal, and the two tubes would open to the exterior together.

It is quite clear that the anus of existing Vertebrata is not in the position of the primitive anus or blastopore of ancestral forms, and it has been commonly supposed that the present anus is a new formation. That the blastopore closes is certain, but it has been recently pointed out by Mr. Weldon² that the present anus occurs along the line of the blastopore; and I hope soon to be able to show that the permanent anus is identical with the blastopore, the temporary closure of which is simply a matter of developmental necessity. However this may be, there can be but little doubt that this relation of the hind end of the neural canal to the alimentary canal has existed in the ancestors of Vertebrates.

¹ Vide self, "On the Origin of Metameric Segmentation, &c.," 'Quart. Journ. Micr. Sci.,' and these "Studies."

² 'Quart. Journ. of Microscopical Science,' 1883; and these "Studies," pp. 5, 6.

To return to the main question, what is the function of the neural canal at this stage? It seems to me that that function must have been in the main a respiratory one. The water entered the canal by the anterior pore, was driven through it by the cilia, and at the hind end passed through the neurenteric canal into the alimentary canal, and so out by the anus. In support of this I appeal to certain well-known physiological and anatomical facts.

In the first place, in the Vertebrata the brain requires more oxygen for its well-being than any other tissue of the body, and in those Vertebrates, e. g. Amphibia and Sauropsida, in which there is only one ventricle, special arrangements are present to ensure a supply of pure arterial blood to the head. In the second place, in the tracheate animals the central nervous system has a specially rich supply of tracheæ. Finally in certain worms, e. g. Nemertines, Aphrodite, the whole nervous system contains hæmoglobin, which may be supposed to exercise a special attraction for oxygen, and hold it in a convenient state for the use of the nerve-cells.

It is interesting to notice here that in most of the animals I have just mentioned, in which there are special arrangements for the respiration of the nervous system, the vascular system is but little developed. It seems probable that the ancestral Vertebrate with the siphon stage of neural canal was without a well-developed vascular system. When this and definite respiratory organs became developed, a new stage in the evolution of the neural canal was reached, in which it lost its respiratory function, this being assumed by the vascular system. As a result of this, the anterior and posterior openings became closed. This brings us to the present condition of a closed central canal, whose function it is not within my province to examine.

Before concluding I wish to point out that a canal leading into the centre of the central nervous system is not confined to the Vertebrata. Such canals are present leading into the cephalic ganglia of adult Nemertines, and in the most con-

spicuous part of the nervous system of *Balanoglossus*, a similar arrangement of a very complicated nature exists.¹ They are also present in the cephalic ganglia of the embryos of most tracheate animals.

¹ From an unpublished research by Mr. W. Bateson, of St. John's College.

On the Fate of the Blastopore and the Presence
of a Primitive Streak in the Newt (*Triton
cristatus*).

By

Alice Johnson,

Demonstrator of Biology, Newnham College, Cambridge.

With Plate XVII.

THE coincidence of the blastopore with the anus in the Newt has already been observed by Mr. Sedgwick.¹ His assertion was, he says, based only upon surface views. He therefore suggested to me that I should attempt to verify it by cutting sections of the embryos, and my results confirm what he has stated.²

I. THE FATE OF THE BLASTOPORE.

A.—At the close of segmentation the blastopore is placed in the normal position at the hind end of the embryo. With the greater growth of the dorsal surface, consequent on the appearance of the medullary folds and formation of the medullary canal, it comes to occupy a place on the ventral surface at some distance from the hind end (vide fig. 11). Its distance from the hind end increases as development goes on (vide figs. 12, 13). The tail

¹ A. Sedgwick, "On the Origin of Metameric Segmentation and some other Morphological Questions," this Journal, January, 1884.

² Some of the main points of this paper have already appeared in a communication made to the Royal Society in June, 1884.

begins to bud out behind it, at a time when about ten mesoblastic somites have been formed, as a small conical knob whose blunt apex points forwards, and the tail has become very distinct in the stage represented in fig. 13, when there are about eighteen somites, and the rudiments of the sense organs, cerebral vesicles, visceral arches, &c., have appeared.

The blastopore leads into the hind gut, whose cavity is here broad, but very shallow (vide fig. 8). At a greater distance from the blastopore the cavity becomes much narrower and no deeper (vide fig. 6), so that it is very difficult to follow it in transverse sections. In the longitudinal sections, however, its continuity is quite apparent (vide figs. 11, 12, 13). Fig. 14 represents a transverse section, showing the open blastopore at a time before the tail is formed, and figs. 15, 16, 17, a series of transverse sections, showing the passage of the blastopore into the hind gut at a considerably later stage with a very distinct tail.

I find no stage at which the blastopore is closed.

B. Historical. — Scott and Osborn¹ describe a posterior dilatation of the medullary canal, the sinus rhomboidalis, which remains open for some time after the rest of the canal is closed. They say that its folds enclose the blastopore, and, therefore, when they come together, a neurenteric canal is formed. Their account of the exact date of the closure of the sinus rhomboidalis is a little obscure, but seems to indicate that it takes place while the number of mesoblastic somites is quite small, and before the rudiments of the visceral arches and of the tail have appeared.

Hertwig² figures an open blastopore at a slightly later stage than this, but he describes it as being situated at the end of a small conical process, which, judging from his surface views of the embryo, one would take to be the tail.

¹ W. B. Scott and H. F. Osborn, "On the Early Development of the Common Newt," this Journal, October, 1879.

² O. Hertwig, 'Die Entwicklung des Mittleren Keimblattes der Wirbelthiere,' Jena, 1881.

Bambeke¹ states that the blastopore disappears before the formation of the medullary folds.

As to the fate of the blastopore in other Amphibia, I conclude from Clarke's² account of the development of *Amblystoma* that it becomes the anus in this form, though the fact is not actually expressed in so many words. He says (p. 7), "At the extreme anal end the (medullary) folds remain separate over a small area, the space formerly occupied by the vitelline plug (the mass of yolk-cells which projects into the cavity of the blastopore and nearly fills it up at an earlier stage), and form a rounded edge about this small cavity or pit" (p. 8). "In a ventral view . . . are seen both the optic vesicles . . . and the anus at the posterior end of the neural tube" (p. 9). "The beginning of the tail also shows distinctly, and its median ridge, at the end of which is the dark cavity of the anus, is now much increased in size." No mention is made of the closure of the blastopore, and Clarke's figures (pl. ii, figs. 9, 10, 12, 14) confirm my deduction.

In *Pelobates*, Bambeke³ states that the anus appears to him to correspond to the place formerly occupied by the "bouchon de Ecker" (vitelline plug). He figures it at a comparatively early stage (vide plate iv, fig. 5).

II. THE PRIMITIVE STREAK.

A.—The first structure to appear on the surface of the ovum after the segmentation has been completed is a groove which generally extends from the blastopore along the greater part of the dorsal surface. This is the "Rückenrinne" of the German observers, the "Sillon médian" or "Sillon primi-

¹ Ch. van Bambeke, "Nouvelles Recherches sur l'embryologie des Batraciens," 'Archives de Biologie,' vol. i, 1880.

² S. F. Clarke, "Development of *Amblystoma Punctatum*," part i, external, 'Studies from the Biological Laboratory of the Johns Hopkins University,' No. 2, 1880.

³ Ch. van Bambeke, "Recherches sur le Développement du *Pelobate brun*," 'Mémoires Couronnés, &c., de l'Acad. Roy. de Belgique,' 1868.

tive" of Bambeke, and may obviously be called the primitive groove. Hertwig¹ says that it is at all stages sharply marked off from the blastopore by an intervening ridge of cells. In my specimens this sometimes occurs, but it happens at least as frequently that the groove is continuous with the blastopore at its first appearance, and I always find them continuous after the formation of the medullary folds.

Transverse sections through an embryo with a primitive groove and before the medullary folds have been formed shows that in the region of the groove the three embryonic layers are continuous with one another (vide fig. 1, which represents a section taken through about the middle of the embryo). It happened in this embryo that the primitive groove was continuous with the blastopore. In the anterior part of the embryo the groove flattened out and gradually disappeared. Fig. 2 represents a section through the groove near its anterior end, and shows that here the epiblast is distinct from the other two layers, the mesoblast still retaining its connection with the hypoblast. The mesoblast has generally been described (viz. by Scott and Osborn, Hertwig, and Bambeke²) as being derived exclusively from the hypoblast, except at the blastopore, from the lips of which it grows. It appears to me, on the contrary, that the greater part of it is derived from the primitive streak as in the higher Vertebrates, for it is seen in fig. 2 that the mesoblast cells, where they are represented as derived from the hypoblast, are much fewer in number than appears in fig. 1, where they are shown growing out from the primitive streak.

The primitive groove in another embryo of a slightly later stage exhibits a deep pit at its anterior end. I am unable at present to state whether any fusion of the layers exists in the region of this pit at this time.

The next step forwards in development consists in the for-

¹ O. Hertwig, loc. cit.

² Ch. van Bambeke, "Formation des feuilletts embryonnaires et de la Noto-corde chez les Urodèles," 'Bulletins de l'Acad. Roy. de Belgique,' 2me série, tome 1, 1880.

mation of the medullary folds. In fig. 2 the dorsal half of the epiblast is seen to be thickened. This occurs first in the anterior part of the body, where also the folds are first clearly formed. They consist of a pair of sharply-marked ridges, bounding a very wide, flat area. The medullary plate, which includes the whole of the dorsal surface, is made up of narrow deep columnar cells. The rest of the epiblast, which formerly consisted of a single layer of columnar cells (vide figs. 1, 2), now begins to divide into two layers of flatter cells. These well-known peculiarities of the medullary plate and general epiblast have already been sufficiently figured by previous observers.

The primitive groove at this period extends from the blastopore throughout the whole medullary plate. The consequent division of the latter into two halves is especially conspicuous in front. It occasionally happens that the groove is absent in the middle region of the body. This was the case with the embryo, transverse sections of which are represented in figs. 3, 4, 5, and in which the medullary folds existed anteriorly, but diminished gradually and vanished behind. Fig. 3 shows the open blastopore, with the three embryonic layers coalescing at its edges in the ordinary manner. In fig. 4 the rounded primitive groove is seen indenting the primitive streak. In fig. 5 the groove is flatter, but the layers are still fused beneath it. The blastopore itself at this stage is narrow and elongated.

In another specimen of the same stage as that just described I find that the primitive groove extends for a short distance in front of the medullary folds. Near its anterior end it becomes rather suddenly considerably deeper and also loses its rounded outline, being instead triangular in section and sharply pointed at its apex. It presents in this region, in fact, an appearance strikingly similar to that of the blastopore, although not communicating with the archenteron. I believe, however, that the epiblast and hypoblast are fused at this point, and it can hardly be doubted that this deep pit, with the fused layers at its apex, represents the front end of the blastopore. It is evidently the same structure as the pit found at the front end

of the primitive groove at an earlier stage, and corresponds in position more or less with the future mouth.

As the medullary folds approach one another the primitive groove becomes gradually obliterated in the narrowing and folding up of the medullary plate, and the primitive streak remains only in the hind region. At the front end of this reduced primitive streak the sides of the medullary plate come together to form a solid mass instead of the thick-walled canal that exists in front. This fact is illustrated in figs. 6, 7, 8, and 9, which are taken from a series of transverse sections through the hind end of an embryo. The medullary canal in passing back round the hind end gradually loses its lumen (vide fig. 6, where the medullary canal is seen above and the solid mass of epiblast cells below). Further forwards on the ventral surface this solid mass becomes fused with the underlying hypoblast cells and the lateral plates of mesoblast. The primitive streak, thus constituted, forms a slightly pronounced ridge on the surface of the embryo (vide fig. 7). Nearer the blastopore the ridge is flatter (vide fig. 8). In fig. 9 the blastopore itself is seen with the continuity of the layers at its lips.

Fig. 10 shows the primitive streak, as seen in transverse section, of an embryo with a distinct tail, rudiments of the visceral clefts, &c. In figs. 11, 12, and 13 the primitive streak of different stages is shown in longitudinal section, but it cannot then be distinguished so clearly.

I have been unable to find at any stage the neurenteric canal mentioned by Scott and Osborn.

In the course of the development, the medullary canal is gradually differentiated backwards out of the primitive streak, and the hind gut, from being curved as seen in fig. 13, becomes straight.

The arrangement of the layers in the primitive streak of the Newt at the stage represented in figs. 6—9 resembles closely that described by Professor Balfour in the tail of the embryo *Lepidosteus*.¹

¹ F. M. Balfour and W. N. Parker, "On the Structure and Development of *Lepidosteus*." 'Phil. Trans. of the Roy. Soc.,' part ii, 1882.

B. Historical.—The great breadth and flatness of the medullary plate at its first appearance is a well-known characteristic of Amphibian embryos. They are further distinguished at this period from the embryos of other Vertebrates by the division of the medullary plate into two symmetrical halves by means of the dorsal or primitive groove. This feature, as well as the continuity of the primitive groove with the blastopore, has been noticed by almost all observers of Amphibian embryology. Hertwig¹ alone denies the continuity of the two structures, but it has been described by Bambeke² in Triton and Axolotl; by Clarke³ in *Amblystoma*; by Ecker⁴ in the Frog; by Götte⁵ in *Bombinator*; and by Bambeke⁶ in *Pelobates*. Prévost and Dumas⁷ figure the primitive groove in the middle of the medullary plate of the Frog, but do not mention its blastopore.

The only other Vertebrate, as far as I know, in which a similar disproportionately broad medullary plate and a like relation of the primitive groove to the medullary plate and blastopore have been described, is the Sturgeon. Kowalevsky, Owsjannikoff, and Wagner⁸ describe in the embryo Sturgeon a specially broad medullary plate, in the middle of which is an opaque streak, which they call the "Primitivstreif," though they do not assert that any fusion of the layers exists there. A "Primitivrinne" runs down the centre of the "Primitivstreif," ending in the blastopore (vide their figures on pp.

¹ O. Hertwig, loc. cit.

² Ch. van Bambeke, "Nouvelles Recherches, &c.," loc. cit.

³ S. F. Clarke, loc. cit.

⁴ A. Ecker, "Icones Physiolog.," 1851—1859.

⁵ A. Götte, "Die Entwicklungsgeschichte der Unke," Leipzig, 1875.

⁶ Ch. van Bambeke, "Recherches sur le développement du *Pélobate brun*," loc. cit.

⁷ Prévost and Dumas, "Deuxième mém. s. l. génération. Développement de l'œuf des Batraciens," 'Ann. Sci. Nat.,' ii, 1824.

⁸ A. Kowalevsky, Ph. Owsjannikoff, and N. Wagner, "Die Entwicklung d. Störe," 'Vorläuf. Mittheilung. Mélanges Biologiques tirés du Bulletin de l'Acad.,' Imp., St. Pétersbourg, vol. vii, 1870.

175, 176). In Salensky's¹ account, however, no such structure as a primitive groove is mentioned or figured.

The solid condition of the hind end of the medullary canal, such as I find in the Newt, has been described by Strahl² for the Lizard, and by Gasser³ for the Bird.

III. SUMMARY OF FACTS AND GENERAL CONSIDERATIONS.

In the Newt (1) the anus of Rusconi, or blastopore, becomes the actual anus of the adult.

(2) A primitive streak exists on the dorsal surface in front of the open blastopore.

(3) The primitive groove extends along the whole of the dorsal surface from the open blastopore, and for a short distance in front of the medullary folds.

(4) The front end of the primitive groove deepens into a distinct pit, at the apex of which there is, almost certainly, a fusion between the hypoblast and epiblast.

The Newt affords another instance of the variability of position of the last open part of the blastopore in different groups of the Chordata.

In *Amphioxus*, the blastopore is posterior, and gives rise to a neurenteric canal on the formation of the medullary folds and closure of the medullary canal.

The same is the case with the *Ascidians*. In *Elasmobranchs*, the blastopore is converted into a neurenteric canal on the closure of the medullary folds. Behind this, there is a yolk blastopore, which closes without leaving a trace.

No neurenteric canal is known in *Teleosteans*, and an invagination, giving rise to a blastopore, has not been described.

¹ W. Salensky, "Recherches sur le développement du *Sterlet*," 'Archives de Biologie,' vol. ii, 1881.

² H. Strahl, "Beiträge zur Entwicklung von *Lacerta agilis*," 'Arch. f. Anat. u. Phys.,' 1882.

³ Gasser, "Der Primitivstreifen bei Vogelembryonen," 'Schriften d. Gesell. zur Beförd. d. gesammten Naturwiss. zu Marburg,' vol. ii, supplement i, 1879.

In *Petromyzon*, an invagination takes place. The blastopore remains open for a long time, though not permanently. The medullary canal is formed first as a solid cord, which becomes continuous with the hypoblast at the lip of the blastopore, thus forming the rudiment of a neurenteric canal.

In *Acipenser*, the invagination blastopore is converted into a neurenteric canal.

In *Lepidosteus*, there is no open blastopore of the ordinary kind, formed by means of an invagination, but Professor Balfour says: "In the region of the tail, the axial part of the hypoblast, the notochord and the neural cord fuse together, and the fused part so formed is the homologue of the neurenteric canal of other types. Quite at the hinder end of the embryo, the mesoblastic plates cease to be separable from the axial structures between them" ('Comp. Embryology, vol. ii, p. 93). This arrangement seems to be comparable with the primitive streak and neurenteric canal at its front end, such as is found in the higher Chordata.

In Amphibians generally, the invagination blastopore gives rise to a neurenteric canal. In the Newt, however, the invagination blastopore becomes the anus. A primitive streak extends along the dorsal surface in front of the blastopore, and I believe that there is no neurenteric canal. The primitive groove, which extends in front of the medullary folds, has a deep pit at its anterior end. In *Amblystoma* also, as mentioned above, the blastopore probably becomes the anus.

In Reptiles, there is an invagination blastopore, which becomes a neurenteric canal. Behind this point there is a primitive streak. The anus is formed along the line of the primitive streak, which extends at least as far forwards as the opening of the allantois into the alimentary canal in the Lizard,¹ and probably in all types having an allantois. Strahl² states that in the Lizard, the invagination begins in the middle of the primitive streak, near, but not at, its front end. By the

¹ W. F. R. Weldon, "Note on the Early Development of *Lacerta muralis*," this Journal, January, 1883.

² H. Strahl, loc. cit.

time that the hypoblast has been perforated by the invagination, the differentiation of the layers has extended as far back as the blastopore. Therefore, when a neurenteric canal is formed, this exists at the front end of the now reduced primitive streak.

In Birds, the invagination blastopore occurs comparatively late in development, e. g. it is most fully developed in the Duck with twenty-six mesoblastic somites and a medullary canal closed except at the extreme hind end. A neurenteric canal is found at a later stage. The primitive streak exists only behind the invagination blastopore and its corresponding structure, the neurenteric canal. At the latter, the hypoblast is fused with the epiblast and mesoblast, but remains separate from these two layers throughout the rest of the primitive streak.

In Mammals, the invagination blastopore begins as a pit in the epiblast at the front end of the primitive streak.¹ It then extends downwards and perforates the blastoderm completely. When the medullary groove is formed, it constitutes a neurenteric canal, piercing the floor of the hind end of the groove, but, before the medullary folds close, its ventral opening into the archenteron has become obliterated, and its upper part alone remains.

The view that the primitive streak represents part of the original blastopore is now so generally accepted that it may be assumed here for purposes of argument.

No reason has been suggested for the various behaviour of the blastopore of the Chordata in these different cases. It is sometimes a simple opening which gives rise to a neurenteric canal and then vanishes altogether. In other instances, it is elongated and composed of a primitive streak with an opening (which becomes a neurenteric canal) generally at its front end, but in one form (the Lizard) in the middle, or the opening may be (in the Newt) at the hind end of the primitive streak and persist without having any connection with a neur-

¹ W. Heape, "On the Germinal Layers and Early Development of the Mole," 'Proc. Roy. Soc.,' 1881.

enteric canal. Sometimes the blastopore merely consists of a primitive streak with no opening at all.

In all cases, except the Newt, the opening is restricted to the embryonic stages, and may be described as an embryonic structure. As to the variations, we can only say, for want of a more definite reason, that they are for purposes of embryonic convenience.

It is obvious, from all the facts adduced, that the original Vertebrate blastopore was elongated, but its present condition shows that great changes have taken place, since, even in the embryo, part or parts of the opening have been obliterated, these parts varying in different embryos. It is obvious, too, that the anus, at any rate, is derived from the original blastopore, and therefore is probably not an entirely new formation, acquired within the group, but is homologous with the anus of the primitive ancestral form.

My results show also that the primitive streak (i. e. blastopore) extends much further forwards than was supposed. In fact, the pit found at the front end of the primitive groove in the Newt corresponds in position more or less with the future mouth as has been remarked. This points to the probability of a connection between the blastopore and mouth, and so supports Mr. Sedgwick's¹ view that the blastopore of the Chordata was an elongated dorsal slit, the ends of which gave rise to the mouth and anus.

In *Peripatus*,² too, it is known that the blastopore is an elongated structure, the middle part of which closes, while the ends become respectively the mouth and anus of the adult.

The fusion of the embryonic layers is most distinct at the hind end of the embryo. I believe that it exists also at the front end of the primitive groove in the Newt. In the middle region of the body its existence is doubtful, but the fact that the primitive groove extends along the dorsal surface from the

¹ A. Sedgwick, loc. cit.

² F. M. Balfour, "Anatomy and Development of *Peripatus capensis*," this Journal, April, 1883.

open part of the blastopore to the anterior pit seems to prove that the blastopore as a whole is dorsal and not ventral.

A slight additional argument in favour of this view may perhaps be found in the much greater nearness of the archenteron to the dorsal surface than to the ventral in early stages of development before the yolk has been absorbed. It seems natural that the cavity should exist near the surface from which the involution to form it originally sprung.

It has already been mentioned that, in the Lizard, the primitive streak extends in front of the anus on the ventral surface as far as the opening of the allantois into the alimentary canal. Scott and Osborn described, at a comparatively late stage (with rudiments of external gills, &c.) in the Newt, a very distinct fusion of the hypoblast and epiblast in the middle ventral line behind the mouth. I have myself observed the fusion which they say is connected with the early formation of the thyroid body. Can this also be part of the primitive streak? If so, neither the mouth nor anus represent the extreme ends of the blastopore.

A possible connection between the two methods of formation of the mesoblast in Vertebrates, viz. as outgrowths from the primitive streak or lips of the blastopore, and as outgrowths from the hypoblast, is suggested by the theory of an elongated dorsal blastopore. We may suppose that, at a time when the blastopore was a long narrow open slit, the archenteron was a large cavity opening into it in the median line, and the mesoblast consisted of a pair of pouches opening into it on each side for its whole length. When the blastopore became closed and a separation between the epiblast and hypoblast ensued, the mesoblast naturally retained its connection with the latter, since it was functionally from the beginning an appendage of the archenteron. Of course, where the primitive streak existed the mesoblast would keep as far as possible traces of its original condition, but in regions where the primitive streak was obliterated the mesoblast could only proceed from the hypoblast.

In conclusion, I wish to express my very sincere thanks to

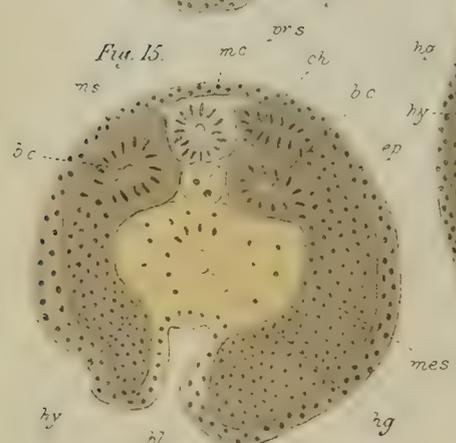
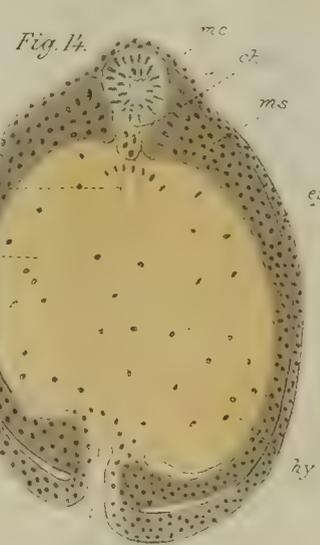
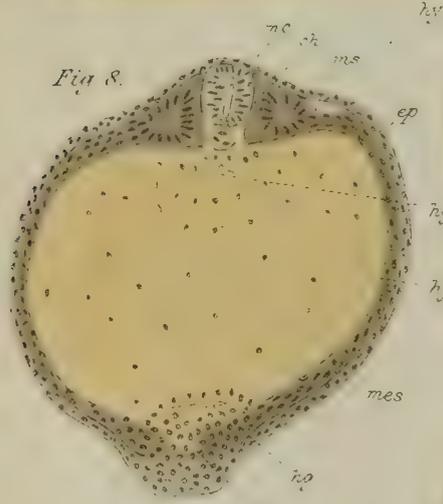
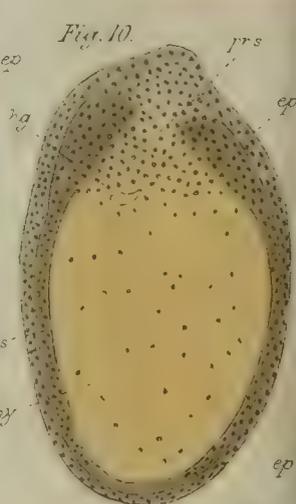
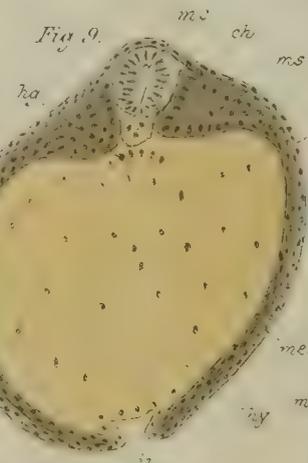
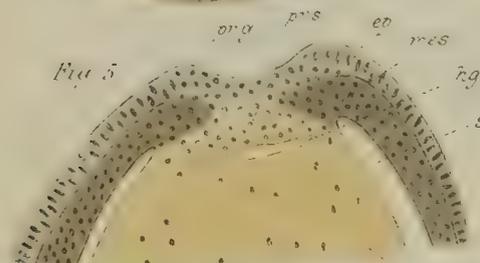
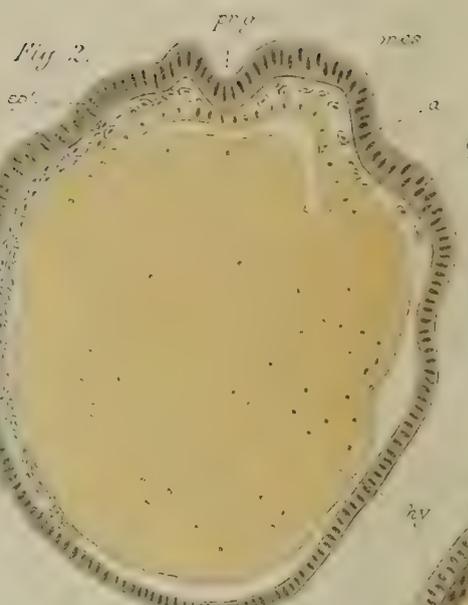
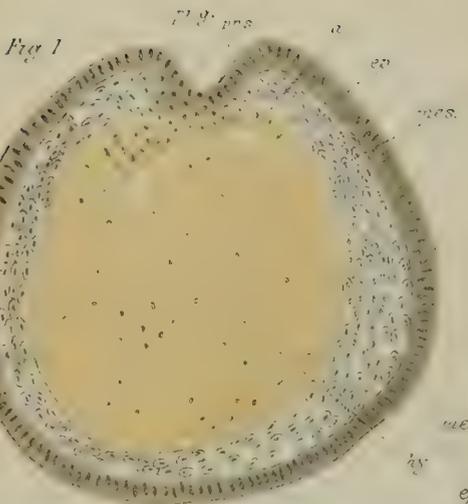




Fig. 6.

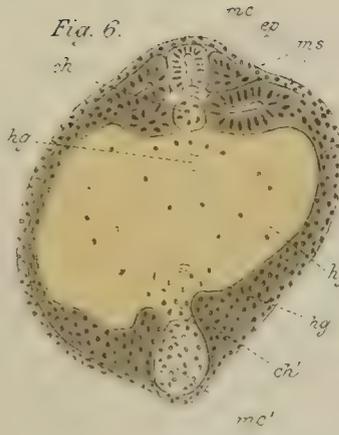


Fig. 7.

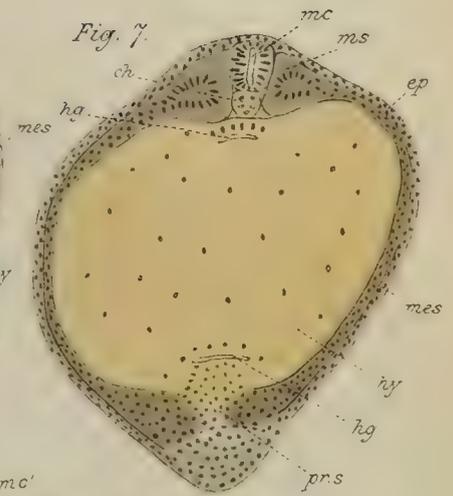


Fig. 12.

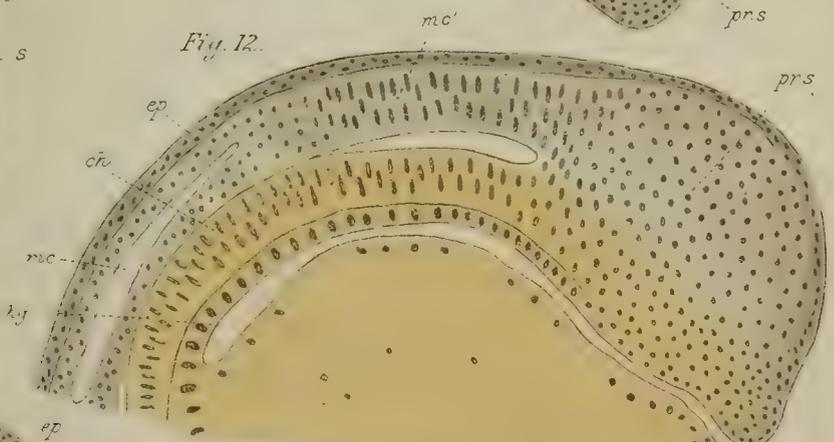


Fig. 11.

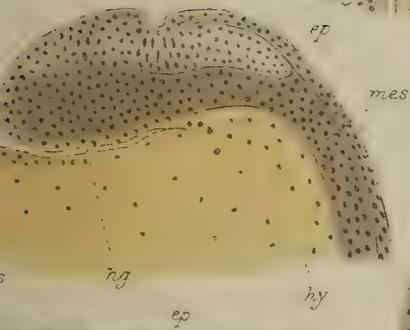


Fig. 16.

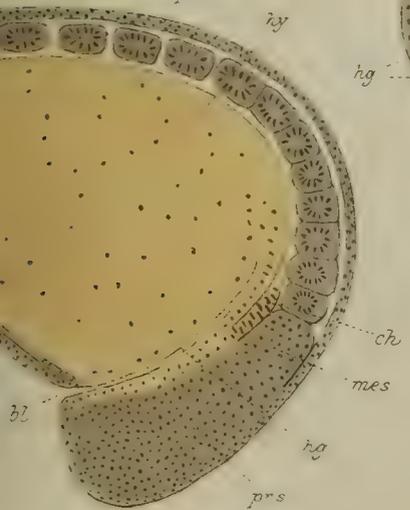
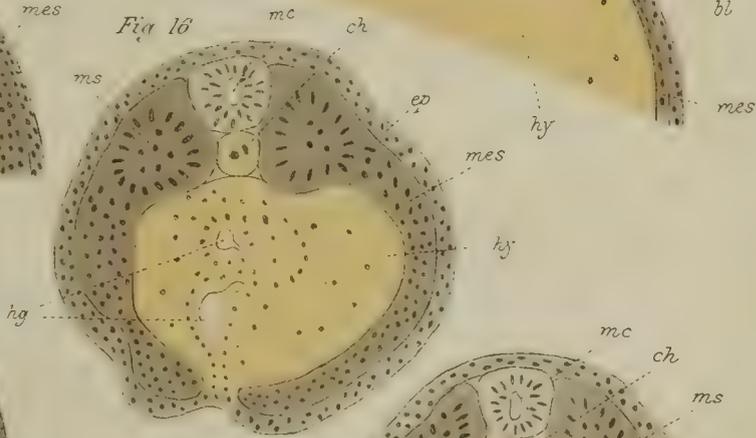
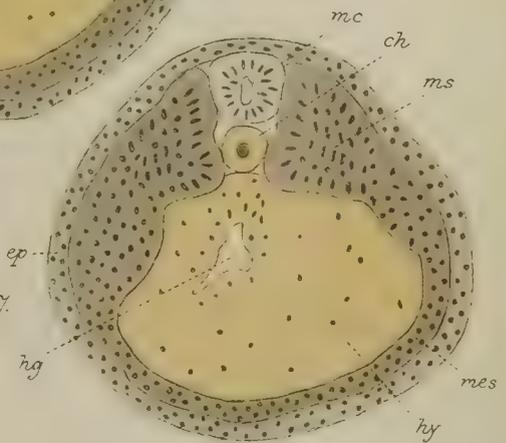


Fig. 17.



Mr. Sedgwick for his kindness in helping me both in my work and in the preparation of this paper.

EXPLANATION OF PLATE XVII.

Illustrating Miss Johnson's paper on "The Fate of the Blastopore and the Presence of a Primitive Streak in the Newt."

List of References.

a. Archenteron. *au.* Auditory vesicle. *b.c.* Body-cavity. *bl.* Blastopore. *ch.* Notochord. *ch.'* Rudiment of notochord. *ep.* Epiblast. *ep'*. Thickened epiblast of medullary area. *f.b.* Fore-brain. *f.g.* Fore-gut. *h.g.* Hind-gut. *hy.* hypoblast. *m.c.* Medullary canal. *m.c.'* Solid medullary canal. *mes.* Mesoblast. *m.s.* Mesoblastic somite. *o.v.* Optic vesicle. *pr.g.* Primitive groove. *pr.s.* Primitive streak. *s.* Space between hypoblast and mesoblast. *v.c.* Outgrowth of fore-gut to form visceral cleft.

FIG. 1.—Transverse section through embryo before the formation of the medullary folds. The section is taken through about the middle of the embryo.

FIG. 2.—Transverse section through the same embryo, taken at some distance further forwards.

FIGS. 3, 4, 5.—Transverse sections through embryo in which the formation of the medullary folds has just taken place, and the medullary area is still very broad. Fig. 3 passes through the blastopore, and is the most posterior of the series. Fig. 4 is taken through the dorsal surface at some distance in front of the blastopore. Fig. 5 is the most anterior of the series near the front end of the posterior part of the primitive groove.

FIGS. 6, 7, 8, 9.—Transverse sections through an embryo with several mesoblast somites. Fig. 6 is the most posterior, and fig. 9 the most anterior of the series.

FIG. 10.—Section through the primitive streak of an older embryo, with rudiments of the visceral clefts, tail, &c. The section is oblique, i. e. between the transverse and horizontal planes, consequently the primitive streak appears deeper than usual.

FIG. 11.—Longitudinal section through the hind part of an embryo with the medullary folds just closed. The section is slightly oblique.

FIG. 12.—Longitudinal section through the hind part of an embryo with about twelve somites.

FIG. 13.—Longitudinal section through an embryo with about eighteen somites.

FIG. 14.—Transverse section through an embryo before the formation of the tail, showing the open blastopore.

FIGS. 15, 16, 17.—Transverse sections through a considerably older embryo than that of fig. 14, showing continuity of blastopore with hind-gut. Fig. 15 is the most posterior, and fig. 17 the most anterior of the series.

The light grey colour signifies the epiblast and organs derived from it, the dark grey signifies mesoblast, and the yellow signifies hypoblast and organs derived from it.

On the Suprarenal Bodies of Vertebrata.

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Morphology in the University.

With Plates XVIII and XIX.

THE suprarenal bodies of Vertebrates are, as is well known, made up of two sets of elements, sharply distinguished from one another, both by their adult structure, and by their mode of origin in the embryo. The substance which from its position in the mammalian suprarenal is known as "medullary" is now almost universally admitted to consist of metamorphosed nerve-cells, which arise from one or more of the ganglia of the sympathetic system. As to the origin of the remainder, however, the so-called "cortical" substance, little is certainly known. In Elasmobranchs, Balfour¹ describes the homologue of this substance as "making its appearance . . . as a rod-like aggregate of mesoblast cells, rather more closely packed than their neighbours, between the two kidneys near their hinder ends;" but he leaves it an open question, whether these cells arise from the general indifferent mesoblast surrounding them, or whether they are derived from any of the adjacent organs of the embryo.

These observations of Balfour were followed, in 1882, by two

¹ "Elasmobranch Fishes," p. 246.

important papers by Braun¹ and Mitsukuri,² the one dealing with the development of the suprarenals in lizards, the other in mammals.

In lizards, Braun describes the cortical substance as arising "as a thickening in the walls of the vena cava inferior." In the earliest stage figured by him, a large mass of cortical blastema is already established, as seen in Pl. 1, fig. 4 of his paper. In this figure, as in all the others given by Dr. Braun, it is noticeable, as he himself says, that "the flattened, nucleated endothelium (of the blood-vessel) is easily to be distinguished" from the adjacent tissue, and that it shows no sign of proliferation. It is therefore difficult to conclude from this account that the suprarenals arise as appendages of the blood-vessels themselves, Braun's observations throwing little more light upon the real origin of the cortical substance than did the earlier ones of Balfour.

In the same way Mitsukuri, treating of mammals, finds the first rudiment of the cortical substance in a little knot of isolated mesoblast cells "on each side of and ventral to the aorta, on the inner side of the Wolffian bodies, and dorsal to the mesentery."

Gottschau, in a later paper³ has described in mammals phenomena nearly in accordance with those observed in lizards by Braun,—emphasising more than Mitsukuri the connection between the cortical substance and the adjacent blood-vessels.

From none of these observations can we learn anything of the mode of origin of the blastema described, each author taking up its history at a point when the cells composing it have already lost any connection which they may primitively have possessed with another embryonic organ. Janosik⁴ has attempted to trace the earlier history in mammals, and has

¹ "Bau u. Entwickl. d. Nebennieren bei Reptilien," Semper's 'Arbeiten,' Bd. v.

² "On the Development of the Suprarenal Bodies in Mammalia," 'Quart. Journ. Mic. Sci.,' 1882.

³ 'Archiv. für Anat. u. Phys.,' 1883.

⁴ 'Archiv für Mikr. Anat.,' 1883.

been led to believe that the blastema of Gottschau, Mitsu-kuri, and others arises as a series of (segmental?) outgrowths from the peritoneum, in the angle between it and the root of the mesentery and the peritoneum. As, however, very few figures are given with this paper it is not easy to form an idea of the exact nature of the events described.

This state of things led me to believe that it might be worth while to examine carefully embryos younger than those used by any previous observers, and so to trace the earlier history of the cortical blastema. This I have been able to do, during the summer of the present year, in the chick, in *Lacerta muralis*, and in *Pristiurus*. As my observations are most complete in the case of *Lacerta*, I begin with an account of the development in that type. In order fully to understand the development of the suprarenal body, it will be necessary to follow the development of the glomeruli of the mesonephros, which has been described by Braun (*loc. cit.*) After the formation of the segmental vesicles and Wolffian duct each segmental vesicle gives off from its outer margin a solid column of cells, which joins the Wolffian duct, and soon acquires the  shape characteristic of the young segmental tubes in so many Vertebrates. After this cord of cells has united with the Wolffian duct, the lumen of the segmental vesicle extends into it, and it takes on all the characters of a segmental tubule. After this has happened, one wall of the persisting segmental vesicle becomes pushed in by a plexus of blood-vessels, and forms a glomerulus.

But while the wall of the glomerulus is being thus invaginated, a proliferation of the cells composing it occurs at the side opposite to the point of attachment of the segmental tube, that is, on the inner margin of the glomerulus.

In fig. 1, I have attempted to represent the condition of things in one of the anterior glomeruli of an embryo with about twenty protovertebræ. The section passes nearly through the centre of the glomerulus, which is seen to be only partially invaginated; and I may here call attention to the manner in which, in lizards at least, the invagination seems to take place

before the entrance of the blood-vessels, none of which are to be seen in the section figured. The epithelium is much more columnar than at a later stage, and is regularly one cell thick on the outer side, while on the side undergoing invagination it is more or less regularly composed of two layers of cells; but at every point except one the whole glomerulus is bounded by cells of a definitely epithelioid character, having no processes, and showing no indication whatever of any tendency to proliferation. At the inner margin, however, the case is different; here the limiting cells are irregular in shape, and can in no way be separated, by any sharp line of demarcation, from the cells forming the mass (*s. r. b.*), which is seen to be attached to the inner wall of the glomerulus. This mass gives rise both to the connecting tubules between testis and epididymis and to the cortical substance of the suprarenals. At present it is seen to extend for a short distance dorsalwards, between the segmental tubule (*s. t.*) and the vena cava (*v. c.*), and then to bend rather sharply ventralwards towards the generative ridge, the anterior end of which (*W. r.*) is seen in the section. As a contrast to the continuity between the cell mass in question and the cells bounding the cavity of the glomerulus I would especially call attention to the distinctness of the line of demarcation between it and the endothelium of the vena cava, at the point where the two are in contact—a distinctness which, persisting, as we shall see it to do, through all stages of the development of the suprarenal blastema, renders it extremely difficult to believe that the endothelium is in a state of proliferation, or that there is any real connection between it and the suprarenal blastema.

The small blood-vessel (*b. v.*) which is seen in the figure is also perfectly sharply separated from the adjacent tissues.

The section represented in fig. 2, from an embryo about 4.5 mm. long, with twenty-four protovertebræ, shows a further advance in the development of the suprarenal blastema and its associated glomerulus. The section, which passes through the entrance of a segmental tube into the glomerulus, shows the completion of the invagination, and the entrance of blood-

vessels (diagrammetrically indicated by shading). The epithelium of the glomerulus is everywhere, except on its inner side, formed of a single layer of cells, which are much flatter than in the preceding stage, but on the inner side the cells pass, as before, without any definite line of demarcation, into the suprarenal blastema, which is still composed of a compact mass of polygonal cells, without any distinction being visible between the part which is going to form suprarenal body and that which is going to form a seminiferous tubule. In this section the distinction between the endothelial cells of the various blood-vessels and the tissues surrounding them is even better marked than in the one last described.

The appearances which I have attempted to describe are seen first in the more anterior, then in the hinder glomeruli of all that region of the mesonephros which is coextensive with the generative ridge, and in one or two glomeruli in front of it.

The blastema which I have described grows, in the succeeding stages, in two directions: dorsalwards between the cardinal vein (or vena cava) and the tubules of the mesonephros, and ventralwards into the prominence of the Wolffian ridge. In such a section as that shown in fig. 3, for example, which is taken from the posterior part of the mesonephros of an embryo of 8 mm., two distinct regions may now be distinguished, a region (*s. r. b.*) dorsal to the point of origin from the glomerulus, the cells composing which will go to form the suprarenal, and a region (*s. str.*) going from the glomerulus ventralwards into the generative ridge, which is the rudiment of the testicular network. No histological difference can as yet be detected between the one region and the other, the whole blastema being composed of a mass of polygonal cells with rounded nuclei, the characters of which are everywhere identical.

In an embryo of 10 mm. (figs. 4 and 5), a slight distinction between the two parts is for the first time apparent, though the histological characters of adult suprarenal cells are not acquired for some time. Of the two sections figured, that shown in fig. 4 is taken in front of the Wolffian ridge; in it,

therefore, the blastema attached to the glomerulus gives rise only to suprarenal tissue. For this figure, I have purposely chosen a section in which the contact between the suprarenal rudiment and endothelium of the vena cava was as close and as extensive as possible, in order to show the distinctness which, in spite of their close apposition, exists between the two structures, and to contrast once more this distinctness of the vena cava endothelium with the irregular way in which the cells of the glomerulus wall are merged in the blastema. This section is also interesting from another point of view. One of the arguments used by Dr. Braun, in order to disprove the existence of any real connection between the rudiment of the testicular network and that of the suprarenal, is that the segmental rudiments of the former structures are well developed before the appearance of any suprarenal tissue at all. Dr. Braun believes that the whole of the outgrowth from each glomerulus becomes converted into a seminiferous tubule. But if this be so, what can be the function of such an outgrowth in front of the testicular region?

In fig. 5 is seen a section through the beginning of the generative ridge: the suprarenal and seminiferous rudiments are still continuous, but the one is a little more deeply stained, and its component cells are a little smaller than the other. As before, the endothelium of the surrounding blood-vessels forms a distinct layer over the blastema, the cells of which are quite sharply defined and clearly recognisable.

The upward growth of the suprarenal rudiment, already well marked in fig. 5, is still better seen in fig. 6, from the middle of the trunk of an embryo of 13 mm.—almost the oldest in which a connection between suprarenal and seminiferous tubules can be seen. In an embryo of 18 mm. (fig. 7), the separation has already taken place, and the suprarenal is cut off by blood-vessels from all adjacent structures, though it remains now, as always before, perfectly distinct from the endothelium of the vessels themselves. This stage is only very slightly younger than the youngest figured by Braun, as fig. 4, Pl. I. of his paper shows; the

chief difference between his figure and mine being that he has, having overlooked the earlier stages, been led to an erroneous form of opinion as to the mode of origin of the tissue which he figures. From this point onwards, however, his observations as to the histological differentiation of the cortical substance, and the entrance into it of the medullary ganglion cells are so complete that it is needless to attempt to add anything to his description.

In *Pristiurus*, as in other forms, the early history of the suprarenals has only been traced from a point at which a mesoblastic rudiment, distinct from all other organs, already existed. This is the stage at which Balfour, in the passage already quoted, begins his account of their development. I propose, therefore, to trace the history of this blastema in *Pristiurus*, which is the only Elasmobranch in which I have observed it.

In figs. 9 and 10 are shown two consecutive sections through a *Pristiurus* embryo 8 mm. in length, at a stage corresponding to Balfour's Stage I—the stage immediately preceding that in which he begins the history. Both these sections pass through the opening into the body cavity of the same segmental tube, which is seen to give off, just after the narrowing of its funnel shaped opening into the body cavity, a small process (*s. r.*), which projects towards the root of the mesentery. In fig. 9, which passes through the middle of this process, it is seen to have a very considerable lumen. In fig. 10 it is cut tangentially, and the lumen is therefore not apparent.

In figs. 11 and 12, from a slightly older embryo, this diverticulum of the segmental tubule is seen to have obtained a considerable size, and to project quite to the middle line over the root of the mesentery. It is not seen in the figure to be joined by a similar structure from the opposite side, because the section copied was so oblique that the right hand side was intervertebral. In the next following section, however (fig. 13), the wall of the outgrowth of the other side is cut.

In an embryo of between 9 and 10 mm. the outgrowth has become solid, and lies just over the root of the mesentery, as shown in fig. 14; further, at this stage the outgrowths have

so coalesced with those in front and behind that an intervertebral section, such as that shown in fig. 15, still passes through them.

One feature of the sections of this age, which I do not understand completely, is the shifting of the position, with regard to the segmental funnel, of the point of attachment of the suprarenal outgrowth; while in the preceding stage (see fig. 12) the outgrowth was external to the primitive ova, opening distinctly into the segmental funnel, it is now attached to the peritoneal epithelium at the root of the mesentery internal to the primitive ova. While I am unable to account for this apparent change of position, I see no reason for doubting the identity of the structure I have called *s. v.* in figs. 14 and 15 with that similarly named in the preceding figures.

In the next stage, finally, which is a young embryo of Balfour's Stage IV, we find (fig. 16) the unpaired rod of mesoblast described by him lying at the root of the mesentery, but still attached segmentally (see the left hand side of the figure) to the segmental funnel.

I have unfortunately no stage intermediate between this and the stage last described, but it seems obvious that the unpaired blastema existing at this stage must be produced by the fusion of the paired outgrowths of the earlier stages.

An important point with regard to this blastema in *Pristiurus*, which has apparently been overlooked by Balfour, is that it extends throughout the whole length of the mesonephros.

It is well known that in an adult Elasmobranch there are two sets of suprarenal bodies: one a series of paired, more or less regularly segmental bodies, attached to the dorsal wall of the cardinal vein on each side in the mesonephric region, and the other one unpaired, median body, lying between the two halves of the metanephros.

Balfour was of opinion that the bodies of the anterior set, though they show in the adult a division into cortical and nervous positions as distinct as that which exists in the suprarenals of higher Vertebrates, were yet derived entirely from sympathetic ganglia. The presence, in the anterior end of the

body, of a blastema such as I have described seems to throw doubt on the correctness of such a view; though I have unfortunately been unable, owing to want of material, to prove by examination of later stages the share which this blastema takes in the formation of the paired anterior suprarenals.

In the chick, as might perhaps have been expected, from the highly-modified development of the whole kidney, the mode of origin of the suprarenal blastema differs in many important points from that which has been described for the dogfish and for the lizard.

Before the fourth day of incubation there is no trace of any suprarenal rudiment whatever. By about the end of this day, however, certain large cells, the rudiments of the cortical substance, make their appearance in the indifferent mesoblast at the inner side of the mesonephros. The exact mode of origin of these cells I have been unable to determine. At their first appearance they lie, singly or in groups of two or three, in the mesoblast between the aorta and the kidney, being distinguished from the surrounding cells by their rounded, unbranched form, their larger size, and the clearness of their protoplasm. During the end of the fourth day, and the early part of the fifth, they increase in number, either by division or by addition from the surrounding mesoblast, till in an embryo of about the middle of the fifth day of incubation, they form groups of a considerable size, which present in section the appearances seen in fig. 17. The cells seen in this section, though they are more numerous than at the time of their first appearance, have not appreciably changed their relations to the surrounding parts. They are seen to lie surrounded entirely by branched mesoblast cells without any connection, either with the epithelium of the adjacent glomeruli, or with the walls of any blood-vessels. In this isolated condition the suprarenal cells remain during the fifth and sixth days, travelling, however, gradually towards the mesonephric glomeruli, and at the same time increasing in number, and tending to arrange themselves in irregular branched columns, having in section an elliptical outline. During the seventh day they attach them-

selves to the epithelium of the glomeruli, so as to appear as in fig. 18. In this figure the epithelium of the glomerulus is seen to be distinct from the suprarenal for a short distance; but in a part of the section I was unable, after a tolerably careful examination, to convince myself of the existence of any distinct layer of epithelial cells separating the cavity of the glomerulus from the adjacent blastema.

Such a section as that shown in fig. 18 may be seen in almost any glomerulus in the region of the suprarenal during the seventh day. On the eighth day the appearance of the blastema changes. While still retaining its connection with the glomeruli (fig. 19) it has increased considerably in size, and its component cells have acquired most of the histological characters which they present in the adult. The individual cells are large, polygonal, and distinctly marked off one from the other; their protoplasm, which does not stain very readily with carmine or hæmatoxylin, is clear or very finely granular, and their nuclei are clear, oval, or elliptical, with well-defined contours and a number of coarse granules in their interior. The most characteristic feature in the blastema of this age is, however, the definite arrangement of the cells into columns, giving them, more than at any earlier stage, the appearance of the cortical substance of an adult suprarenal.

I have already said that the blastema during the eighth day remains attached to the glomeruli; such appearances as those seen at *x* in fig. 19, which are very frequent, tempt one strongly to believe that at this time the number of the cells composing it may be added to by proliferation from the glomerulus epithelium; but I have not been able to satisfy myself that this is the case.

From this time the changes in the cortical blastema, so far as I have followed them, do not differ in any important particulars from those described by Braun in *Lacerta muralis*.

A noticeable feature throughout the whole of the early history of the organ under consideration in the chick, is the very distinct separation between the cortical blastema and the blood-vessels, the original blastema-cells being at a great

distance from any vessel, and the later tissue only approaching one when it has so greatly increased in size as to have pushed all the intervening mesoblast, so to speak, on one side. There is no possibility of believing, in this case at least, that the walls of the blood-vessels have the slightest share in the production of the cortical blastema.

The great difference between the results of the investigations of previous observers and those which have just been described, is sufficiently obvious. If, however, the accuracy of my observations be admitted, we have a much more rational explanation of the phylogeny of the suprarenals than is possible if we adopt the view of Braun, and others;—an explanation which receives support, both from the anatomical relations of the adult organs, and with those of the corresponding organs in Myxinoids and Teleosteans.

In *Bdellostoma*, I have already¹ attempted to show that the head kidney has become modified so as to form an organ functionally analogous to the suprarenals; while in Teleosteans, a most remarkable series of modifications, affecting every region of the kidney, has been described by Balfour and Emery; a series which seems to me to supply every stage needful to complete our conception of the passage from such a form as *Bdellostoma* to that of a higher vertebrate. Balfour showed² that the head kidney of many adult Teleosteans consisted, not of renal tissue, but of a mass of parenchymatous "lymphatic" material, richly supplied with vessels, whose function, whatever it might be, was certainly not that of a normal kidney. He afterwards found the same kind of modification to exist in the head kidney of the Teleosteoid Ganoids.³

Though the observations of Balfour left it highly probable that the "lymphatic" tissue described by him was really a result of the transformation of part of the embryonic kidney,

¹ Quart. Journ. Mic. Sci., April, 1884.

² Quart. Journ. Mic. Sci., 1882.

³ "On the Structure and Development of *Lepidosteus*," *Phil. Trans.*, 1882.

he did not investigate the details of its development. This was afterwards done by Emery,¹ with the following results:—

In those Teleostei which he has studied, Professor Emery finds that at an early stage the kidney consists entirely of a single pronephric funnel, opening into the pericardium, and connected with the segmental duct, which already opens to the exterior. Behind this funnel, the segmental duct is surrounded by a blastema, derived from the intermediate cell mass, which afterwards arranges itself more or less completely into a series of solid cords, attaching themselves to the duct (see fig. 8). These develop a lumen, and become normal segmental tubules, but it is, if I may be allowed the expression, a matter of chance, how much of the blastema becomes so transformed into kidney tubules, and how much is left as the "lymphatic" tissue of Balfour, this "lymphatic" tissue remaining either in the pronephros only, or in both pro- and mesonephros.

We have here, as it seems to me, an explanation of the reason why the suprarenals, while arising from the pronephros in Myxinoids, are mesonephric in origin in the higher Vertebrates. The same causes which led to the degeneration of the original renal pronephros (causes among which the specialisation of the pericardium, and the development of the air-bladder and lungs may have played a considerable part)—the same causes which led to the establishment of the mesonephros as the chief seat of renal secretion may, and indeed must, have rendered advantageous the suppression of any glandular organ in the pronephric region; and thus, when, in consequence of the change of function of the Wolffian duct more and more of the mesonephros became useless as a kidney, it is easy to understand how some of its component parts underwent in their turn the same change of function as had been undergone by the anterior part of the renal organ at an earlier stage in its evolution, stages in the completion of this process remaining

¹ 'Atti dell' Accademia dei Lincei.,' 1882.

both in the commencing modification of the Teleostean mesonephros on the one hand, and on the other in the suprarenal of Amphibia, with its own "portal" circulation, and its close connection with the renal tissue.

EXPLANATION OF PLATES XVIII & XIX,

Illustrating Mr. W. F. R. Weldon's Paper "On the Suprarenal Bodies of Vertebrata."

Complete List of Reference Letters.

Al. Alimentary canal. *Ao.* Aorta. *Bv.* Blood-vessel. *gl.* Glomerulus. *g. ep.* Glomerulus epithelium. *pe. ep.* Peritoneal epithelium. *Mes.* Mesentery. *v. c.* Vena cava. *s. t.* Segmental tubule. *s. str.* Testicular tubule. *s. r. b.* Suprarenal blastema. *W. r.* Wolffian ridge.

The figures were in all cases drawn by the aid of a Zeiss's camera lucida.

FIG. 1.—Transverse section through a glomerulus of an embryo of *Lacerta muralis* with twenty-one protovertebræ.

FIG. 2.—Similar section through an embryo with twenty-three protovertebræ.

FIG. 3.—Similar section through an embryo 8 mm. long.

FIG. 4.—Similar section from an embryo 10 mm. long.

FIG. 5.—Similar section from an embryo of 11 mm.

FIG. 6.—Similar section from an embryo of 13 mm.

FIG. 7.—Similar section from an embryo of 18 mm.

FIG. 8.—Transforming blastema of teleostean kidney, copied from Emery.

FIGS. 9 and 10.—Two consecutive sections through an embryo of *Pristiurus melanostomus* of 8 mm.

FIGS. 11—13.—Consecutive sections from an embryo of *Pristiurus* of 8½ mm.

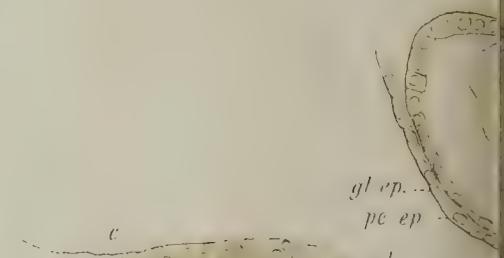
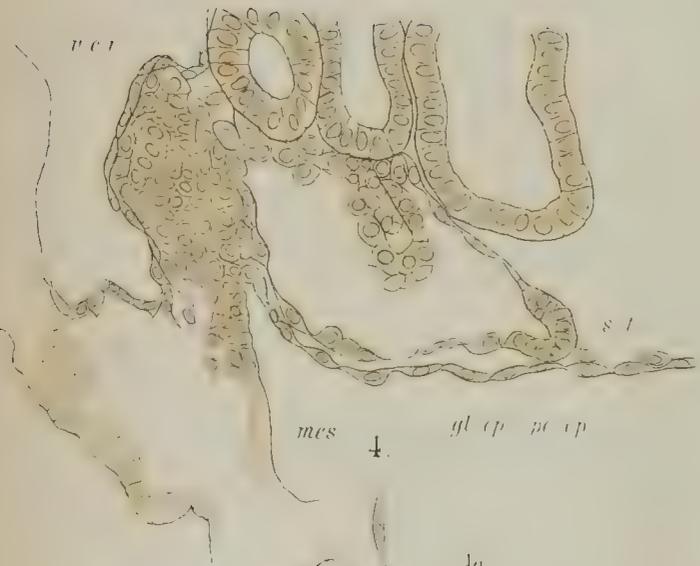
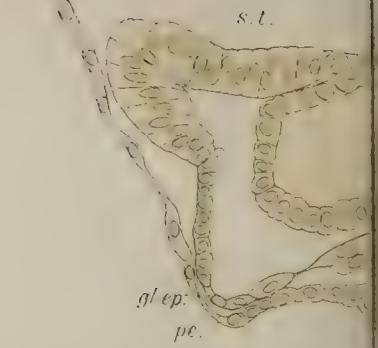
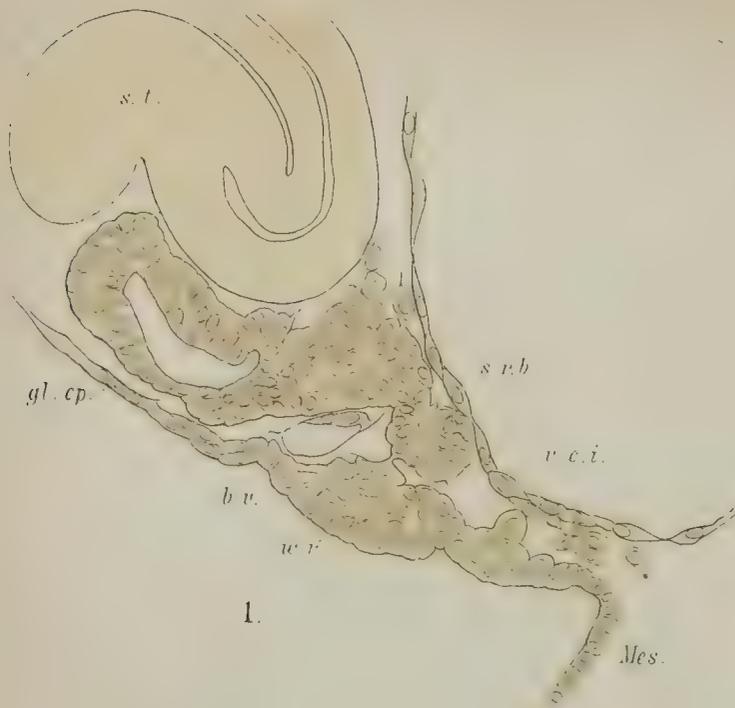
FIGS. 14 and 15.—From an embryo of *Pristiurus* of 10 mm.

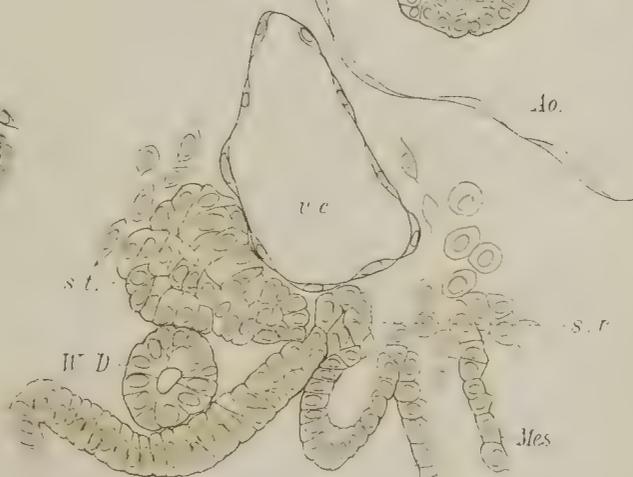
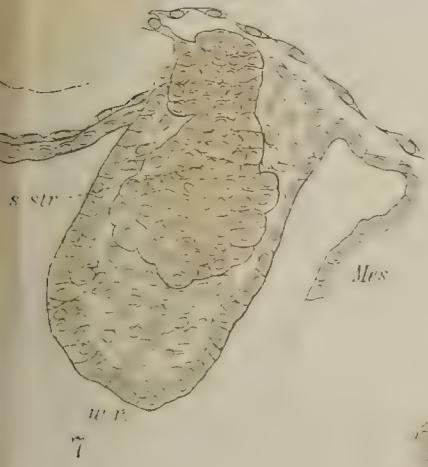
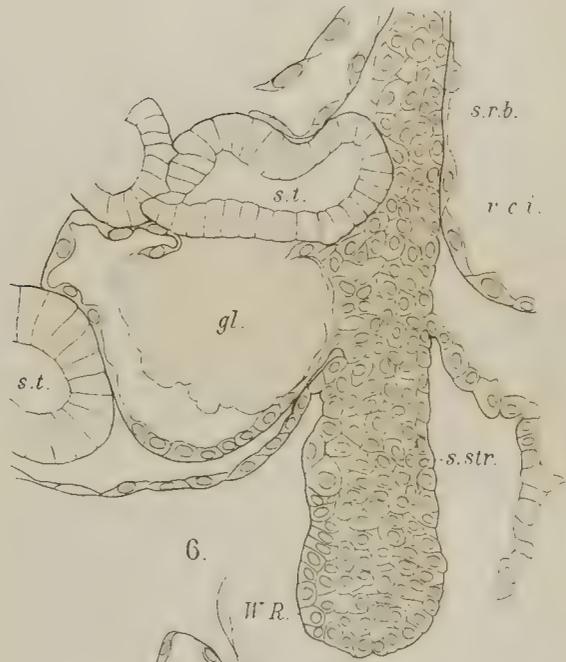
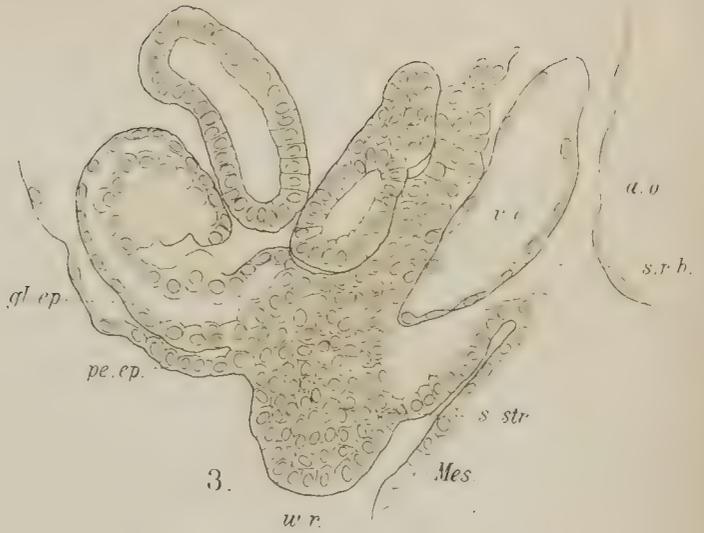
FIG. 16.—From an embryo of *Pristiurus* slightly older than that figured in figs. 14 and 15.

FIG. 17.—From a five-day chick.

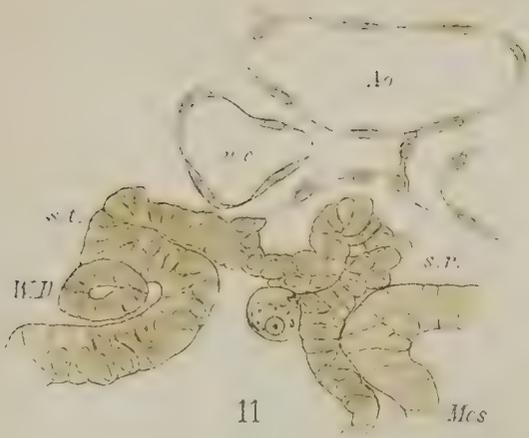
FIG. 18.—From a seven-day chick.

FIG. 19.—From a nine-day chick.





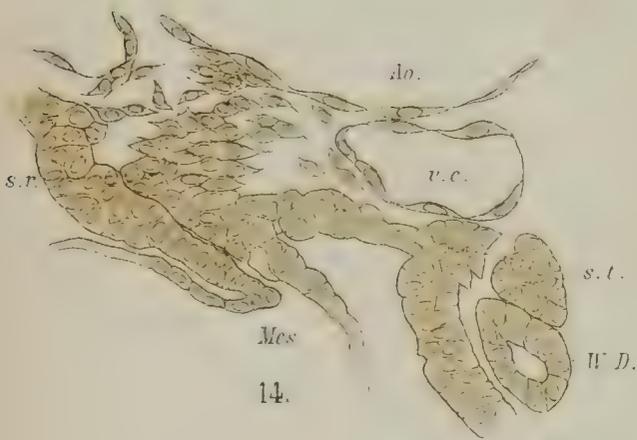
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14



15



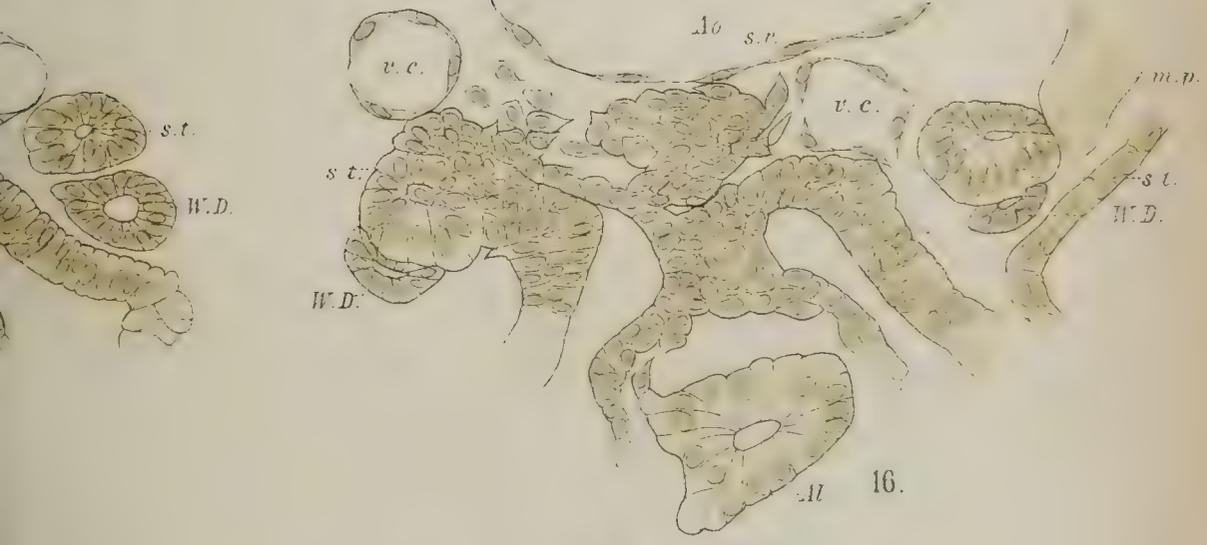
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18



13.



16.



19.

On a Peculiar Sense Organ in *Scutigera*
coleoptrata, one of the Myriapoda.

By

F. G. Heathcote, B.A.,
Trinity College, Cambridge.

With Plate XX.

IN the spring of the year I was fortunate enough to get a fair number of *Scutigera* in the South of Europe. In making an examination of their anatomy I found a sense organ which seemed to me to be of sufficient interest to render a more complete examination desirable. This organ is placed on the ventral surface of the head at a short distance behind the mouth and near the base of the mandibles. Its external appearance under a low power of the microscope (Zeiss's objective A A) is shown in fig. 1.

GENERAL FEATURES.

The organ which was first mentioned by Latzel, consists of a chitinous sac with a slit-like opening (fig. 1, *eo.*). The opening is placed between the base of the mandibles and the maxillæ. The sac has a somewhat complicated form which will be best understood by reference to four diagrams (see Plate XX, figs. A, B, C, D).

The first of these shows a rough outline of the appearance of the organ from the ventral side; the second, third, and fourth being diagrammatic sections through the dotted lines *AB*, *CD*, and *EF*. *B* is a transverse section through the anterior portion of the organ. It shows the main sac communicating with the

exterior by a narrow neck and two lateral recesses opening into the neck and placed ventrally to the main sac parallel with the ventral surface of the head. The section *C* taken through the median portion of the organ exhibits similar relations, excepting that the median dorsal wall of the sac is projected into the interior in two longitudinal folds which make a partial division of the sac into three portions, two deep, wide lateral pouches, and one deep narrow recess between them. This latter I shall speak of as the median recess. A third section, *D*, is taken through the hinder part of the organ. Here the lateral recesses and the slit-like opening to the exterior are absent and the two dorsal folds almost completely divide the main sac in three portions.

It is worthy of note that the effect of the median and lateral recesses is to produce a freely projecting lip or edge on the dorsal and ventral aspects of each pouch.

The general shape of the interior of the organ is therefore posteriorly that of two pouches projecting into the interior of the head, while between them is a median dorsal recess formed by the folds above described, which constitute the inner walls of the pouches where they approach one another. Anteriorly the division into two pouches is not so perfect, but there are two deep lateral ventral recesses. The slit-like opening to the exterior begins at the anterior end and extends about a third of the length of the whole organ.

The chitinous exoskeleton is continued into and lines the whole organ. It is not, however, of uniform thickness. In the median and lateral recesses and on the folds constituting the lips of the pouches it is smooth, but in the pouches themselves it is thrown into a number of folds and bears a large number of chitinous hairs (fig. 2, *h.*) which project into the lumen of the pouches. The folds form alternate ridges and depressions, so that when looked at from the surface through a microscope the chitinous lining of the pouches has a reticulated appearance (fig. 8). The hairs, whose length is about that of the diameter of each pouch, are of peculiar form (fig. 9). Each consists of a stout elliptical basal portion, the inner end of which is inserted

into the chitinous lining of the pouch and indeed projects for a short distance on the inner side of the latter. The outer end is prolonged into a long fine hair.

GENERAL FEATURES OF THE INTERNAL ANATOMY.

The hypodermic layer of cells or matrix lying beneath the exoskeleton accompanies the chitin round the lateral recesses, and at the edge of the folds which form the lateral lips of the two pouches becomes continuous with a thick layer of sensory epithelium which lines the greater part of the two pouches (fig. 2, *s. e.*). On reaching the dorsal lips of the pouches, which lips bound laterally the median recess, the epithelium loses its sensory character and again becomes simple hypodermis. On the dorsal part of the median recess the epithelium again becomes sensory in character. The nerve supply is furnished by two short thick nerves (fig. 2, *N.*) which arise from the front portion of the subœsophageal ganglion. The two nerves enter the sensory epithelium, one to each pouch, near the posterior part of the organ, and there breaks up into a number of fibres which become lost in the epithelium. The form of the organ as indicated by the division into two pouches (fig. 2, *p.*) and the double nerve supply seem to me to show conclusively that it is double, and that each of the two pouches with its other parts is to be regarded as constituting a separate sense organ.

HISTOLOGY.

The histology of the cellular tissues demands a more detailed account. The cells forming the matrix from which the exoskeleton is renewed after each moult are large, rather columnar in their character, and have a well-defined nucleus. They are closely applied to the chitin and accompany it up to the end of the lateral recesses (refer to fig. 3, *hy.*) where the folds forming the ventral lips of the pouches begin. Here the chitin is thrown into folds somewhat like those which characterise the surface of the pouches. The hypodermic cells here lose their regularity of outline and follow the chitin into the

folds and irregularities into which it is thrown (fig. 3, *hy.*). Their nucleus is larger and stains more deeply. At the lateral ventral lip (fig. 2, *lvl.*) the cells are more elongated and more closely packed together, and gradually take the character of the sensory epithelium which forms the greater part of the lining of the pouches. These sensory cells are long and columnar and at their outer ends are prolonged into a blunt projection of less diameter than the rest of the cell (fig. 7, *oe.*) and about one third the length of the whole. At the folds which bound the median recess the cells lose their sensory character and take the form of the ordinary hypodermic cells. The mass of sensory cells at the top of the median recess which are continuous with the hypodermic cells are of a character distinct from those described as lining the pouches. They are of irregular elongated shape and resemble ganglion-cells, the inner end being sometimes bifurcated (fig. 6, *Bi.*). The sensory epithelial layer is of considerable thickness (fig. 2).

I have hitherto spoken of the epithelial layers simply as investing the chitinous pouches with their hairs, but I will now consider the means by which the hairs and cells come into relation. There is no doubt that the terminal parts of the sense cells project into the depressions (fig. 3) in the chitin, caused by the folds spoken of above, and that each chitinous hair is inserted into the chitin immediately outside this projecting part of a sense cell. I am also inclined to believe, though, owing to the small amount of material at my command my evidence on this point is not conclusive, that the bases of the chitinous hairs, i. e. the part which projects on the inner side of the chitinous lining, have a small cavity in their basal parts, into which a threadlike prolongation of the sense cell projects.

I have invariably found foreign bodies in the median and lateral recesses, and as the latter are in communication with the exterior they may possibly be grains of dirt or sand, but I think that they may be concretions.

CONCLUSIONS.

The active predatory habits of this Myriapod and its power of swift locomotion would seem to render well-developed sense organs a necessity to it; in fact it has faceted eyes in place of the simple eye-spots of most Myriapods.

The organ above described must, I think, be included among the great number of widely dissimilar organs usually classed together as auditory, and may be compared to the tympanic organ of insects.

The auditory organs of insects have been investigated principally by v. Siebold ('Archiv für Naturg.,' 1844), Leydig ('Müller's Arch.,' 1855 and 1860), v. Hensen ('Zeitschr. f. wiss. Zool.,' tom. xvi, 1866), and v. Graber ('Denkschr. der K. Akad. der Wissensch.,' Wien., 1875). The tympanic organ of the Acridiidae consists essentially of a tympanic membrane supported by a chitinous ring. Places in the tympanic membrane are thickened, so as to form solid chitinous pieces of peculiar form, the internal surface of which is covered with indentations in which the extreme ends of the sensory apparatus end (Fr. Leydig, 'Müller's Archiv,' 1855, p. 401). The auditory nerve spreads out on these chitinous pieces and forms a ganglion, from which fibres ending in peculiar sense cells are given off. A trachea lies close to the ganglion internally to it, and not unfrequently swells to a vesicle.

On comparing the organ of *Scutigera* with such an organ there is found to be a great similarity in the general plan. Each pouch in *Scutigera* represents the insect tympanum. In both cases we have a thick nerve breaking up into a number of sensory elements, which end in depressed spaces in the chitinous membrane. With regard to the chitin hairs which project through the chitin in *Scutigera*, I think it will be worth while to consider Hensen's investigations on the auditory rods of insects (Hörstifte, v. Hensen, l. c.). He makes an interesting comparison between these structures and the auditory hairs of the crustacean auditory sac, and draws the con-

clusion that the two structures present a very great morphological resemblance. If his arguments hold good it seems to me permissible to compare the hairs of *Scutigera* to those in the auditory sac in Crustacea, and also to the auditory rods (Hörstifte) in insects.

There is one point, however, in which the organ of *Scutigera* differs greatly from the tympanic organ, viz. in the absence of a tracheal vesicle. I think it doubtful, however, whether this tracheal vesicle is an essential part of the insect auditory organ. The swelling of the tracheal trunk seems not to take place in all cases (Leydig, l. c.), and Hensen, in giving what he considers the most probable hypothesis as to the action of the tympanic organ, says: "Die tracheen schwingungen sind ohne Bedeutung." Balfour, in his short account of the auditory organ of terrestrial insects ('Comp. Emb.,' ii, 423), does not mention the tracheal vesicle.

I have examined this sense organ of *Scutigera*, both by dissection and by means of sections. I found that the tissues were best preserved by a mixture of corrosive sublimate and acetic acid. The difficulty of cutting the chitin in sectioning was overcome by embedding in very hard paraffin.

My investigations were entirely carried on in the Cambridge Morphological Laboratory.¹

¹ Since forwarding this paper (November, 1884) to the editor of this Journal my attention has been drawn to a paper by Dr. Haase in Schneider's 'Zool. Beiträge,' 1884, upon "Schlundgerüst und Maxillarorgan von *Scutigera*."

As I am on the point of leaving England on a long voyage it is now too late for me to make an extensive reference to this work, but I may add that in my opinion Dr. Haase's observations do not necessitate any alterations in the foregoing paper.

DESCRIPTION OF PLATE XX,

Illustrating Mr. F. G. Heathcote's Paper "On a Peculiar Sense Organ in *Scutigera coleoptrata*, one of the Myriapoda."

Letters used in all the Figures.

m. Mouth. *f.* Furrow in chitin. *e. o.* External opening of sense organ. *o.* Sense organ. *mxl.* 2nd maxilla. *N.* Nerve. *s. e.* Sense epithelium. *h.* Chitinous hairs. *hy.* Hypodermis. *p.* Pouch. *lr.* Lateral recess. *Mr.* Median recess. *lvl.* Lateral ventral lip. *Mdl.* Median dorsal lip. *ch.* Chitin. *Bi.* Bifurcated end of cell at top of median recess. *oe.* Outer end of cell. *lh.* Base of chitinous hairs projecting into interior. *bp.* Basal part of chitinous hair. *fh.* Free end of hair.

Fig. 1 was drawn for me by Mr. Chapman; all other figures were drawn by myself with the aid of Zeiss's camera lucida. Fig. 2 is combined from three sections.

FIG. 1.—Ventral view of head of *Scutigera coleoptrata*. The sense organ is seen through the chitin. *m.* Mouth. *f.* A furrow in the chitinous exoskeleton, marking out two irregular areas just in front of the organ. *e. o.* Opening of organ to the exterior. *o.* Organ seen through the chitin. *oc.* Eye. *mxl.* 2nd maxilla.

FIG. 2.—Transverse section through the organ, showing the nerve (*N.*), sense epithelium (*se.*), chitinous hairs (*h.*), hypodermis (*hy.*), median recess (*Mr.*), and lateral recesses (*lr.*); also the two pouches (*p.*). (Zeiss's c c objective.) Owing to the action of the reagents used the sensory epithelium has shrunk away from the chitinous lining of the sac. *lvl.* Lateral ventral lip. *Mdl.* Median dorsal lip.

FIG. 3.—Transverse section through the region of the lateral recess, showing the transition from the hypodermis to the sensory epithelium. *hy.* Hypodermic cells. *se.* Sense cells. *lr.* Lateral recess. *ch.* Chitin. *h.* Chitinous hairs projecting into the pouch. *lvl.* Lateral ventral lip of pouch. (Zeiss's F objective.)

FIG. 4.—Transverse section, taken so as to show the hypodermic cells (*hy.*) joining the sense cells (*se.*) in the region of the median recess. (Zeiss's F objective.) *hy.* Hypodermic cells. *se.* Sense cells. *ch.* Chitin.

FIG. 5.—Transverse section through the anterior part of the organ, showing the hypodermic cells becoming continuous with the sense cells (*se.*). The

section is in front of Fig. 4. (Zeiss's D objective.) *se.* Sense epithelium. *ch.* Chitinous lining of the organ. *hy.* Hypodermis.

FIG. 6.—Tailed cell from sense epithelium at top of median recess. *Bi.* Bifurcated end. *oe.* Outer end. (Zeiss's F objective.)

FIG. 7.—Sense cells from epithelium (*se.*). *oe.* Outer end of cell. (Zeiss's F objective.)

FIG. 8.—Surface view of chitinous lining of pouch seen from the internal side, showing the projecting bases of the chitinous hairs. *bh.* Base of hair projecting through the chitin. (Zeiss's water immersion L objective.)

FIG. 9.—Chitinous hair under high power. (Zeiss's L water immersion.) *bp.* Basal part of hair. *Bh.* Part of the basal piece which projects through the chitinous lining of the pouch towards the interior. *h.* The hair itself projecting into the interior of the pouch.

Fig. 1.



Fig. 5.

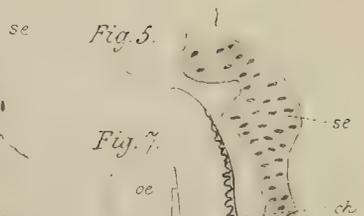


Fig. 4.



Fig. 7.

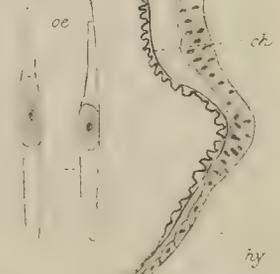


Fig. 6.



Fig. 8.



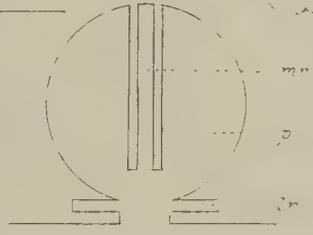
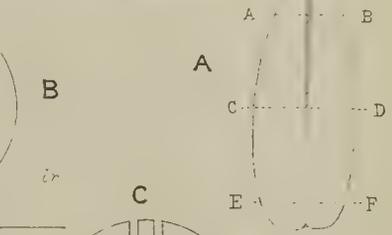
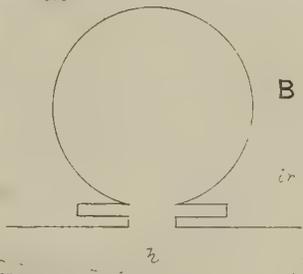
Fig. 9.



Fig. 2.



Fig. 3.



The Development of the Mole (*Talpa Europea*),
the Ovarian Ovum, and Segmentation of the
Ovum.

By

Walter Heape, M.A.,

Demonstrator of Animal Morphology in the University of Cambridge.

With Plate XXI.

THE RIPE OVARIAN OVUM.

THE position of the ripe ovarian ovum in the ovary is betrayed by the rounded semi-transparent Graafian follicle in which it lies, projecting prominently on the surface of the ovary.

If an ovary containing such a follicle be held firmly with a pair of forceps on a slide, and the follicle be pricked with a needle, or better still, sharply gashed with the point of a fine scalpel, the ovum spirts out on to the slide together with a not inconsiderable amount of clear transparent fluid, the liquor folliculi.

In accordance with the degree of ripeness of the ovum thus obtained it is more or less completely invested by a mass of epithelial cells, in the midst of which it lay in the discus proligerus within the follicle.

These epithelial cells are radially arranged round the ovum (fig. 1). The cells of the innermost layer are more or less elongated and their inner end, tapering somewhat, rests upon a thick transparent membrane which surrounds the ovum, the so-called zona radiata (the zona pellucida of the older observers).

The shape of the cells of this inner layer varies according to age, as van Beneden has observed (No. 4), but they invariably have the aspect of an epithelial investment. To this layer of cells the misleading term of *membrana granulosa* has been applied.

THE ZONA RADIATA.

The zona radiata in fully ripe ova (*vide* figs. 1 and 2) is a clear transparent membrane with a granular outer border upon which the surrounding cells of the discus proligerus rest (fig. 1).

The inner portion of this membrane is so transparent that the outlines of the epithelial cells may clearly be seen through it.

The origin of the granular outer portion has not been satisfactorily traced; it may possibly, according to Balfour (No. 1), be due to the presence of the remains of the primary vitelline membrane, within which the zona radiata has been subsequently produced. On the other hand, the appearance may be due to the irregularity of the surface of the zona radiata itself, this latter circumstance being in its turn occasioned partially by the close adhesion of the surrounding cells of the discus (fig. 6), partially by the open mouths of numerous canals which pass radially through it, and to which I shall call attention directly (fig. 7).

I have not myself attempted in this paper to trace the development of the ovarian ovum or its membranes, and must therefore at present leave this question without further discussion.

The thickness of the zona varies in the two specimens represented (figs. 1 and 2) between $\cdot 008$ and $\cdot 001$ mm. The two ova themselves were both completely surrounded by the cells of the discus proligerus, but in the one drawn in fig. 2 the greater portion of these cells has been carefully detached.

The radially striated appearance of the zona has long been shown to be due to a vast number of fine canals passing radially through it. These canals I find open on the inner side of the

zona by a slightly dilated mouth, while on the outer side of the zona they communicate with the exterior by a considerably wider opening (fig. 7). Into the external openings of these canals I have been able to trace prolongations of those cells of the discus which are immediately in contact therewith (fig. 7), and there appears to me no room for doubt that the contents of these follicular cells are thus rendered available for the nutriment and growth of the ovum.

Owing to the extreme minuteness of the canals it is quite possible that they are only rendered visible by the protoplasm of the follicular cells, which is less transparent than the zona itself, passing through them, and the fact that careful observers have not succeeded in detecting these pores would be accounted for by the cessation of the nutrient process at the time of observation. I may add I have observed the radial canals through the zona in optical sections of various whole ova, as well as in many actual sections of ova situated within the Graafian follicle.

I have before mentioned that the close investment of the ovum with follicular epithelium cells is in accordance with the degree of ripeness of the ovum itself. When the latter is fully mature only a very small number of, and in some instances no, epithelium cells are carried out with it upon the rupture of the follicle. Thus the attachment of the epithelium to the zona ceases when the ovum becomes mature, and no further nutriment is required, and this is of itself some further proof of the nutrient function of the follicular epithelium cells.

I myself never detected any follicular cells within the zona, such as has been described by Lindgren (No. 15), von Sehlen (No. 21), and Virchow (No. 22); nor have I seen any trace of a micropyle in the zona, such as M. Barry (No. 3) and others held to exist.

THE VITELLINE MEMBRANE.

Within the zona radiata and enclosing the ovum itself in all those ripe ovarian ova examined by me, is a second very thin

membrane, the vitelline membrane (*vide* Reichert No. 18, Meyer No. 17, and van Beneden No. 4). In the ovum drawn in fig. 1, this membrane may be seen where a space exists here and there between the zona and the ovum.

In fig. 2 no space was to be distinguished with the magnifying power used (Zeiss D) for the drawing, but in fig. 7, which is a drawing of a portion of the circumference of the same ovum with a higher magnifying power (Zeiss, imm. 3), a narrow space is clearly shown between the ovum and the zona, and a very fine membrane is there discernible closely covering the ovum. This membrane is, however, most clearly visible in fig. 8, which is the drawing of an ovum in which maturation has taken place; in this specimen there is a considerable space between the vitelline membrane and the zona, the former being rendered still more evident on account of the contraction of the material of the ovum itself within the vitelline membrane. The space between the vitelline membrane and the zona radiata I propose to call the circum-vitelline space.

The development of the membranes, about which there has been considerable discussion, I propose to consider in a future paper.

The Yolk.

The ripe ovarian ovum itself is composed of food-yolk of two kinds—(1) homogeneous, partially transparent, vesicular bodies, (2) minute highly refractive granules of various sizes,—and of a network of protoplasm which divides the yolk into rounded or cubical masses such as I have endeavoured to represent in figs. 2 and 7. The two kinds of yolk are similar to those described by most of the observers of Mammalian ovarian ova. It is worthy of remark, however, that I found no globules in the Mole's ovum similar to those described by Beneden and Julin (No. 6), and figured by those authors in their paper (No. 7) on the ova in Cheiroptera.

The difference in the density of the yolk in various Mammalian ova is very remarkable and would, I suspect, if examined

with regard to the early phases of development, throw some light upon the curious differences which then occur.

Kolliker (No. 14, 2nd edit., p. 44) and Schulin (No. 20), declare that the human ovum is markedly deficient in yolk vesicles when compared with the ovum of the Cat or the Cow. Bischoff (Nos. 8, 9, 10, 11), in his figures of the ova of the Rabbit, Dog, Guinea-Pig, and Deer, shows that the Deer's ovum is not filled with such a dense mass of yolk as is that either of the Dog or Rabbit, while the ovum of the Guinea-Pig is remarkably transparent, a statement in the latter case with which Reichert's (No. 18) and my own observations fully coincide (*vide* fig. 21). The Mole's ovum must be classed in this particular with that of the Rabbit and Dog, while the Bat's ovum, it appears, is similar to that of the Guinea-Pig.

The network, which has as far as I know hitherto only been observed in Mammalian ova by Schäfer (No. 19) in young ovarian ova of the Rabbit, was very distinct in the ovum represented in fig. 2. A similar appearance was noted in other ova, but in a considerable number no such network could be detected. There appears to me, however, good reason to believe that the appearance is due to a protoplasmic reticulum in the meshes of which the food material lies.

THE NUCLEUS.

In all those ova in which the nucleus was observed it was placed excentrically, the density of the yolk being so great it could not be distinguished when lying in the centre of the ovum. It was found to be either circular or oval in optical section, and bounded by a distinct membrane. In the ovum represented in fig. 2 the nucleus is indicated by a circular ring; its contents could not, however, be observed owing to the density of the supervening yolk, the network before spoken of being seen overlying the nucleus.

In figs. 3, 4, 5, I have drawn the nuclei of three ova which I obtained from the female from which the ovum drawn in

fig. 2 was taken. I tore open these ova and isolated their nuclei; the one represented in fig. 3 was flattened by withdrawing the fluid in which it was immersed from beneath the coverslip, the other two are, as nearly as may be, not under the influence of pressure. In all of them a homogeneous nuclear substance bounds a central clear space in which lies the nucleolus. Besides the nucleolus a small number of large and small highly refractile irregular-shaped bodies are contained within the nucleus.

In fig. 4 the nucleolus, which is not bounded by a membrane, consists mainly of an aggregated mass of minute granules, a single larger granule being embedded in the midst of these. A ring of four very large irregular granules surrounds the nucleolus, and a few fine granules are contained in the peripheral nuclear substance.

In fig. 5 the boundary of the nucleolus is more distinct, and the transparent space surrounding it is well marked. A few small and medium-sized granules are contained within the nucleolus, while a number of small particles are suspended in the nuclear substance.

Fig. 3 shows still further differentiation. The nucleolus is free from granules, is contained within a definite sharply-marked outline, and within the nucleolus itself an appearance of radial striation may be noticed. A ring of large granules (broken by pressure) surrounds the nucleolus, and a number of smaller particles are distributed peripherally.

It appears, therefore, from an examination of these three nuclei, that a single nucleolus only is present, and that a variable number of larger or smaller or of both-sized granules are also contained within the nucleus. The nucleolus is situated in a transparent central portion of the nucleus, while in the peripheral homogeneous nuclear substance a number of minute highly refractile granules are suspended. A few larger irregular-shaped granules may be arranged close around, but distinct from the nucleolus, while the latter may itself contain smaller granules. Whether or not the isolated granules are to be regarded as nucleolar material is a question I do not pretend

to decide, but the appearance of the nucleoli in figs. 4 and 5, considered in connection with the researches of Grüber (No. 12) on the nuclei of Protozoa, would suggest that such is the case.

Mature Ovarian Ovum.

The phenomena of the maturation of the ovum I have not had an opportunity of observing in all its phases, but I have been fortunate enough to obtain a fully mature ovarian ovum (or one almost in a mature condition) which has been represented in fig. 8.

In this latter the ovum lies freely within the zona radiata and is separated from it by a considerable space, the circum-vitelline space in which, according to v. Beneden, is a fluid, the circum-vitelline fluid. The vitelline membrane is here distinctly seen on account of the contraction of the substance of the vitellus.

The ovum itself is very dense and contains a number of dark granules not observed in less mature ova; it is separated from the vitelline membrane by a narrow space excepting (1) at certain points where pseudopodia-like processes of the vitellus project across the space and are attached to the vitelline membrane, and (2) at one spot where no contraction of the ovum has occurred. At this latter point the vitellus is more transparent than elsewhere, and the nucleus may there be seen in close approximation to a dark oval body lying immediately outside the vitelline membrane, while a second more transparent oval body in which is a central dark mass may be seen lying in the midst of the circum-vitelline space. These two bodies are the polar bodies (*p. b.*), the second of which has but just been produced; while the nucleus seen within the ovum is the female pronucleus (*f. p.*).

It is possible to describe the vitellus as composed of a cortical more clear, and a medullary granular portion such as Beneden (No. 5) describes in the mature ovarian ovum of the Rabbit, but the boundary of these layers is by no means easy to define. The light-coloured space in which the nucleus is

situated is continuous undoubtedly with the cortical portion (*vide* Beneden, loc. cit.).

When fully mature the vitellus again swells out and there is no space seen between the ovum and the vitelline membrane. At the same time the distinction between cortical and medullary portions ceases to be visible, and the female pronucleus probably retires to the centre of the ovum, judging from its behaviour in other types, and is no longer to be seen owing to the density of the yolk. In this condition the ovum is fully ripe and is ejected, by the bursting of the follicle, into the funnel-shaped opening of the Fallopian tube.

Beneden (No. 5) describing the process of the formation of polar bodies in the Rabbit's ovarian ovum, concludes that the germinal vesicle is ejected to form those bodies, and that the ovum becomes therefore a non-nucleated cell, while Balfour (No. 2, vol. i, p. 61) in criticising this statement suggests that further observations "will demonstrate that part of the germinal vesicle remains in the ovum to form the female pronucleus."

The latter supposition, I would venture to think, is justified by the observations above recorded, and I would suggest that it is possible the supposed "Monerula" condition of the ovum described by van Beneden was due to the fact that the opacity of the ovum and the retirement of the nucleus to its central portion at the time the observation was made, prevented it from being seen.

IMPREGNATION.

Impregnation takes place in the upper portion of the Fallopian tube.

In fig. 10 an ovum is represented which was obtained from the upper end of the oviduct; it has not yet divided into segments, but spermatozoa have found their way within the zona radiata and two nuclei (the male and female pronuclei) may be seen approaching one another.

The vitellus is irregularly granular (for the sake of clearness this condition has not been represented in the figure) and is

closely surrounded by the vitelline membrane. The circum-vitelline space is narrow, and within this space a number of spermatozoa and also two polar bodies were observed. The ovum appears to have expanded considerably since the maturation stage when the circum-vitelline space was wide, for in the ovum represented in the figure the polar bodies are greater in diameter than is this space, and thus cause a depression on the surface of the ovum.

As to the number of spermatozoa which actually enter the substance of the ovum I have no more evidence than appears in the drawing (fig. 10), in which if my interpretations are correct, a single male pronucleus is present. No movement was observed among the spermatozoa within the peri-vitelline space; they appear to be attached there, and indeed in the case of a similarly-conditioned ovum when the zona was removed, these spermatozoa remained fixed to the vitellus and were not pulled away with the zona.

I have always failed to observe either the presence of cilia or a rotation of the ovum within the zona such as Bischoff describes.

THE SEGMENTATION.

The first segmentation furrow gives rise to two oval segments of which one is generally somewhat larger than the other, although the difference in size may be quite inconsiderable, or there may be no difference at all, as is practically the case in the ovum figured (fig. 11), the one segment being 20.25×15.5 , the other 19.75×16 .

The vitellus in both segments is finely granular and presents no difference in character in either segment.

The nuclei are distinct, numerous spermatozoa are contained within the circum-vitelline space, and two polar bodies are visible.

The zona radiata, with its rough granular outer border, is distinctly striated.

The measurements of the segments of several other ova of this stage are given in the table on p. 213.

Four segments now make their appearance by the division of the first two (fig. 12). Each of the segments is of different size, and indeed in every ovum which I have examined of this stage with one exception, such is the case. (For measurements *vide* table p. 213.)

Spermatozoa and polar bodies are still to be seen in the circum-vitelline space and have been found in ova as old as fifteen segments, although the former in fewer numbers and both considerably shrunk.

From this point the segmentation continues entirely irregularly, and the segments formed are of various sizes. Figs. 13 to 19 are sketches of ova with six, seven, eight, nine, seventeen, and larger numbers of segments. A table of the measurements of the segments of several of them will be found on p. 213.

Throughout I have been unable to discover that the segments are arranged in any definite manner, and have not found it possible to distinguish the slightest difference in the contents or in the density of any segments during the process of segmentation. In size the segments also appear to me to bear no relation the one to the other.

Segmentation is carried on during the passage of the ovum down the Fallopian tube, and is completed by the time the former reaches the uterus.

After the close of segmentation, and when the ovum has descended into the uterus, but not until then, the segments are clearly divided into two layers. The arrangement is as follows:—A single layer of cubical hyaline segments completely surrounds, except at one point, an inner mass of rounded or polygonal densely granular segments. The gap in the outer layer of hyaline segments is filled up by one of the granular segments (fig. 20). The cause of this sudden change is not absolutely clear, but I would suggest the following hypothesis as a probable explanation.

I have little hesitation in stating that not only have the outer layer of segments become more hyaline than heretofore, but the segments of the inner mass have become denser, and

contain larger granules and more granules than they hitherto have done; and I would suggest that the yolk material originally contained in all the segments alike, has been transmitted from those occupying the outermost layer to those lying within, in order to allow the former segments to perform the function, and exhibit such activity as is now required of them.

In order to make my meaning clear I will briefly state what these changes are; for a detailed account of this subject, however, I must refer the reader to a former paper (No. 13). Very shortly after the segmented ovum enters the uterus it dilates into a vesicle—the “blastodermic vesicle.” In the early stages of this formation the change is due entirely to the activity of the outer layer of segments; first by a flattening out, and secondly by the multiplication of these cells; the inner mass meanwhile remaining passively attached to one point on the circumference of the vesicle.

Later the cells of the inner mass assist in the formation of the vesical wall, and eventually the whole of the inner mass, with the exception of a very small number of cells which form hypoblast, become so disposed. The outer layer of segments and the largest portion of the inner mass of segments, therefore, together form the epiblast of the blastodermic vesicle.

Eventually the epiblast of the embryo is formed from a portion of the wall of the vesicle, the hypoblast of the embryo from a small number of the inner mass-segments, while the mesoblast has its origin from both epiblast and hypoblast layers.

Primarily, therefore, the blastodermic vesicle is formed by the energy of the outer layer of segments, and I would suggest that the differentiation of the outer and inner segments, the one from the other, after the ovum enters the uterus, is due to the transmission of yolk contained in the outer segments to the inner segments, this transmission being performed in order that the changes about to take place in the constitution of the ovum may more readily be performed.

Van Beneden, in his description of the Rabbit's ovum in 1875 (No. 5) describes the first two segments formed as the

one larger and hyaline, the other smaller and containing a more dense vitelline material. The hyaline segment he calls the epiblastic, the more opaque segment the hypoblastic sphere. He then describes the order of the subsequent segmentation phenomena, and declares that the segments derived from the primary hyaline epiblastic sphere gradually grow round those formed from the primary hypoblastic sphere, and there results a structure precisely similar to that described above (p. 210), which he calls the "metagastrula" stage. This metagastrula Beneden compares with the gastrula of lower types, and he derives the epiblast of the blastodermic vesicle and of the embryo from the outer "epiblastic" spheres, and the hypoblast and a portion of the mesoblast from the inner "hypoblastic spheres."

There can be little doubt, however, that Beneden's account of the derivation of the layers is incorrect, and that the greater portion of the inner segments, as well as the whole of the outer segments, give rise to epiblast. When this is considered, and when the probable homologies of the primitive streak are recollected, any comparison of the so-called "metagastrula" of the Mammalian ovum with the gastrula of lower types is found to be impossible, and the significance of whatever differences may exist in the two primary segments is rendered unimportant.

In the absence of any figures in Beneden's paper I have been unable to compare the appearance of the segments he describes in the Rabbit's ovum with those I have examined in the Mole, but I have myself examined segmenting ova of the Rabbit, and have isolated the segments the one from the other, in order the more clearly to compare them, and in no case have I been able to distinguish the slightest difference in the density or constitution of these segments.

If my observations are correct, then, the differentiation of the segmentation spheres into two layers in the fully segmented ovum is not a primary differentiation such as Beneden discerns, but a secondary differentiation due to the peculiar circumstances of nutrition and development attending the formation of the Mammalian embryo.

SUMMARY.

The membranes surrounding the ripe ovarian ovum are two: (1) a single outer, thick, zona radiata, with a granular peripheral and a transparent inner portion, pierced radially by fine canals through which nutriment is obtained by the ovum from the follicular cells (of the discus proligerus) immediately in contact with the zona: (2) an inner very delicate vitelline membrane which closely covers the ovum itself; and between these membranes is a space, the circum-vitelline space. The confirmation of Reichert's (No. 18), Meyer's (No. 17), and van Beneden's (No. 4) observations as to the presence of the inner delicate vitelline membrane appears of some interest as many embryologists are still sceptical of its existence, while the relation of the follicular cells with the radial canals of the zona supports the view as to the source of the nutriment of the ovarian ovum. On the other hand the fact that nothing was seen comparable to a micropyle in the zona, such as M. Barry (No. 3), and Meissner (No. 16), described, nor any follicular cells within the zona such as Lindgren (No. 15), von Sehlen (No. 21), and Virchow (No. 22), have observed, is some further proof that the conditions of the material investigated by these authors was abnormal.

The yolk contained within the ovum, which is of two kinds: viz. (1) homogeneous vesicular bodies, (2) minute highly refractile granules, is contained within the meshes of a protoplasmic reticulum; it is dense and contains no large globules such as Beneden (Nos. 6 and 7) describes in the Bat's ova. The rounded or oval nucleus contains a single centrally placed nucleolus and a variable number of smaller or larger granules, which may possibly be considered as nucleolar material.

During maturation the vitellus becomes divided into a medullary granular, and a cortical non-granular portion, the circum-vitelline space between the zona radiata and the vitelline membrane is enlarged, while the vitellus itself contracts away from the vitelline membrane excepting (1) here and there where pseudopodia-like processes connect the two, and (2) at

one spot where the polar bodies are formed. At this latter place two polar bodies may be seen in the specimen figured, outside the vitelline membrane, whilst the nucleus remains as the female pronucleus lying in the peripheral portion of the ovum. Finally, the vitellus again expands and the nucleus retires to the centre of the ovum and is no longer to be seen. Assuming that these observations are correct, Beneden's description of the ejection of the vesicle to form the polar bodies and the subsequent non-nucleated condition of the ovum must be considered erroneous.

Impregnation appears to be effected by a single spermatozoon, although a considerable number of spermatozoa find their way through the zona and may be seen lying passively in the circumvitelline space.

The segmentation occurs while the ovum travels down the Fallopian tube. Two and then four segments are formed, after which the course of segmentation is irregular. The segments themselves are of irregular size and do not appear to be divisible into two kinds (epiblastic and hypoblastic) as Beneden describes. After its entrance into the uterus, a division of the segments into an outer hyaline layer and inner deeply granular mass takes place, and I would suggest the hypothesis that the vitelline matter which was originally contained in all segments alike has been transmitted from the outer segments to the segments lying in the interior of the ovum, in order that the former segments may the more readily and actively multiply and flatten out to form the wall of the blastodermic vesicle. The epiblast of the vesicle and of the embryo is derived from the whole of the outer layer and by far the largest proportion of the inner mass of segments. The hypoblast is derived from the small remaining portion of the inner mass and the mesoblast, subsequently, from both epiblast and hypoblast layers. This being the case, the division of the segmentation spheres, by Beneden, into epiblast and hypoblast spheres from the time when the first two segments were formed, is incorrect; and at the same time the theory of a comparison of the metagastrula stage with the gastrula of other animals is likewise untenable.

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Fig. 1.



Fig. 2.

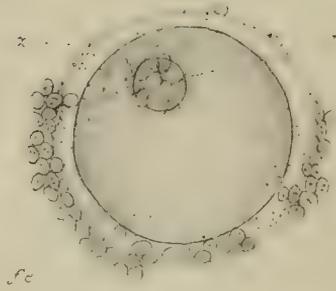


Fig. 3.



Fig. 4.

Fig. 9.

Fig. 11.



Fig. 10.

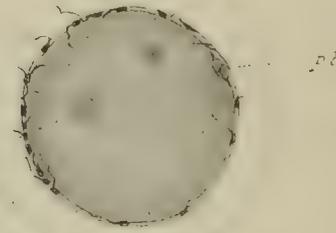


Fig. 8.

Fig. 12.

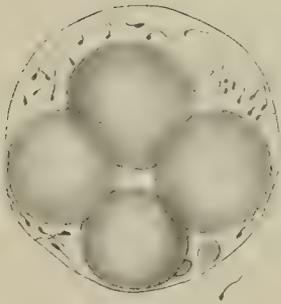


Fig. 13.



Fig. 14.

Fig. 17.



Fig. 18.



Fig. 19.



Fig. 5.



Fig. 6.



Fig. 7.



Fig. 15.

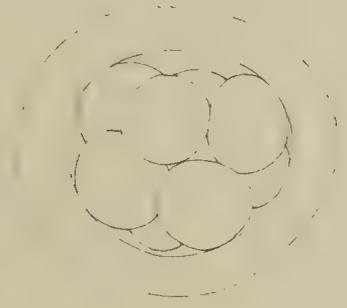


Fig. 16.

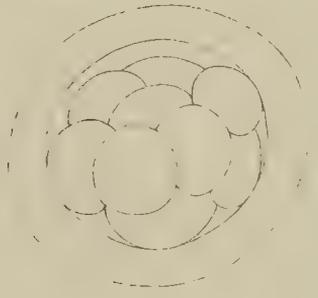


Fig. 20.

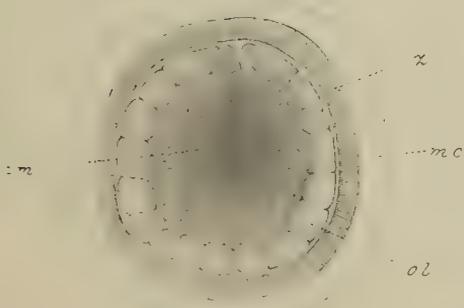
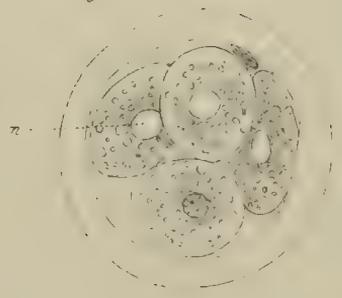


Fig. 21.



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EXPLANATION OF PLATE XXI,

Illustrating Mr. W. Heape’s Paper on “The Development of the Mole (*Talpa Europea*), the Ovarian Ovum, and Segmentation of the Ovum.”

Reference Letters.

c. v. s. Circum-vitelline space. *f. e.* Follicular epithelium. *f. p.* Female pronucleus. *g.* Granules within nucleus. *m. c.* Mucous coat. *m. p.* Male pronucleus. *n.* Nucleus. *nc.* Nucleolus. *p. b.* Polar body. *r. c.* Radial canals. *sp.* Spermatozoa. *v. m.* Vitelline membrane. *y.* Yolk. *z.* Zona radiata.

All the figures are drawings of the ova of the mole, except Fig. 21, which represents a guinea-pig’s ovum. Figs. 13—19 have been copied for me by Mr. H. A. Chapman.

FIG. 1.—Ovarian ovum not yet ripe, surrounded by follicular epithelial cells, *f. e.* The outline of these cells is to be seen through the transparent zona, *z.* The outer edge of the zona is granular. A vitelline membrane may be distinguished here and there. (Zeiss D, occ. 2.)

FIG. 2.—A ripe ovarian ovum. A few follicular epithelial cells only remain attached to the zona. Network of protoplasm permeating the vitellus (Zeiss D, occ. 2.)

FIGS. 3, 4, and 5.—Nuclei of three mature ovarian ova, similar to that drawn in Fig. 2. Single nucleolus, *nc.*, and large and small granules, *g.*, in each nucleus. Yolk vesicles, *y.*, and granules surrounding nucleus in Fig. 4. (Zeiss F, occ. 2.)

FIG. 6.—A portion of the circumference of ovum represented in Fig. 2,

showing the uneven surface of the zona, *z.*, and its granular outer border. The radial canals, *r. c.*, passing through the zona, and the circum-vitelline space between the vitellus and the zona, *c. v. s.* (Zeiss, imm. No. 2.)

FIG. 7.—Small portion of the zona of the same ovum, more highly magnified. The follicular epithelial cells, *f. e.*, are here seen to be prolonged into processes which enter the radial canals, *r. c.*, passing through the zona. The vitelline membrane, *v. m.*, surrounding the ovum is here shown. (Zeiss, imm. No. 3.)

FIG. 8.—Mature ovarian ovum. Vitellus has contracted, and a large circum-vitelline space, *c. v. s.*, left between vitelline membrane, *v. m.*, and zona, *z.* Vitellus has also contracted within the vitelline membrane, excepting where amœboid-like processes connect the two, and at a spot where a polar body, *p. b.*, is seen lying against but outside the vitelline membrane. A second polar body lies freely in the circum-vitelline space. The female pronucleus, *f. p.*, is present within the ovum. (Zeiss D, occ. 2.)

FIG. 9.—More highly magnified portion of the same ovum, showing two polar bodies, *p. b.*, outside, and female pronucleus, *f. p.*, within the vitelline membrane.

FIG. 10.—Impregnated ovum. Male and female pronuclei, *m. p.* and *f. p.*, are visible within the ovum. Two polar bodies and numerous spermatozoa, *sp.*, in the circum-vitelline space. (Zeiss D, occ. 2.)

FIG. 11.—Ovum segmented into two.

FIG. 12.—Ovum segmented into four.

FIG. 13.—Ovum segmented into six.

FIG. 14.—Ovum segmented into seven.

FIG. 15.—Ovum segmented into eight.

FIG. 16.—Ovum segmented into nine.

FIG. 17.—Ovum segmented into fifteen.

FIGS. 18 and 19.—Ova segmented into a number of segments.

The ova represented in Figs. 10—19 were all obtained from the Fallopian tubes of moles.

FIG. 20.—Fully segmented ovum obtained from the anterior end of the uterus of a mole. The segments are now divided into an outer layer of hyaline segments, *o. l.*, and an inner mass of densely granular segments, *i. m.* There is one spot on the circumference of the ovum where the hyaline segments are not continuous, and here one of the granular segments is interposed. The layer of hyaline material *m. c.*, outside the zona, is a coating of mucous material which has collected there since the ovum entered the uterus.

FIG. 21.—The ovum of a guinea-pig, segmented into four to show the large yolk granules and the transparent appearance of the segments.

The Early Development of *Julus Terrestris*.¹

By

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With Plates XXII & XXIII.

My investigations, the results of which are contained in the following paper, were begun in June, 1882. I collected a number of Chilognatha and kept them in glass jars, the bottoms of which were covered with damp earth. I soon found that *Julus terrestris*² was the species best suited for my purpose, as though the eggs presented some difficulties not present in the eggs of other species, yet they were of a convenient size and were easily to be procured in great numbers.

I fed the animals on sliced apples and occasionally on green leaves, and this diet seemed to suit them well, for I never failed to get several clumps of eggs in the breeding season, though it is only this summer that I succeeded in getting them in any number. The breeding season of these animals lasts from the end of May till the end of August, though the weather has a considerable influence on the time when they begin and leave off breeding. I have observed copulation, which takes place exactly as described by Cuvier ('Régne animal,' 3rd edit., 1836, vol. ii, p. 330). I was unable to determine how long a time elapses after copulation before the eggs are laid, but believe it to be short. About four days before laying her eggs the

¹ The numbers in brackets in the text refer to the list of papers at the end.

² The species was kindly identified for me by Mr. T. D. Gibson Carmichael, F.L.S., as *Julus terrestris*, Leach.

female constructs a sort of globular case of mud for them. The bottom of this was, in the case of my animals, formed by the bottom of the glass, while at the top was a small round hole which was closed up after the eggs were laid. Four days after the case was begun the eggs were laid, each case containing a clump of about a hundred eggs fastened together by a sticky substance. By breaking away the top of the case I was able to take out as many eggs as I wanted for examination, and covering the remainder carefully with earth they proceeded with their development without injury, though if exposed to the air for about a quarter of an hour they shrivelled and were destroyed.

Methods.

The principal difficulties with which I had to contend in the preparation of the ova were, in the first place, the hard chitinous chorion, and, secondly, the great amount of food-yolk.

With regard to the first of these difficulties, I tried to remove the chorion by Bobretski's method, but I failed completely in this. I also tried to burst the chorion by endosmosis of various fluids. Perenny's fluid burst the chorion quickly, but as soon as the shell was burst in one place the contents rushed out, destroying the embryo. The state of preservation of the tissues so preserved was not satisfactory, nevertheless I gained some valuable series in this manner. I also tried various strengths of nitric acid with unsatisfactory results. I was therefore obliged to cut the ova with the chorion still on, soaking them thoroughly in the hardest paraffine and cutting rather thick sections. With regard to the preservation of the tissues I tried a great variety of fluids and also the method of preserving by heat described by Mr. Patten in his paper (12) on the development of Phryganids; but I found that I got the best results from corrosive sublimate, osmic acid, and picric acid. The last of these fluids, in some cases, burst the shell after the contents were hardened and thus enabled me to gain excellent series of sections.

The staining of my sections was a matter of much difficulty.

Borax carmine stained well in the earlier stages, while the ovum was still in the ovary, and also in later stages, when the embryo was far advanced in development; but in the intermediate stages, between about the tenth day and hatching, was wholly useless; staining the yolk-spherules equally with the nuclei. Hæmatoxylin was better, staining the nuclei deeply; but it also stained the smaller yolk-spherules so as to make it a difficult matter in some cases to distinguish between them and the nucleoli. The best fluid was alum-carmine prepared after Grenacher's method. This fluid has the advantage of staining the nuclei and nucleoli with a different tinge to that of the yolk-spherules, and the result was most satisfactory. The difficulties in the way of observing the course of development were many and were only overcome by cutting a great number of sections, only about one series in twenty being perfectly satisfactory.

The warmth of the weather had a great influence on the rate of development; one clump of eggs, for instance, was hatched on the twelfth day after being laid, while another was not hatched till the twenty-fifth. As the shorter period seemed to be the most usual, I worked out a clump of eggs which hatched on the twelfth day, and preserved a number each day, using the results as a standard by which to estimate the progress of development in other ova.

I propose in the present paper to begin with the ovum in the ovary after it has attained a fair size and to trace its development up to the time of hatching, leaving for a future paper its further development to the adult animal.

The Ovarian Ovum.

The ovum within the ovary is surrounded with a follicular envelope derived from the cells of the ovary. It has a large nucleus and a single large nucleolus, within which it is usually possible to make out two or three vesicular spaces. The body of the ovum stains slightly. The nucleus is large and distinct, stains slightly, and when viewed under a high power ($\frac{1}{15}$ oil

immersion by Reichert) consists of a network of protoplasm, chromatin granules, and more fluid protoplasm.

The nucleolus is round, very distinct, and stains very deeply. At a slightly later stage a deeply stained mass appears in the body of the ovum; this is possibly equivalent to the yolk-nucleus described by Carus in Spiders (4). It increases and finally forms a very distinct ring within the body of the ovum, as shown in fig. 1, *r*. It is a semi-fluid mass which stains deeply but does not show any structure. I have not observed any appearances like those described by Balbiani in his account of the yolk-nucleus of *Geophilus* (10). This mass of deeply staining, structureless material is the first food-yolk formed in the course of development of the ovum. As the latter increases in size, the ring of deeply staining material breaks up and becomes more equally distributed throughout the ovum in the form of small globules, which are more deeply stained than the rest of the cell-substance, though not so deeply as the ring before mentioned. These globules increase in size and gradually take the appearance of yolk-spherules, such as are present in all subsequent stages up to a very late period of development. Yolk-spherules continue to be formed in the protoplasm of the ovum up to a considerably later stage; such spherules invariably stain deeply while quite small, though the large spheres stain but slightly. I do not consider that the process of formation of the first food-yolk differs in any essential from that of the formation of the yolk-spherules at a later stage. The fully developed ovum within the ovary is shown in fig. 2; it is of an oval form with a thick milk-white shell, which is formed from the follicular envelope of the earlier stages. The body of the ovum consists of a great number of yolk-spherules, which are embedded in and separated from one another by strands of protoplasm which constitute a network extending throughout the ovum. At the periphery is situated the nucleus in which is a single large, deeply staining nucleolus. Examination with a high power lens ($\frac{1}{15}$ oil immersion, Reichert) shows the nucleus to consist of a network of solid protoplasm, enclosing a more fluid protoplasm in its meshes, and of chro-

matin granules which are present in small numbers (fig. 15). Within the deeply staining nucleolus, several vesicular spaces are present. I am unfortunately unable to read Russian, but from an examination of the figures of a Russian paper by Repiakoff, published in 1883, on the development of *Geophilus*, I imagine that the ovum of *Geophilus* at this stage is of similar structure.

I have been unable to observe anything of the impregnation of the ova, which probably takes place immediately before deposition.

My earliest stages occur late on the same day on which the ova are laid; sections through such ova show (fig. 3) that the protoplasmic network and yolk-spherules remain as before, but the nucleus is no longer at the periphery, but is situated in a mass of protoplasm in the centre of the ovum. This mass of protoplasm is of irregular shape, but its long axis corresponds with that of the ovum. From it amœba-like processes radiate in all directions, forming a protoplasmic network throughout the egg (fig. 17, *a*, *b*). The nucleus is no longer a distinct vesicle, but its position is marked by the chromatin granules alone. There is no nucleolus.

Early on the second day the nucleus and the central mass of protoplasm divide into two parts. The division of the protoplasm is not, however, complete, the two resulting masses with their nuclei remaining connected by a network of protoplasm. This is shown in figs. 4 and 16. The two first segmentation masses separate till they are some distance apart, though still connected by strands of protoplasm; they then divide, so that we now have four segments all connected together. This process is carried on until there are a considerable number of these segmentation masses present, and early on the third day the first formation of the blastoderm begins. At the close of segmentation the ovum consists of a number of these segmentation masses, resulting from the division of the original central mass of protoplasm. Each of these masses has a dense central portion, in which is situate the nucleus, while the outer portion is broken up into innumerable processes, which connect the masses together and permeate the yolk in every direction.

In fig. 17, *a*, *b*, I have shown the protoplasmic network under a high power. Early on the third day some of the segmentation masses make their appearance on the outside of the ovum at different parts, and there undergo rapid division, the resulting cells spreading out to form the blastoderm in a manner very similar to that which takes place in Amphipods (14). In figs. 6, 18, I have shown this process taking place.

The large flat-shaped cells which form the first beginning of the blastoderm differ considerably from the segmentation masses from which they originate. Their outline is clear and distinctly marked; their nucleus is very distinct, of an oval shape, with its long axis pointing in the direction of the long axis of the cell. A section through an ovum in this stage, when seen through a low power, shows the blastoderm cells as flat, pavement-like cells, with a long-shaped nucleus. An oil immersion lens, however, shows further details. Each cell is directly continuous with the neighbouring blastoderm cells, and also with the cells which remain in the yolk, by means of fine processes of protoplasm. There is also a difference observable in the cells within the yolk, which at this stage constitute the endoderm. Their outline is far more distinct; their nucleus is round, deeply stained, and rather smaller than at an earlier stage.

Fig. 6 shows a single segmentation mass appearing at the surface of the ovum, and about to divide to give rise to blastoderm cells.

Fig. 18 is part of a transverse section through an ovum at a slightly later stage seen, under a high power; it shows a segmentation mass which has divided, giving rise to several blastoderm cells, while some of the cells arising from the original segmentation mass remain behind in the yolk as endoderm, but are still connected with the blastoderm cells by processes.

At the stage represented in the last-mentioned section the blastoderm is present in isolated patches on the surface of the ovum. At the close of the blastoderm formation, then, the ovum consists of an external layer of flat cells—the ectoderm—with deeply stained nuclei, these cells being continuous on the

one hand with one another, and on the other with the cells in the interior of the yolk by means of fine processes of protoplasm. The cells in the interior of the yolk are the direct descendants of the first segmentation masses. They constitute the endoderm. Their fate is various. Some of them are employed in the formation of the keel, which I am about to describe in the next section ; that is, in the formation of the splanchnic and somatic layers of the mesoderm. Another part is employed in the formation of the endodermal lining of the mesenteron, while a third part remains in the yolk after the mesenteron is formed, and gives rise to mesoderm cells, which are employed in the formation of various muscles and of the circulatory system. These cells will be mentioned again in the last part of this paper.

The flat surface cells enclosing the yolk constitute, as already stated, the ectoderm, and give rise to the usual ectodermal derivatives.

With regard to the retention of the primitive connection of the cells of the ovum until this stage, nothing of the sort has, I believe, been described before, except by Sedgwick in *Peripatus* (17). The most important part is, it seems to me, not the connection of cell to cell, but the connection of layer to layer by means of processes of the cells.

Formation of the Mesoderm.

About the middle of the fourth day several of the stellate endoderm cells approach the ectoderm, in the middle line of what will eventually be the ventral surface of the embryo. Such cells are shown in figs. 7, 8, 19. Fig. 7 is an earlier stage than that shown in fig. 8. That the cells are really endodermal, and are not divided off from the ectoderm, is, I think, conclusively proved by the shape of the cells which at this period compose the ectoderm. They are flat and thin and the nucleus is long and oval, and lies in the direction of the long axis of the cell. I cannot believe that they would divide in the direc-

tion of their long axis ; and, in fact, before they do begin to take part in the formation of the mesodermic keel, they undergo an alteration, which I shall describe. When first the endoderm cells just mentioned begin to come together in the middle line near the ectoderm their appearance is somewhat peculiar ; their nucleus is small, round, and deeply stained ; their form is stellate and their outline very distinct.

Processes pass from them to the ectoderm cells. This is shown in fig. 19, which is a transverse section through an ovum on the fourth day, taken in a plane such as to cut through the first beginning of the keel. When a fair number of these cells are assembled in the middle ventral line a change takes place in the cells of the ectoderm just outside them. The latter become more rounded, while their nuclei, instead of being long and oval, become round. In fact they undergo an alteration which causes them to resemble the cells which I have described as assembling immediately below them. This alteration is shown in figs. 19 and 20, which are transverse sections through the first beginning of the keel.

The ectoderm cells in the middle line, after altering their shape as I have described, increase by division, and take a considerable share in the formation of the keel. The cells in the middle line, both ectoderm and endoderm, continue to increase, and are joined by more cells from the hypoderm, and eventually on the fifth day we find a keel in the middle ventral line, something like that described by Balfour in his paper on the development of *Agelena labyrinthica* (16). Both ectoderm and endoderm have taken part in the formation of the keel.

When the keel is fully formed the cells of which it is composed are large, somewhat irregular in shape, and have a large nucleus. They are all directly connected together, though, owing to their being closely packed together, it is difficult to see anything of their connections, except where one cell has been somewhat separated from the others. The keel is of considerable thickness, being about six or more cells deep in its thickest part.

The keel is shown in transverse section in fig. 9 *a*, and fig. 20. At the end of the sixth day the keel is still present but an alteration is taking place in the cells of which it is composed. They are no longer round and thick, but are becoming elongated in the direction parallel to the surface. At the same time they continue to multiply and spread themselves out, so as to form two definite layers within the ectoderm (fig. 10). These are the splanchnic and somatic layers of the mesoderm. The cells of the ectoderm and of the somatic mesoderm are still connected, and also the cells of the splanchnic and somatic mesoderm.

On the eighth day the mesoderm extends round a great part of the embryo—rather more than half way round. The keel has almost disappeared (fig. 11).

On the ventral surface the cells are no longer flat but have assumed a columnar form. Their nuclei are now oval in shape, their long axis pointing, as does that of the cells to which they belong, towards the interior of the ovum. This is in fact the first formation of the ventral plate and is shown in fig. 10. While these changes are going on the remnants of the keel are disappearing. The mesoderm now becomes thicker on each side of the ventral line. This is shown in fig. 21. Both layers are concerned in this thickening, and at these points the two layers become indistinguishable. Outside the thickenings, that is, farther away from the middle ventral line, the two layers are closely applied to each other and to the epiblast as before. The effect of these changes is that the greater part of the mesoderm is now arranged in two parallel longitudinal bands along the ventral surface of the embryo; these bands being connected in the middle line by a thin portion consisting of two layers (fig. 22). Fig. 21 is a transverse section through the ventral half of an ovum at this stage.

The two longitudinal bands now begin to be constricted off into the mesodermal somites. The latter are formed from before backwards and their position corresponds with that of the future segments of the body. The number of somites thus formed is eight, corresponding to the eight segments with

which the embryo is finally hatched. The somites are at first solid, but a cavity appears in them at a later period.

The ectoderm of the ventral plate now alters its character, the cells becoming more pointed and much more closely packed together.

From the Formation of the Stomodæum and Proctodæum to the Hatching of the Embryo.

Early on the ninth day the stomodæum is formed as an invagination of the ectoderm near one end of the ventral surface. Shortly after the first formation of the stomodæum the proctodæum appears as a shallow, somewhat wide invagination near the end of the ventral surface.

The body segments, already established by the segmentation of the mesoderm, now become more apparent, each being marked by a deep transverse furrow in the ectoderm (figs. 24, 25, 28). Fig. 12 is a section taken longitudinally through the embryo, and shews the stomodæum, the proctodæum, the eight mesodermal segments, and a single ectodermal furrow close behind the stomodæum. Fig. 24 shows this first furrow under a higher power. (Zeiss c.)

The endoderm cells are still scattered within the yolk, but they are gradually becoming collected in the median line just below the mesoderm. The stomodæum and proctodæum become more deeply invaginated, extending a considerable distance into the yolk and at the same time the endoderm cells begin to form the mesenteron, arranging themselves round a central lumen. Fig. 27 shows the formation of the proctodæum and the hypoblast cells beginning to form the mesenteron.

At the end of the ninth day, then, the embryo is of a long oval shape, with a deeply invaginated stomodæum at the anterior end and a proctodæum not quite so deep at the other; the mesoderm is divided into eight segments; a deep furrow in the ectoderm marks off the first segment which will eventually become the head, and the mesenteron is almost formed.

The changes which take place on the tenth day result in the embryo assuming its definite shape. These changes consist of the completion of the ectodermal segmentation, the formation of the nervous system, and the formation of the ventral flexure. Eight segments, including the head, are marked off from one another by ectodermal furrows, the last segment being a long one, from which the anal segment will eventually be divided off. Each of these eight mesodermal somites has now acquired a cavity. This is shown in fig. 28, which is a vertical longitudinal section through the second segment on the tenth day.

The two layers are distinguishable, the somatic being chiefly concerned in the formation of the muscles of the limbs.

The ventral flexure now begins to be formed between the seventh and eighth segments. Its first appearance, shown in figs. 29, 30, is seen quite clearly from the outside through the chorion. Metschnikoff has described it as occurring on the tenth day in *Strongylosoma*, which hatched on the seventeenth day, in a more advanced stage than *Julus terrestris* is at the time of hatching.

The ventral flexure is first formed by a deepening of the transverse furrow which forms the division between the seventh and eighth segments. It is therefore first formed nearer the anal end of the embryo. As the furrow deepens and the embryo increases in size, the last segment grows in length. The furrow does not deepen in a direction at right angles to the long axis of the embryo, but in a slanting direction, as shown in fig. 14. The effect of this is that the end segment is bent round against the head segment. The eighth segment just referred to is considerably longer than any of the others except the head, and the tissues show a considerable difference to the tissues in other parts of the body. Even on the eleventh and twelfth days, when the nervous system is far developed in all other parts of the body, in the eighth segment the tissues are imperfectly differentiated, the nerve-cord not showing any ganglia but lying on the ectoderm as a thin cord not quite separated from it. At a later period of development the anal segment is constricted off from this

segment, and from its anterior part the future segments formed in later life are developed. Just before the first appearance of the ventral flexure when the body segments are fully formed, the embryo develops a cuticular envelope over the whole surface of the body. This may be seen during the first formation of the ventral flexure surrounding the body but hanging loosely from it. This envelope is the so-called amnion of Newport.

Just before the first trace of the transverse furrow which marks the beginning of the ventral flexure has made its appearance, the nervous system begins to be formed. The first traces of this consist in a thickening of the ectoderm on each side of the middle line. This is soon followed by the formation of a shallow furrow between the thickened parts; this longitudinal furrow corresponds with that described by Metschnikoff in *Strongylosoma*. Fig. 31 shows the furrow and the ectodermal thickenings. Fig. 32 shows a later stage where the nerve-cords are almost separated from the ectoderm. The bilobed cerebral hemispheres are formed first and the nerve-cords are formed from before backwards, the posterior portion not being complete till a considerably later stage of development.

The nerve-cords are widely separated, but are connected by a thin median portion. In later embryonic life they are closely approached to one another and almost form one cord.

On the eleventh day the embryo has increased considerably in size. The ventral flexure is complete and the animal lies with the long end segment folded closely against the rest of the body, the end of the tail being against the stomodæum. The nervous system is now completely separated from the ectoderm, and the ectoderm has now assumed its adult appearance. It now separates a second membrane like that which I have already described as occurring on the tenth day.

These two membranes I regard as equivalent to two moults of the animal. The nerve-cords have considerably altered its appearance; it has sunk deeply into the interior of the body except in the end segment and now lie closely beneath the

mesenteron. They are divided into ganglia, one pair being present for each segment of the body; from each ganglion a nerve is given off to the corresponding body segment. The sub- and supra-œsophageal ganglia are almost formed.

The splanchnic layer of mesoderm covers the mesenteron, the stomodæum, and proctodæum. The median part of the somatic mesoderm lies above the nerve-cord, between it and the gut; from thence it passes downwards to the body wall. This arrangement is shown in fig. 34, which is a transverse section through an embryo of the twelfth day.

Within the yolk, which is still present in great quantity in the body-cavity, there are present a number of cells remaining over from the hypoderm after the formation of the mesodermic keel, and the mesenteron. These cells eventually give rise to the circulatory system, to the muscles of the segments, in part at any rate, and to other muscles; they are therefore mesoderm cells. The lumen of the mesenteron is now continuous with that of the stomodæum and of the proctodæum.

Fig. 14 shows a longitudinal vertical section through an embryo of this age.

On the twelfth day the Malpighian tubes grow out of the proctodæum. Their lumen is from the first continuous with that of the proctodæum. They end blindly and are enveloped by the splanchnic mesoderm.

Fig. 34 is a transverse section through an embryo on the twelfth day. The section is taken through a ganglion in the posterior part of the body. It shows the two ganglia united by a narrow median part and each giving off a nerve to the ventral part of the body, where the rudiments of a pair of limbs can already be traced. The Malpighian tubes are also shown. This section also shows the body cavity divided into four compartments by means of thin layers of mesoderm. Late on this day the animal is hatched with only the rudiments of its appendages, and I propose to reserve a full description of the stage till a future time.

Literature.

But little work has been done on the early development of Chilognatha. According to Newport, De Geer was the first to watch the development of Julidæ (6). He observed that *Julus* and *Polyxenus* were hatched with three pairs of limbs and a fewer number of body segments than is possessed by the adult animal.

Savi was the next observer. In 1817, in a paper quoted by Newport (11) and which I have not been able to obtain, he said that *Julus* was hatched without limbs. The next observer was Waga. In 1840, he, in a paper quoted by Newport (11), states that the young Julidæ are completely apodal at the time of hatching. Gervais (8), the next observer, in 1844, gives a great deal of fresh information about the later development of Chilognatha, but has little to say with regard to the earlier stages before hatching. He tells us, however, that *Glomeris marginata* has three pairs of limbs before hatching; that *Polydesmus complanatus* has also three pairs when hatched.

Fabre (7) in 1855, investigated the development of *Polydesmus*, and describes it as having three pairs of limbs and eight body segments, including the head segment, at the time of hatching. He also investigated *Julus aterrimus*, and describes it as hatching on the fifteenth day, being then apodous and without any organ or appendage, and the shape of the body being reniform; five days afterwards, he tells us, that he observed the first traces of body segmentation, and that seven days after hatching the animal consisted of eight body segments and possessed three pairs of limbs.

Metschnikoff found that the young of *Julus Morreletti* were hatched with three pairs of limbs (9), while Newport found that in *Julus terrestris* the just hatched young only possessed the rudiments of three pairs of limbs, and faint traces of the antennæ. My own investigations, which were carried

out on the same species as Newport's, confirm his account. In my opinion the conclusion to be drawn from these different accounts is that in different species of Chilognatha, and even in closely allied species of Julidæ, the hatching of the embryo takes place at very different stages of development.

In 1841, Newport published his paper on the organs of reproduction and the development of the Myriapoda (11). This is the first paper containing any real information of the early stages in the development. On the first three days he describes the appearance of the yolk-spherules as seen through the chorion, and describes the whole contents of the egg as becoming firmer. On the fourth day he saw "a little granular mass on one side of the shell" which he was inclined to regard as the future being. He made no further observations till the nineteenth day, when he describes the ventral flexure of the embryo within the shell. On the twentieth day he was able to make out six body segments. On the twenty-fifth day the embryo was hatched.

I am inclined to think that the little granular mass which he describes on the fourth day was the first beginning of the blastoderm.

Nothing more was written on the early development of the Myriapoda till 1874, when Metschnikoff published his paper (9), which contains the greater part of what we know of Chilognath development. His fullest observations were made on *Strongylosoma*. He describes the segmentation, the formation of the blastoderm, the formation of the ventral plate, the ventral flexure of the embryo, the segmentation of the mesoblast, and of the body, and gives a full description of the later stages. As I shall have to discuss his paper in detail I will not attempt to give a fuller account of it here.

In 1877, Stecker published a paper (13) in which he describes the development of *Julus fasciatus* and several other species of Chilognatha. His account does not agree either with mine or with that of Metschnikoff. As his account has been fully criticised by Balfour (2), I will not refer to it here at greater length.

The above is a short account of the early literature of Chilognath development in the first stages of development, and as with the exception of Metschnikoff's paper the only bearing they have on my own work is to show that Chilognatha, even in very closely allied species, are hatched at different stages of development, I shall not refer to them again, with the exception of Metschnikoff's paper, which I shall mention further in the next section of my paper when discussing the bearing of my own work.

Summary.

With regard to the segmentation I have described, it will be seen that it differs considerably from that seen by Metschnikoff (9), who describes it as total; the ovum being divided into two, four, &c., segments. I saw nothing of such a division, nor does Newport, who observed the eggs of the same species as I did, record any such appearances. Newport's observations were made on the eggs of a species found in Madeira; that is in a hot climate; and as regards segmentation were not carried on by means of sections. As the amount and distribution of the food-yolk has a great influence on the segmentation, I think it probable that in my species the segmentation differs slightly from that in the species investigated by Metschnikoff. The difference, however, consisting in the external segmentation of the ovum is not, I think, a very important one. The segmentation of *Julus terrestris*, as I have described it, shows a remarkable resemblance to that found in Amphipods by Ulianin (14). He describes an external segmentation by means of shallow furrows formed in the surface of the ovum, which is composed in great part of food-yolk; in each space thus marked out, a large amœba-like mass of protoplasm provided with a nucleus is present; the division of these protoplasmic masses coincides with the formation of the furrows. When the blastoderm is just about to be formed the furrows disappear. At the close of segmentation, then the ovum is exactly like the ovum of

Julus terrestris inasmuch as the segments are represented by protoplasmic masses each of which is provided with a nucleus.

The formation of the blastoderm, as I have described it, agrees in the main with that given by Metschnikoff for *Strongylosoma*. According to this author, on the fifth day isolated masses of cells make their appearance on the surface of the ovum and spread themselves round it to form the blastoderm. He was unable to trace the origin of these masses of cells. What he saw was precisely what I have described in the earlier part of this paper.

The formation of the blastoderm in *Julus* is, then, such as is generally found in tracheate development.

The cells which at the conclusion of the blastoderm formation in *Julus* remain within the yolk, represent the endoderm, and have apparently been overlooked by Metschnikoff.

The mode of formation of the mesoderm almost exactly resembles that described by Balfour (16) for Spiders. According, however, to this author the greater part of the cells of the keel or ridge are derived from the ectoderm, whereas in *Julus* the ectoderm furnishes the greater part of them. Balfour states that the keel in Spiders is probably the homologue of the mesoblastic groove of the insect blastoderm. Patten (12) describes a median longitudinal furrow in the ventral plate of Phryganids which gives rise to the mesoblast and to part of the endoderm.

In *Peripatus* (17) the mesoblast originates from the primitive streak, i. e. from the indifferent tissue behind the blastopore, which can be called neither ectoderm nor endoderm. I think that all these structures are homologous.

With regard to the cells which, as I have already mentioned, are employed, neither in the formation of the keel nor at a later period in the formation of the mesenteron, but remain in the body cavity as mesoderm cells directly descended from endoderm—Balfour states that in *Agelena*, after the establishment of the hypoblast the cells remaining in the yolk are not entirely hypoblastic, since they continue for the greater part of the

development to give rise to fresh cells, which join the mesoblast. This is exactly what happens in *Julus*.

Metschnikoff has described the formation of the bands of mesoblast and their division into somites, but his figures are difficult to understand, as he has not drawn either the cell outlines or the nuclei.

The formation of the ventral flexure has been described by Metschnikoff, and, as I have already mentioned, was first seen by Newport. The flexure is, as I have before said, formed between the sixth and seventh post-cephalic segments; that is, it marks off from the rest of the body the long eighth segment in which the tissues are very imperfectly differentiated, and from which the anal segment has yet to be cut off. It is from this imperfectly differentiated segment that the future additional body segments are formed in the later stages of development.

The mesenteron of the adult animal is, as was pointed out to me by the late Professor Balfour, marked with a series of constrictions corresponding with the external segmentation of the body, but no trace of such constrictions has as yet appeared.

The wide separation of the nerve-cords in the embryo has, so far as I know, not been pointed out by any author.

I propose to reserve for a future paper a more full description of the development of the nervous system, the circulatory system, and the segmentation of the embryo, as well as the account of the appendages and other points connected with the further development of the embryo.

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EXPLANATION OF PLATES XXII & XXIII,

Illustrating Mr. F. G. Heathcote’s Paper on “The Early
Development of *Julus terrestris*.”

Complete List of Reference Letters.

bl. Blastoderm. *c. in mes.* Cavity in mesoderm. *ch.* Chorion. *ceph. seg.* Cephalic segment. *c. p.* Central mass of protoplasm. *dors. ec.* Dorsal ectoderm. *ec.* Ectoderm. *f.* Follicular envelope. *gl.* Ganglion. *en.* Endoderm. *lon. fur.* Longitudinal furrow. *m.* Mesoderm. *Malp. t.* Malpighian tube. *mes. b.* Mesodermal bands. *mem. ex.* Membranous envelope. *mesen.* Mesenteron. *mes.* Mesoderm. *mes. hy.* Mesoderm cells directly derived from endoderm. *m. k.* Mesodermic keel. *nu.* Nucleus. *nucl.* Nucleolus. *p. netw.* Protoplasmic network. *pr.* Proctodæum. *proc.* Process. *r.* Ring. *r. ap.* Rudimentary appendage. *rem. k.* Remainder of keel. *seg.* Segment. *s. m.* Segmentation mass. *som. m.* Somatic mesoderm. *sp. m.* Splanchnic meso-

derm. *stom.* Stomodæum. *sub. gl.* Subœsophageal ganglion. *suprac. gl.* Supracœsophageal ganglion. *y. h.* Yolk hypoblast cell. *y. sp.* Yolk-spherules. *v. e.* Ventral ectoderm. *v. f.* Ventral flexure. *v. p.* Ventral plate.

FIG. 1.—Section through an ovum while still in the ovary. (Zeiss, c.) *nucl.* Nucleolus. *nu.* Nucleus. *r.* Deeply-stained ring of first food-yolk. *f.* Follicular envelope of ovum.

FIG. 2.—Section of ovarian ovum shortly before laying. (Beck $\frac{2}{3}$ in.) *nuc.* Nucleolus. *nu.* Nucleus. *y. s.* Yolk-spherules.

FIG. 3.—Section through ovum on first day, shortly after laying. *ch.* Chorion. *y. sp.* Yolk-spherules. *c. p.* Central mass of protoplasm. *nu.* Nucleus.

FIG. 4.—The central mass of protoplasm has divided into two. *s. m.* Segmentation mass. *Nu.* Nucleus.

FIG. 5.—Section through an embryo on the third day. *bl.* Blastoderm. *s. m.* Segmentation masses.

FIG. 6.—Section through an embryo on the third day, rather earlier than Fig. 5. A segmentation mass has just appeared at the surface.

FIG. 7.—Early on the fourth day. *ec.* Ectoderm. *en.* Endoderm.

FIG. 8.—Fifth day, showing first formation of mesodermal keel. *ec.* Ectoderm. *en.* Endoderm.

FIG. 9 *a.*—Sixth day, transverse section through keel. *ec.* Ectoderm. *m. k.* Mesodermal keel.

FIG. 9 *b.*—Section through anterior end of same embryo.

FIG. 10.—Sixth day, keel spreading out into mesoderm. *ec.* Ectoderm. *en.* Endoderm. *m'.* Somatic mesoderm. *m.* First beginning of splanchnic mesoderm. *rem. k.* Remainder of keel.

FIG. 11.—Seventh day. *en.* Endoderm. *ec.* Ectoderm. *v. p.* Ventral plate. *s. m.* Somatic mesoderm. *sp.* Splanchnic mesoderm.

FIG. 12.—Vertical longitudinal section through embryo of ninth day. *st.* Stomodæum. *seg. 1.* First body segment. *mes.* Mesoderm. *v. ec.* Ventral ectoderm. *pr.* Proctodæum. *dors. ec.* Dorsal ectoderm.

FIG. 13.—Longitudinal vertical section on tenth day. *st.* Stomodæum. *pr.* Proctodæum. *mesent.* Mesenteron. *mem. ex.* Membranous envelope. *seg.* Segment.

FIG. 14.—Longitudinal vertical section on eleventh day, taken a little to one side of middle line so as to pass through all the ganglia on one side. *suprac. gl.* Supracœsophageal ganglion. *st.* Stomodæum. *pr.* Proctodæum. *mem. en.* Membranous envelope. *mes.* Mesoderm. *mesen.* Mesenteron. *n. gl.* Ganglia of nerve-cord. *n.* Nerve.

The above fourteen figures were drawn under a Zeiss's microscope with a

$\frac{2}{3}$ in. object-glass by Beck, and a No. 2 eye-piece by Zeiss. They form a complete rather diagrammatic series up to the time of hatching.

FIG. 15.—Nucleus of ovarian ovum just before hatching. Drawn under $\frac{1}{15}$ oil imm. Reichert. *nucl.* Nucleolus. *nu.* Nucleus. *y. sp.* Yolk-spherules.

FIG. 16.—Section through dividing segmentation mass on second day. ($\frac{1}{15}$ Reichert's oil imm.) *y. sp.* Yolk-spherules. *nu.* Nucleus. *nu.*² Nucleus of second segmentation mass.

FIG. 17 *a.*—Part of a section through first day ovum, showing protoplasmic network. ($\frac{1}{15}$ Reichert's oil imm.) *y. sp.* Yolk-spherules. *p. netw.* Protoplasmic network.

FIG. 17 *b.*—Part of a section through second day ovum, showing network. *p. netw.* Protoplasmic network.

FIG. 18.—Part of a transverse section through third day embryo, showing segmentation mass dividing to form blastoderm. *bl.* Blastoderm cells. *y. h.* Yolk hypoblast. *nu.* Nucleus. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 19.—Part of transverse section on fourth day, to show formation of mesodermal keel. *ec.* Ectoderm. *en.* Endoderm. *nu.* Nucleus. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 20 *a.*—Part of transverse section through sixth day ovum, to show keel. *ec.* Ectoderm. *m. k.* Mesodermal keel. *y. sp.* Yolk-spherules. (Zeiss, D.)

FIG. 20 *b.*—Isolated cells of the keel. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 21.—Part of a transverse section through an ovum on the ninth day early, to show thickened bands of mesoderm. *ec.* Ectoderm. *mes. b.* Mesodermal bands. (Zeiss, C.)

FIG. 22.—Part of transverse section through ninth day embryo, to show median portion between mesodermal bands. *ec.* Ectoderm. *sp. m.* Splanchnic mesoderm. *som. m.* Somatic mesoderm. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 23.—Isolated cells from transverse section on ninth day, to show connection between mesoderm and ectoderm. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 24.—Transverse section through part of an embryo on the ninth day late, to show the mesodermal segments. *stom.* Stomodæum. *seg.*¹ Furrow marking off the head segment. *m. seg.* 1, 2, 3, &c. Mesodermal segments. (Zeiss, C.)

FIG. 25.—Longitudinal section through cephalic section. *st.* Stomodæum. *ec.* Ectoderm. *mes.* Mesoderm. *1 seg.* First segment. (Zeiss, D.)

FIG. 26.—Endoderm cell from an embryo of same date when the mesenteron is being formed. ($\frac{1}{15}$ Reichert's oil imm.)

FIG. 27.—Longitudinal section through the proctodæum in same embryo as Fig. 25. The mesenteron is just being formed. *pr.* Proctodæum. *en.* Endoderm. *m. seg.* Mesodermal segment. (Zeiss, D.)

FIG. 28.—Longitudinal vertical section through first post-cephalic segment of a slightly later embryo than Fig. 27. *som. m.* Somatic mesoderm. *ec.* Ectoderm. *mem. ex.* Membranous envelope. *sp. m.* Splanchnic mesoderm. *cav. in mes.* Cavity in mesoderm. (Zeiss, D.)

FIG. 29.—Longitudinal vertical section through part of a tenth day embryo, to show ventral flexure. *sp. m.* Splanchnic mesoderm. *som. m.* Somatic mesoderm. *v. f.* Ventral flexure. (Zeiss, F.)

FIG. 30.—Longitudinal vertical section through embryo rather later than Fig. 29, to show ventral flexure. *ec.* Ectoderm. *mes.* Mesoderm. *v. f.* Ventral flexure.

FIG. 31.—Transverse section through late tenth day embryo, to show nervous system. *sp. m.* Splanchnic mesoderm. *som. m.* Somatic mesoderm. *ec.* Ectoderm. *ec. th.* Ectodermal thickening. *lon. fur.* Longitudinal furrow between nerve-cords. *en.* Endoderm forming gut. (Zeiss, D.)

FIG. 32.—Ventral part of a transverse section through an embryo of the eleventh day, to show the nerve-cord and the Malpighian tubes. This section is taken in the posterior region, about the sixth segment. *som. mes.* Somatic mesoderm. *gl.* Ganglia. *Malp. t.* Malpighian tubes. *pr.* Proctodæum. *sp. mes.* Splanchnic mesoderm. *y. s.* Yolk-spherules. *v. ec.* Ventral ectoderm. *b. c.* Part of body cavity between the nerve-cord and ventral ectoderm. (Zeiss, D.)

FIG. 33.—Vertical longitudinal section through part of a twelfth day embryo, to show the stomodæum. *sub. gl.* Subœsophageal ganglion. *supræœ.* Supræœsophageal ganglion. *st.* Stomodæum. In this section the supra- and sub-œsophageal ganglia are not cut exactly in the middle line, and so appear smaller than they really are. (Zeiss, D.)

FIG. 34.—Transverse section through a twelfth day embryo in posterior region of body. *gl.* Ganglia of nerve-cord. *r. app.* Rudiment of appendage. *mes.* Mesoderm forming a partition to the body cavity. *pr.* Proctodæum. *Malp. t.* Malpighian tubes. *m. en.* Mesoderm cells in the body cavity derived directly from the endoderm.

All the figures were drawn by myself under a Zeiss's camera lucida.

Fig. 1.



Fig. 3.

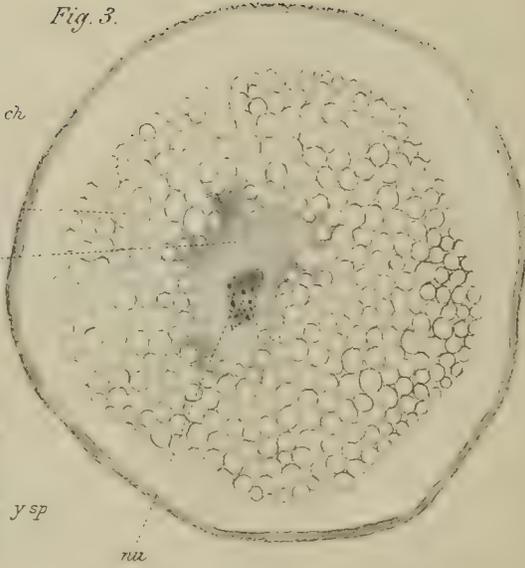


Fig. 4.

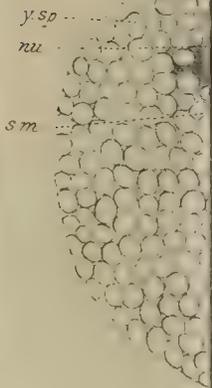


Fig. 2.

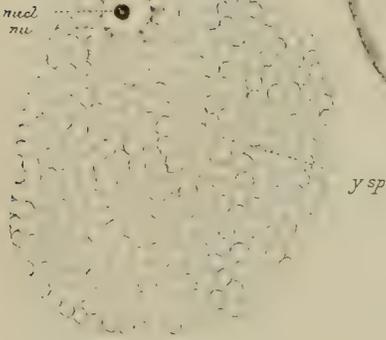


Fig. 9b.

Fig. 9a.

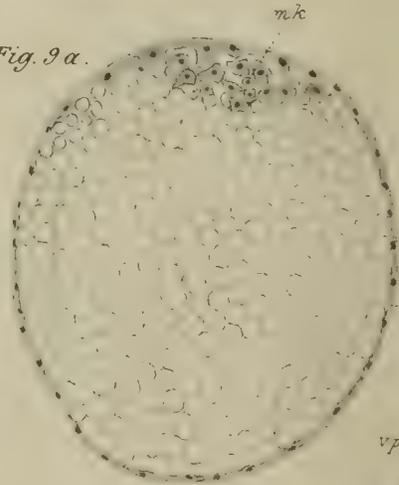


Fig. 7.



Fig. 11.



Fig. 10.

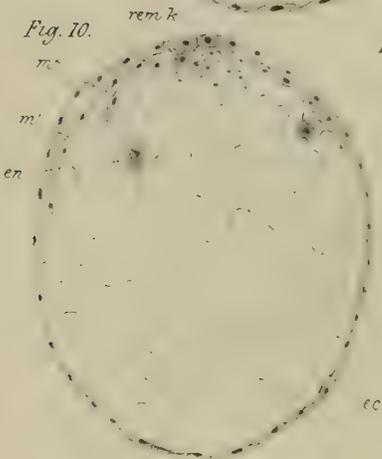
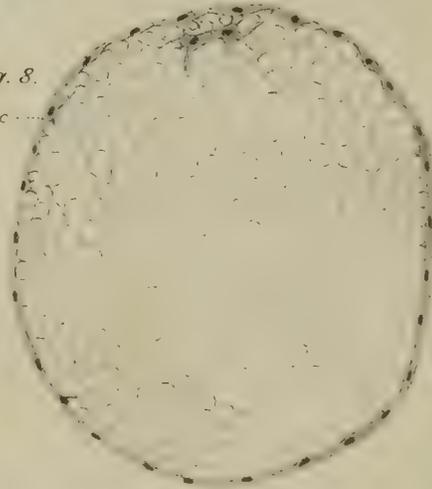


Fig. 8.



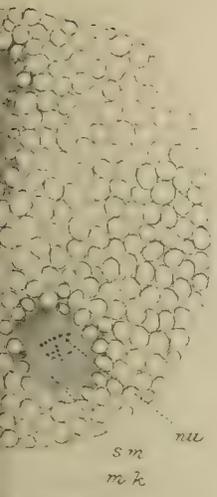


Fig. 5.

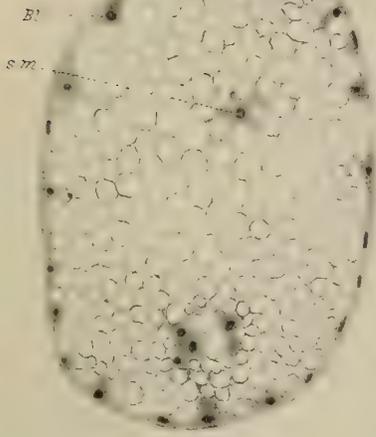


Fig. 6.



Fig. 14.



Fig. 12.

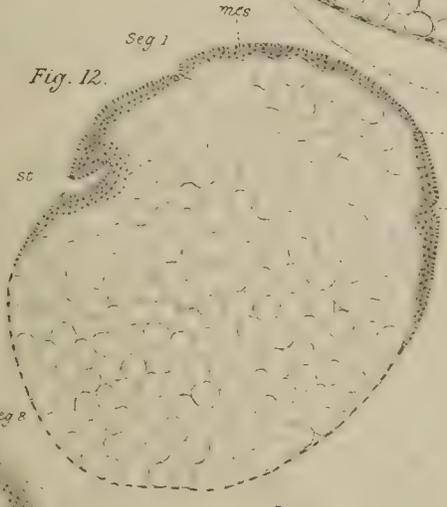


Fig. 13.

Fig. 15.



Fig 16. y sp.

Fig. 17a

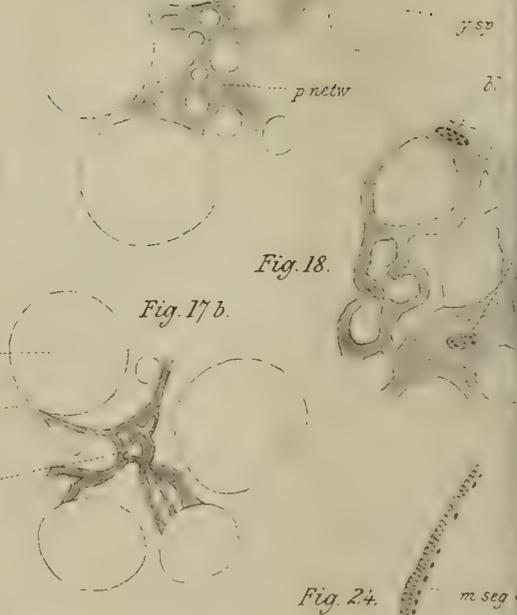
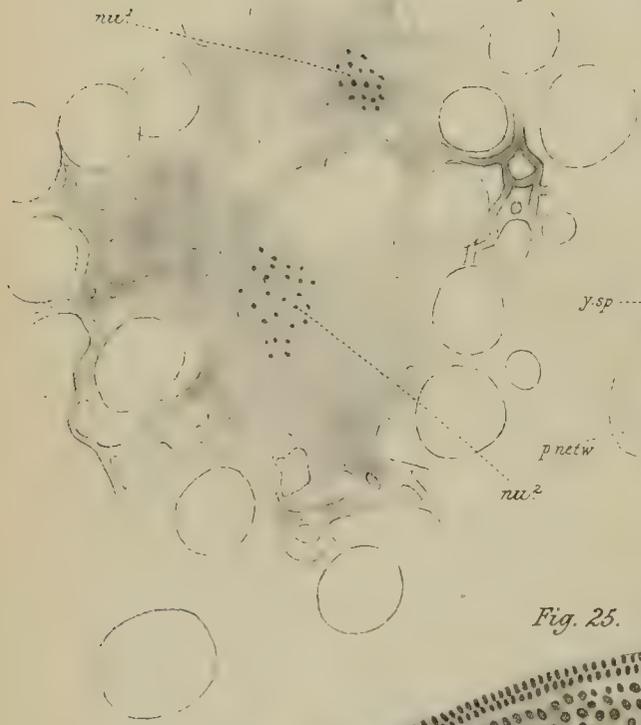


Fig. 18.

Fig. 17 b.

y-sp
p netw
nu.2

Fig. 24.



Fig. 25.

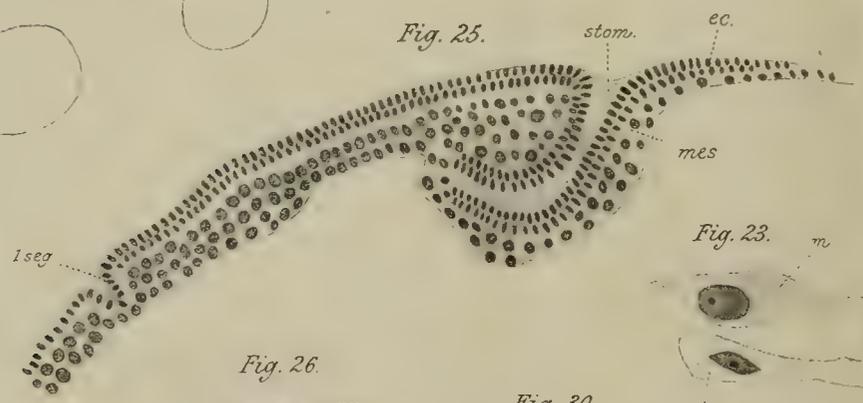


Fig. 23.



Fig. 26.



Fig. 30.



Fig. 27.

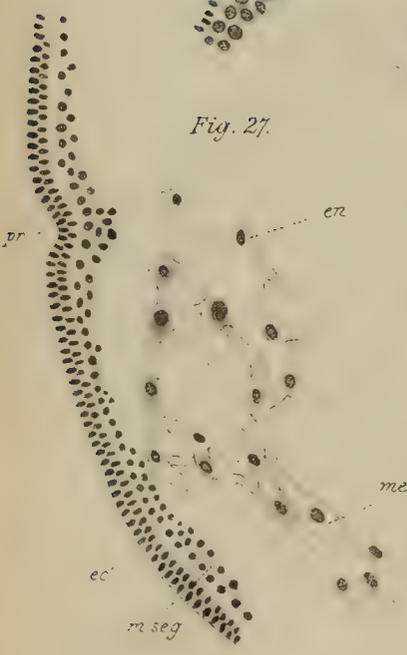
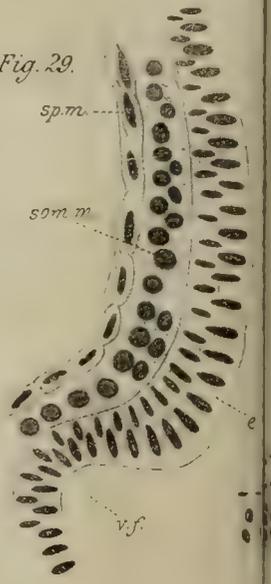
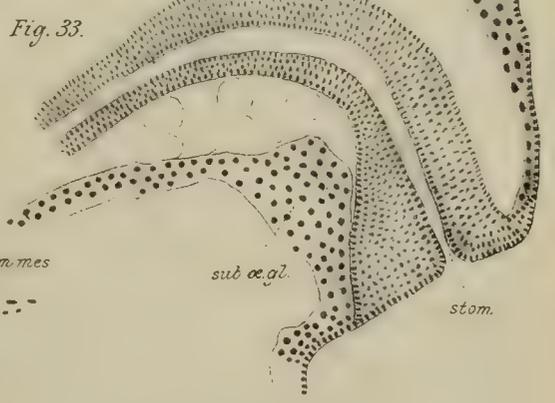
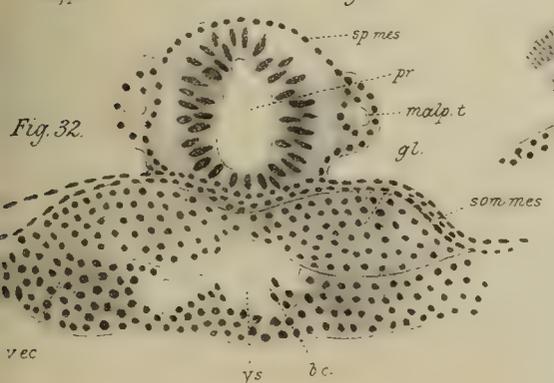
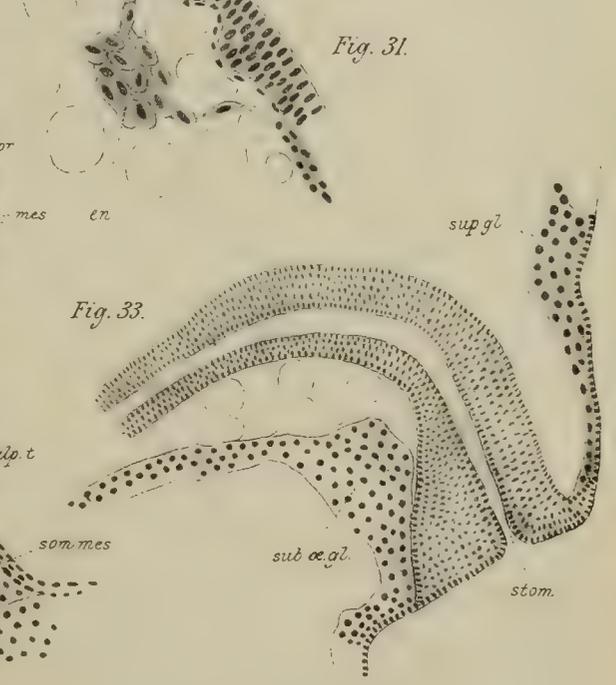
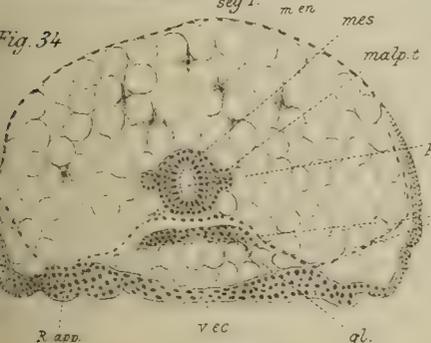
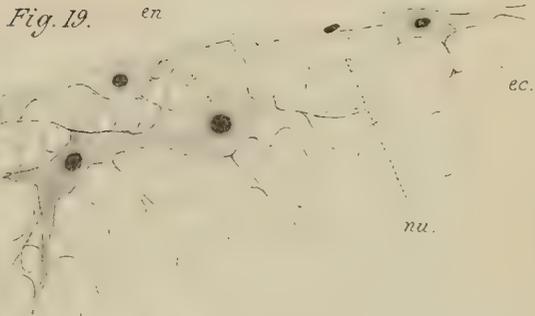


Fig. 28.



Fig. 29.





Notes on the Development of the Newt (Triton Cristatus).

By

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And

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With Plates XXIV, XXV, and XXVI.

THE present paper is a continuation of some observations made by one of us on the early development of the Newt (14). It was then shown that the blastopore of the embryo becomes the permanent anus.

The same discovery has since been made in the Frog by Mr. Spencer (21), in *Petromyzon* by Mr. Shipley (20), and in *Ceratodus* by Mr. Caldwell (6). Dr. Gasser stated the same fact with regard to *Alytes obstetricans* in 1882 (8), in a paper with which the present writers have only recently become acquainted.

THE POST-ANAL GUT.

The existence of a post-anal gut in the embryos of many Vertebrates appears at first sight an important argument against the view of the identity of the blastopore with the anus, because it would naturally be supposed that the blastopore must be at the extreme hind end of the gut. We find, however, that a post-anal gut is present in the Newt embryo, and its relations there, as described below, explain this diffi-

culty. In a transverse section taken a very short distance in front of the blastopore (anus), a portion of the dorsal wall of the gut is partially constricted off (fig. 1), and a little further back becomes completely separate (figs. 2 and 3), and may be traced back into the tail as a solid mass of cells, lying just below the notochord. Near the posterior end of the tail this mass dilates (fig. 5), forming a portion which is probably homologous with the caudal vesicle of the post-anal gut in Elasmobranchs (1), and then fuses with the other structures in the tail at the extreme end (figs. 6, 7).

This solid diverticulum of the alimentary canal appears from its relations to be the post-anal gut, and its point of fusion with the notochord and neural canal no doubt represents the neurenteric canal.

At earlier stages the neurenteric canal, which we believe to be always solid in the Newt, though open for a short time in the Frog, is represented by the point at which the fused layers pass into the blastopore. The neurenteric canal is then, roughly speaking, vertical in direction, since the blastopore is situated at the hind end of the ventral surface. When the tail grows out behind the blastopore, the middle point of the vertical neurenteric canal grows out with it, remaining always at its tip, so that the canal becomes, as it were, drawn out into a loop with dorsal and ventral horizontal limbs. The tail is at first composed of undifferentiated tissue, and the differentiation proceeds as usual from before backwards, the dorsal limb of the loop being the medullary canal, and the ventral the post-anal gut. The two limbs are still connected at the posterior end of the tail by the neurenteric canal.

This mode of development seems to us to show that the tail with the post-anal gut is a secondary structure, developed after the permanent anus. The function of the post-anal gut seems to be to provide material for the growth of the tail during embryonic stages before the blood-vessels have formed. With the appearance of the latter, the post-anal gut gradually atrophies, a remnant of it being attached to the rectum just in front of the anus in a newly hatched larva. At this time it is

seen to occupy the normal position of the post-anal gut, being situated between the dorsal aorta and caudal vein.

In the Frog we find a post-anal gut with a wide lumen behind the blastopore. The lumen gradually narrows towards the hind end, and loses itself in the indifferent tissues of the tail. This stage is somewhat later than that recently described by Mr. Durham (23), with a complete open neurenteric canal. The canal must evidently be drawn out with the growth of the tail, and two regions can then be distinguished in it, the post-anal gut and the neurenteric canal proper. The lumen is obliterated from behind forwards, the neurenteric canal becoming closed first. This would account for the condition we find. Later, the lumen of the post-anal gut is lost, and it becomes a solid structure.

Dr. Gasser gives an account of a post-anal gut in *Alytes* (8) like that of the Newt. The lumen of the alimentary canal is continued a very short way into it, and the rest forms a solid cord in the tail. There is no open neurenteric canal in *Alytes*.

A post-anal gut of the same kind has been described by Mr. Shipley in *Petromyzon* (20).

THE STOMODÆUM AND PITUITARY BODY.

The stomodæum develops as a solid ingrowth of the inner layer of the epiblast just in front of the anterior wall of the fore-gut (fig. 12). The lower part of the ingrowth fuses with the fore-gut (figs. 14, 8, 9) while the upper projects freely and forms the pituitary body (fig. 14). In fig. 8, which represents an oblique transverse section, the relations of the pituitary body to the stomodæum and fore-gut may be clearly seen. It grows upwards and applies itself closely to the infundibulum, curling round it (fig. 14) and forming an indentation in its floor (figs. 38, 37, 36). The extreme end of the pituitary body is shown in fig. 38, where it is hardly distinguishable from the infundibulum.

The stomodæum fuses with the fore-gut at a very early stage, but no actual perforation is formed until a short time after hatching. The region of fusion takes on gradually the

shape of the adult mouth, becoming first elongated transversely, and then horseshoe shaped, with the concavity of the horseshoe directed backwards. The consequence is that, in transverse sections of late stages, the mouth appears to consist of two lateral parts, which are the limbs of the horse-shoe. We find that the pituitary body and stomodæum develop in exactly the same way in the Frog as in the Newt.

The pituitary body has been described as originating from a solid ingrowth of epiblast in Teleosteans by Hoffmann (13), and it seems to arise somewhat similarly in *Lepidosteus* (2). Götte also describes the same method of development in *Bombinator* (9). (See his figs. 127, 128, 250, 252, 292, 293, 298, and 305.)

THE THYROID BODY.

From the hind end of the stomodæum proceeds a solid cord of cells continuous along its dorsal border with the fore-gut (figs. 9, 10, 11). This is the thyroid body. Later a groove is continued into it from the fore-gut, and its hind part becomes a tube by the folding over of the edges of the groove. Subsequently the hind end is completely constricted off from the gut. We have not followed its development further. Scott and Osborn (19) described it as being formed from a fusion of hypoblast and epiblast in the median ventral line. We think that this fusion is the stomodæum, with which the thyroid is continuous at its front end, and that the thyroid itself is developed in a perfectly normal manner.

DEVELOPMENT OF PERIPHERAL NERVOUS SYSTEM.

There is no trace of the peripheral nervous system until the neural canal has completely closed and become separate from the external epiblast. Fig. 15 represents a transverse section through an embryo of a stage just before the closure, showing the epiblast in close contact with the neural canal, with which its two layers are of necessity continuous at this time.

The appearance of the peripheral nervous system is preceded by the formation of a neural ridge. In an embryo in which this is first seen, the neural canal has lost all connection with

the epiblast in the region of the neural ridge, but remains connected with it in the median dorsal line behind the ridge, while still further back the closure of the neural canal is not yet complete. The neural ridge now extends through the head (fig. 16) and the anterior part of the trunk (fig. 13).

It may be here stated briefly that, as far as our observations extend, the development of the spinal nerves is perfectly normal. The neural ridge is prolonged at regular intervals into nerves, which grow down between the medullary canal and muscle-plates. The upper part of each nerve develops a ganglion, and the ventral root is formed later, whether as an outgrowth from the medullary canal or from the ganglion we are unable to say.

After our discovery of the neural ridge, we found that we had been so far anticipated by Bédot (5), who described in detail the development of the spinal nerves in the Newt. Our observations only confirm his on this point.

The Cranial nerves, like the spinal, arise as paired lateral outgrowths of the neural ridge, being completely separate from the epiblast. Figs. 17, 18, and 19 illustrate those outgrowths, which give rise respectively to the 3rd, 5th, and 7th nerves. The 7th and 8th nerves are at first fused, and the common rudiment may be called, for convenience of description, the Facio-auditory nerve.

The Trigeminal nerve (fig. 18) is an outgrowth from the dorsal surface of the brain, and is directed outwards and downwards towards a lateral thickening of the epiblast, which is cut transversely on one side of the section, and more obliquely, so as to appear longer, on the other side.

The Facio-auditory has the same relations to the brain as the Trigeminal, and, like it, is directed outwards and downwards towards a lateral epiblastic thickening. The 9th nerve grows out similarly towards a corresponding epiblastic thickening. These thickenings are situated slightly above the level of the notochord, and are destined to give rise to the mucous canals of the head. It will be most convenient to take the future history of the nerves separately.

The 3rd nerve is seen at a later stage in fig. 20. Its point of attachment has been shifted down the side of the brain, and the nerve is directed forwards towards the eye. We have not ascertained whether or no there is any sensory thickening of the epiblast corresponding to it, but it seems possible that the ciliary ganglion may be fused with the Gasserian, as is stated by Mr. Beard (4) to be the case in the Frog. It would thus not have a separate sense organ of its own.

The Trigeminal nerve grows downwards from the brain till it reaches the level of the sensory epiblastic thickening, and then fuses with it (fig. 21). The point of fusion constitutes the Gasserian ganglion together with the sensory thickening. It is not possible to decide if the epiblast actually takes part in the formation of the ganglion. The mere presence of dividing nuclei in this region, as insisted on by Mr. Beard, seems to us to prove nothing, since all the tissues of the body are actively growing, and consequently contain numbers of such nuclei. We are inclined, therefore, to think that the fusion of the nerve with the epiblast is merely a case of innervation of a sense organ, exactly comparable to what occurs in the nose and ear, and that, in all such cases, the nerve-elements are derived from the brain and the sense elements from the epiblast. Professor Marshall has shown how early this fusion occurs in the case of the ear in the Chick (16).

The root of the 5th nerve is at first attached to the dorsal surface of the brain (fig. 18). Later, the surface of attachment widens out and extends further down the side (fig. 22), and then gradually becomes confined to a small area situated about half way down the brain (fig. 23). The point of attachment is thus shifted downwards, no secondary attachment being formed in this case while the first is lost, as has been described by Professor Marshall in the Chick (16) and in Scyllium (17).

The Gasserian ganglion is for a short time fused into one mass with the sensory epiblast. Soon it begins to sink deeper

into the body, but remains attached to the surface by a cord of cells, which constitutes the dorsal branch (ophthalmic) of the 5th nerve (fig. 24). At the same time a nerve grows down from the ganglion, which soon divides into two branches, a posterior, the inferior maxillary, shown in figs. 24 and 26, and an anterior, the superior maxillary, shown in fig. 24.

The Facio-auditory nerve grows downwards towards its corresponding sensory thickening, and fuses with it at two points, one behind the other. The anterior of these we interpret as the sense organ belonging to the 7th nerve, and the posterior as the ear. There is only a very short distance between them, along which the nerve is not fused. In a later stage, shown in fig. 37, the ear is seen to be clearly distinguishable from the sense organ of the 7th nerve, the ganglion of which is still fused with the skin, while the ear itself is completely separate, forming a simple closed vesicle (fig. 36). The main trunk of the 7th passes on downwards, and fuses with the epiblast of the dorsal wall of the first visceral cleft (figs. 37 and 36). Afterwards, this second connection with the epiblast is lost, and the nerve divides into two branches, one behind and one in front of the first cleft (figs. 26, 31, and 32). At the same time the ganglion on the upper part of the trunk has sunk deeper into the body, remaining attached to the sensory thickening by a cord of cells constituting the dorsal branch (ophthalmic) of the 7th nerve (fig. 31).

The facio-auditory nerve is now attached to the brain by two roots, one behind the other; the anterior is shown in fig. 26, and its connection with the ganglion and præ- and post-branchial branches shows it to be the 7th nerve-root; the posterior passes into the walls of the auditory vesicle (fig. 31), and is therefore the 8th nerve.

The 9th nerve fuses with its corresponding sensory epithelium soon after its origin (fig. 27). The main trunk then passes on and fuses with the epiblast of the 2nd gill-cleft, as shown at a later stage in fig. 28. The root by this time has shifted downwards from the dorsal surface of the brain. The subsequent course of events is exactly the same as in the case

of the 7th nerve. The ganglion retreats further from the surface of the body, remaining attached by the dorsal nerve to the sense organ (fig. 34), and the ventral portion of the main trunk divides into two branches, the post-branchial (fig. 35) behind the second gill-cleft, and the præ-branchial (fig. 33) in front of it.

The Vagus arises from the brain in the same manner as the other cranial nerves, but we have not traced its further development.

DEVELOPMENT OF NERVES IN THE FROG.

We have made a few observations on the development of the nerves in the Frog in some series of sections cut by Mr. Durham, and very kindly lent to us. Our observations, as far as they extend, confirm in every respect what we have described in the Newt. A neural ridge is formed on the dorsal surface of the medullary canal after it has separated from the epiblast, as shown in fig. 30, representing part of a transverse section through the hind region of the trunk of an embryo. In this embryo the neural ridge extended through the trunk, but was less distinct in the head, where the nerves had begun to form as outgrowths from it. Fig. 29 shows the origin of the facio-auditory nerve. Its small size shows that it must be at a very early stage. It is growing on each side from the dorsal surface of the brain towards the auditory vesicle, which is beginning to develop from the inner layer of the epiblast. It seems to us that the whole appearance is inconsistent with the view that the nerve has split off from the epiblast, as Mr. Spencer asserts (21).

HISTORICAL AND CRITICAL.

Our observations are, on the whole, consistent with the account of the derivation of nerves first put forward by Professor Balfour in 1876 (1), afterwards confirmed by Professor Marshall in other types, and since generally accepted. They do not lend any support to the peculiar view of His, as to the presence of a "Zwischenstrang" (11).

Sagemehl (18) derives the spinal nerves in the Frog from a neural ridge, but states that they become detached later from the spinal canal, and subsequently joined to it by the dorsal and ventral roots. Bedot (5) states that in the Newt the connection is never broken, and our researches lead us to agree with him on this point.

Hoffmann (12) describes the spinal nerves in Teleosteans as growing from a neural ridge, but appears to think that the cranial nerves, which arise before the neural canal is closed, are, partially at least, derived from the adjacent epiblast.

O. Hertwig (10), in a few scattered observations on the spinal nerves of the Frog, is inclined to support His' view. More recently the theory of the derivation of the whole or greater part of the cranial nerves from the epiblast has been supported by Mr. Spencer (21) and Mr. Beard (4). This view is a revival of that held by Götte (9). Mr. Spencer asserts that the whole nerve, including root and ganglion, is, in the Frog, split off from the nervous layer of the epiblast. If this be so, all the branches must ultimately be derived from the same source. Mr. Beard confirms him in this statement, and figures one section showing a thickened mass of epiblast continuous dorsally with the still open neural canal, but there is nothing to show that this thickening becomes a nerve. Such a split, as is figured between it and the external layer of epiblast, very often occurs in imperfectly preserved specimens. We find no such thickenings in Newt embryos of similar stages, a typical section of which is shown in fig. 15, and our observations on the Frog lead us to doubt the accuracy of Mr. Spencer's account. We have attempted to show that it is, at all events, not universally true for Amphibia, as Mr. Beard assumes.

Mr. Beard has described in Elasmobranchs (4) a fusion of the typical cranial nerve with the sense organ of its segment. This corresponds with the dorsal fusions found by us in the Newt. The ventral fusion of the nerve with the gill-cleft, as described above in the Newt, corresponds to the second fusion found by van Wijhe in Elasmobranchs (22), and to the ventral

fusion found by Froriep in Mammals (7). Mr. Beard considers that, in Elasmobranchs, all the main branches of the nerve except the post-branchial and the part between the ganglion and the brain are split off from the epiblast. Van Wijhe holds that the epiblast takes some share in the formation of the ganglion at least, while Froriep expresses doubt as to this point, comparing the fusion to the similar fusion of nerve-cells and epithelium cells in the ear. We are strongly inclined to the last view. Professor Marshall (16) has shown how very early the nerve-cells of the ear become indistinguishably fused with it, and there seems no reason why this should not be the case with other sense organs. As to the splitting off of the nerve-trunks from the skin, Mr. Beard's observations and deductions seem to us inconclusive.

In Elasmobranchs Professor Balfour mentioned and figured a fusion between the mucous canals of the head and the nerves supplying them, no line of demarcation existing between the two structures (v. loc. cit., pp. 144, 145, plate xii, fig. 7). He describes this as occurring first in his Stage P, but it is possible that it may take place rather earlier in the Elasmobranchs, as it certainly does in the Newt. Mr. Beard seems to have detected the earlier fusion in Elasmobranchs, and to be unaware that the fact of the fusion was described by Professor Balfour, who found that the nerves were all derived from the brain outgrowths, as we believe to be the case in the Newt. It appears to us that the epiblast in this animal takes no part in the formation of the ganglion or nerve branches, and that the special nerve to the sense organ is an outgrowth from the ganglion, advancing *pari passu* with the withdrawal of the latter from the surface, so that there is at no time any break in the connection between the sense organ and its nerve supply. The withdrawal of the ganglion and formation of the nerve is only a result of the differentiation of the nerve supply into a ganglionic and a fibrous part.

The disposition of these sense organs seems to us a very insecure guide to the segmentation of the head. Mr. Beard considers that the relations of the sense organs to the gill-

clefts shows them to be of segmental value, since they are in some cases situated one above each gill-cleft. At the same time he is obliged to assume the existence of more than one now aborted gill-cleft, in order to account for the number of the sense-organs. If the proof of the segmental value of the sense organs is to depend on the number of the gill-clefts, and the number of the gill-clefts is in turn to depend on the segmental value of the sense organs, it is difficult to discern which is the basis of the argument. Malbranc (15) shows that even in the embryo multiplication of the sense organs by division may occur, so that the number of them seems to be indefinitely variable; and Mr. Beard himself has described such a division in the case of the sense organs of the facial nerve. It seems, therefore, that there was primitively only one such sense organ in this case, and that one cannot depend on the number of the sense organs at any but the very earliest stages, if even then, as indicating segmentation.

SUMMARY OF OBSERVATIONS.

1. A solid post-anal gut is formed behind the blastopore (anus), growing out into the tail, and fusing with the undifferentiated tissues at its posterior end. The fusion of hypoblast and epiblast in this region represents the neurenteric canal.

2. In the Frog the post-anal gut is at first hollow, but afterwards becomes solid.

3. The stomodæum and pituitary body are derived from a solid ingrowth of the inner layer of the epiblast. The hind part of this ingrowth fuses with the front wall of the fore-gut, but the perforation to form the actual mouth does not appear till after hatching. The pituitary body grows upwards as a solid cord, and applies itself to the infundibulum in the ordinary manner.

4. From the hind border of the stomodæum proceeds a solid rod of cells, which constitutes the thyroid body, and is developed from the cells of the middle ventral line of the fore-gut.

5. The development of the peripheral nervous system is preceded by the appearance of a neural ridge, extending along the whole length of the body.

6. The spinal nerves grow out from the neural ridge, and pass downwards between the neural canal and muscle plates.

7. The cranial nerves also grow out from the neural ridge, but are nearer to the surface than the spinal nerves, owing to the absence of muscle plates in the head.

8. When each has attained a certain length it fuses with a thickening of the epiblast, situated some distance above the level of the notochord. (This is the case with the 5th, 7th, and 9th nerves, and probably also with the vagus.)

9. At the point of fusion there is a thickening of the nerve-trunk, forming a ganglion, which afterwards recedes from the surface, remaining, however, attached to the sense organ by a nerve.

10. The main trunk of the nerve passes on, and, in the cases of the 7th and 9th nerves, fuses again with the epiblast of the dorsal wall of the corresponding gill-cleft. Later, the nerve becomes detached from the epiblast, and gives off two branches, one behind and one in front of the gill-cleft.

11. The 5th nerve has no such second (ventral) fusion with the epiblast, but divides below its first (dorsal) fusion into two branches, the superior and inferior maxillary.

12. In the Frog a neural ridge is present at an early stage, just after the closure of the neural canal. The facio-auditory nerve grows out of the brain, and it is therefore probable that the other cranial nerves have the same origin.

N.B.—Our figures are diagrammatic in so far that the outlines of the cells were not perfectly apparent in all sections. This appeared to us to be due to bad preservation, as the better the specimens were preserved the more distinct and complete were the cell outlines. It was generally possible to draw them accurately with a camera and Zeiss obj. *v*, oc. 2. We have therefore represented them throughout as distinct.

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23. DURHAM, H. E.—“Note on the Presence of a Neurenteric Canal in *Rana*,” ‘Quart. Journ. Micr. Sci.,’ June, 1886.

EXPLANATION OF PLATES XXIV, XXV, AND
XXVI,

Illustrating Alice Johnson's and Lilian Sheldon's Paper
“On the Development of the Newt (*Triton cristatus*).”

All the figures represent single sections. They were drawn with a Zeiss's camera lucida and Zeiss's obj. A, oc. 2, except Figs. 1, 2, and 3, which were drawn with obj. B, oc. 2; Figs. 6 and 7 with obj. C, oc. 2; and Figs. 4, 5 and 30 with obj. C C, oc. 2. Fig. 12 was drawn with obj. A, oc. 2, and afterwards reduced by one half; and Figs. 13, 16, 17, 18, 36, 37 and 38 were drawn with obj. A, oc. 2, and afterwards reduced by one third.

N.B.—Grey denotes epiblast, and organs derived from it; brown denotes mesoblast; and yellow denotes hypoblast, and organs derived from it.

Alphabetical List of Reference Letters.

Aud. Ear. *Bl.* Blastopore. *Ch.* Notochord. *F. Aud. rt.* Root of Facio-auditory nerve. *F. B.* Fore-brain. *F. G.* Fore-gut. *Gass.* Gasserian ganglion. *G. VII.* Ganglion of 7th nerve. *G. IX.* Ganglion of 9th nerve. *H. B.* Hind-brain. *H. G.* Hind-gut. *Inf.* Infundibulum. *Lat. V.* Thickening of nervous layer of epiblast to form sense organ corresponding to 5th nerve. *Lat. VII.* Thickening of nervous layer of epiblast to form sense organ corresponding to 7th nerve. *Lat. IX.* Thickening of nervous layer of epiblast to form sense organ corresponding to 9th nerve. *M. B.* Mid-brain. *Mes.* Mesoblast. *N. C.* Neural ridge. *Olf.* Olfactory epithelium. *O. V.* Optic vesicle. *P. a. g.* Post-anal gut. *Pit.* Pituitary body. *Sp. c.* Spinal cord. *St.* Stomodæum. *Thal.* Thalamencephalon. *Thy.* Thyroid body. *V. C. I.* First visceral cleft. *V. C. II.* Second visceral cleft. *III.* Third nerve. *V.* Fifth nerve. *VII.* Seventh nerve. *IX.* Ninth nerve. *III rt.* Root of 3rd nerve. *V rt.* Root of 5th nerve. *VII rt.* Root of 7th nerve. *VIII rt.* Root of 8th nerve. *IX rt.* Root of 9th nerve. *V d.* Dorsal branch of 5th nerve. *VII d.* Dorsal branch of 7th nerve. *IX d.* Dorsal branch of 9th nerve.

V sup. max. Superior maxillary branch of 5th nerve. *V inf. max.* Inferior maxillary branch of 5th nerve. *VII post-br.* Post-branchial branch of 7th nerve. *IX post-br.* Post-branchial branch of 9th nerve. *VII præ-br.* Præ-branchial branch of 7th nerve. *IX præ-br.* Præ-branchial branch of 9th nerve. *x.* Fusion of 7th nerve with epiblast of gill-cleft.

FIGS. 1—7.—Series of transverse sections through an embryo, to show the relations of the post-anal gut to the hind-gut; Fig. 1 being the most anterior, and Fig. 7 the most posterior.

Fig. 1. A little in front of the blastopore, to show the origin of the post-anal gut from the hind-gut.

Fig. 2. Showing the post-anal gut completely separated from the hind-gut.

Fig. 3. Through the blastopore.

Fig. 4. Behind the blastopore.

Fig. 5. Showing dilatation of the solid post-anal gut near the hind end of the tail.

Fig. 6. Showing fusion of the post-anal gut with the notochord and the neural canal.

Fig. 7. Showing fusion of the mesoblast with the other layers near the extreme end of the tail.

FIGS. 8—11 are taken from one series of transverse sections through the anterior end of an embryo, to show the origin of the stomodæum, the pituitary body, and thyroid body. Fig. 8 being the most anterior, and Fig. 11 the most posterior.

Fig. 8. Showing the origin of the stomodæum and pituitary body, and the fusion of the former with the anterior wall of the fore-gut. It also shows the root of the facio-auditory nerve, and its ventral fusion with the epiblast.

Fig. 9. Showing the hind end of the stomodæum.

Fig. 10. Showing the anterior end of the thyroid body as a solid rod of cells attached to the ventral wall of the fore-gut.

Fig. 11. Showing the thyroid body near its posterior end.

FIG. 12.—Longitudinal vertical section through the head end of an embryo, to show the origin of the stomodæum and pituitary body as a solid ingrowth of epiblast in front of the fore-gut.

FIG. 13.—Transverse section through the trunk of an embryo shortly after the closure of the medullary canal, to show the neural ridge.

FIG. 14.—Longitudinal vertical section through the head end of a somewhat older embryo than that from which Fig. 12 was taken, to show the relations of the stomodæum and the pituitary body to the fore-gut, infundibulum, and notochord.

FIG. 15.—Transverse section through the trunk of an embryo shortly before the closure of the medullary canal, showing the epiblast continuous dorsally with it.

FIG. 16.—Transverse section through the head end of an embryo at a stage shortly after the closure of the medullary canal, to show the neural ridge in the brain. Owing to the cranial flexure, all three divisions of the brain are cut through.

FIG. 17.—Transverse section through an embryo slightly older than that from which Fig. 16 was taken, showing the origin of the 3rd nerve as a paired outgrowth from the neural ridge in the mid-brain.

FIG. 18.—Transverse section through the same embryo as that from which Fig. 17 was taken, showing the origin of the 5th nerve from the neural ridge in the hind-brain. The lateral thickening of epiblast on each side is shown.

FIG. 19.—Transverse section through the hind-brain, to show the origin of the 7th nerve as a paired lateral outgrowth of the neural ridge. The lateral thickening of epiblast, which will give rise to the ear and sense-organ of the 7th nerve, is shown on each side.

FIG. 20.—Transverse section through a somewhat older embryo, showing that the root of the 3rd nerve has shifted to the sides of the mid-brain.

FIG. 21.—Transverse section, showing the attachment of the Gasserian ganglion to the epiblastic thickening forming the sense-organ corresponding to the 5th nerve.

FIG. 22.—Slightly oblique transverse section, to show the shifting of the root of the 5th nerve; its attachment is seen to extend continuously from the summit of the brain to a point some way down its side.

FIG. 23.—Transverse section through an older embryo, to show the shifting of the root of the 5th nerve. The nerve is now connected only with a small area of the side-wall of the brain.

FIG. 24.—Transverse section through a still older embryo, showing on the right side the superior maxillary and dorsal branches of the 5th nerve growing out from the Gasserian ganglion. On the left the Gasserian ganglion and inferior maxillary are shown.

FIG. 25.—Transverse section through a young embryo, showing on the left the root of the facio-auditory nerve and its fusion with the epiblast; on the right the auditory epithelium and ventral continuation of the nerve.

FIG. 26.—Transverse section through the same embryo as that from which Fig. 24 was taken, but slightly posterior to it. It shows on the right the Gasserian ganglion and inferior maxillary branch of the 5th nerve; on the left the root, ganglion, and præ-branchial branch of the 7th nerve.

FIG. 27.—Transverse section through a young embryo, showing the root of the 9th nerve and its fusion with the lateral thickening of epiblast corresponding to it. On the right the nerve is seen passing on to the 2nd visceral cleft.

FIG. 28.—Transverse section through a somewhat older embryo. It shows on the right the root, ganglion, and main branch of the 9th nerve, the last fusing with the epiblast of the dorsal wall of the 2nd visceral cleft. On the left only the main branch and its fusion are seen.

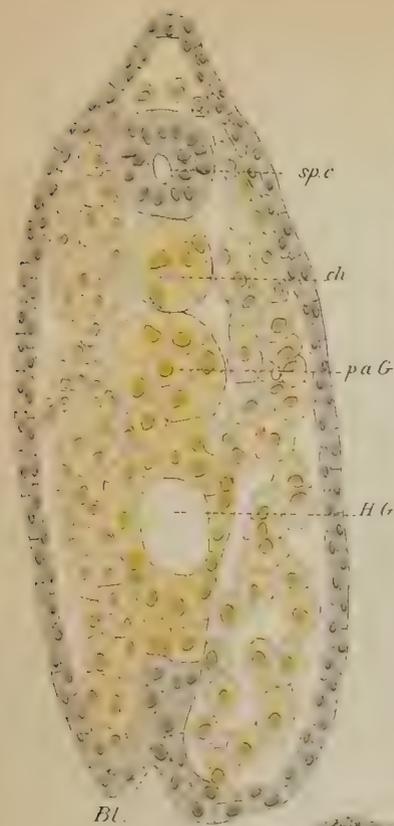


Fig. 1.

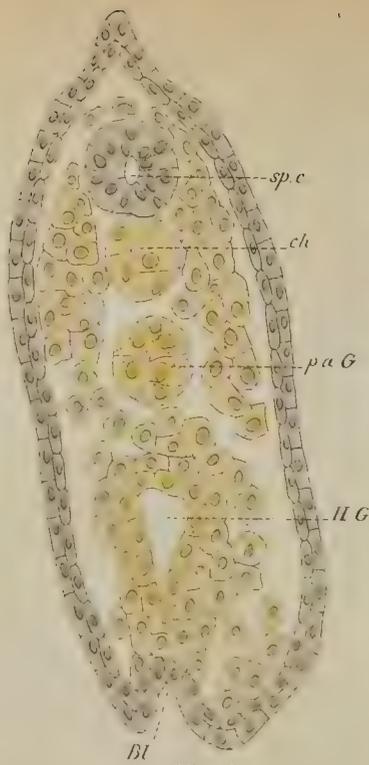


Fig. 2.

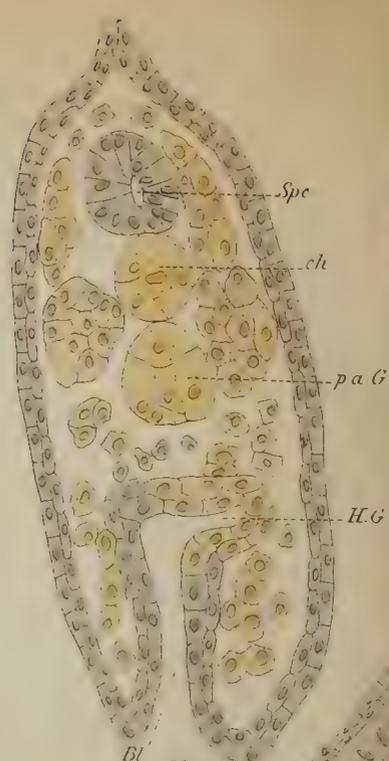


Fig. 3.



Fig. 8.



Fig. 9.

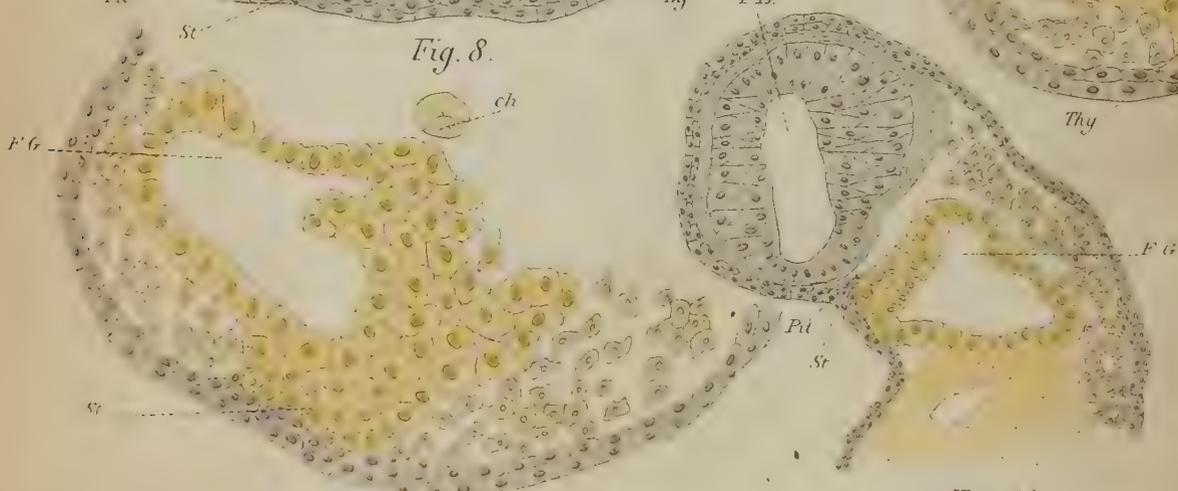


Fig. 12.

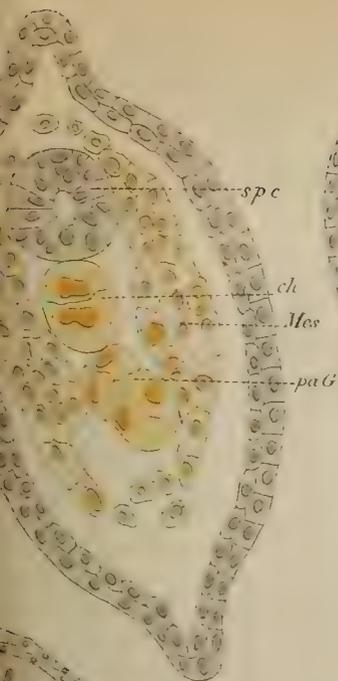


Fig. 4.



Fig. 5.

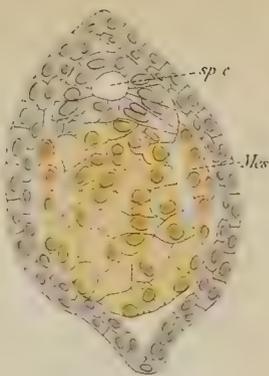


Fig. 6.



Fig. 7.

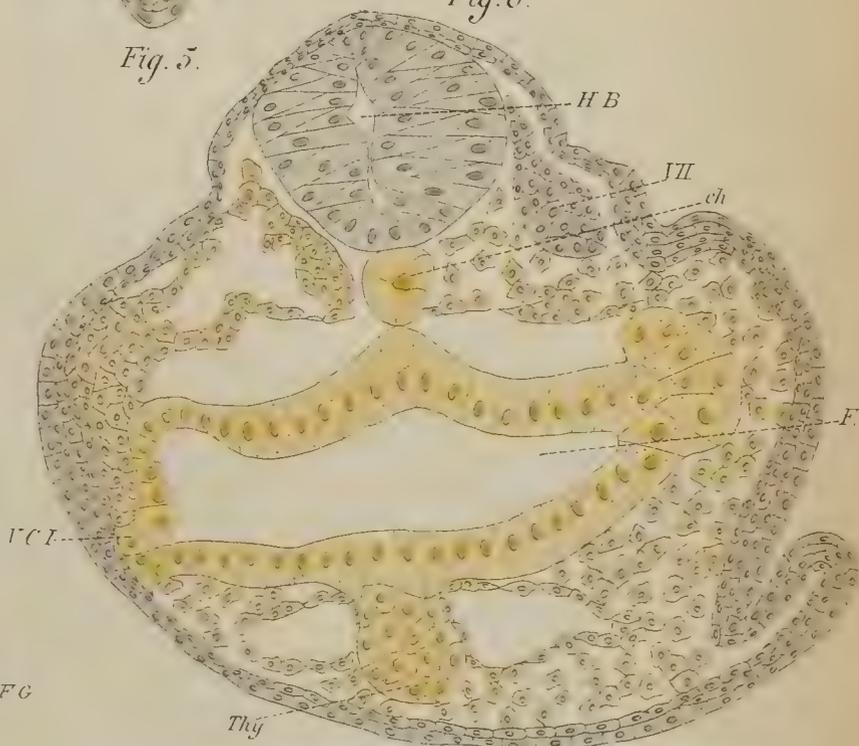


Fig. 11.

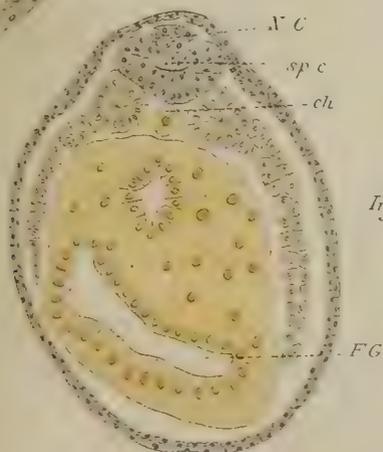


Fig. 13.

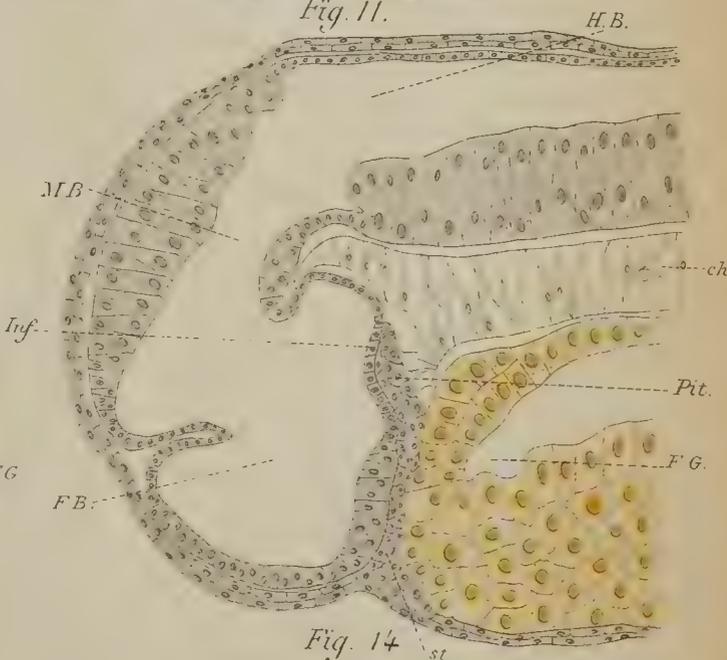


Fig. 14.



Fig. 15.

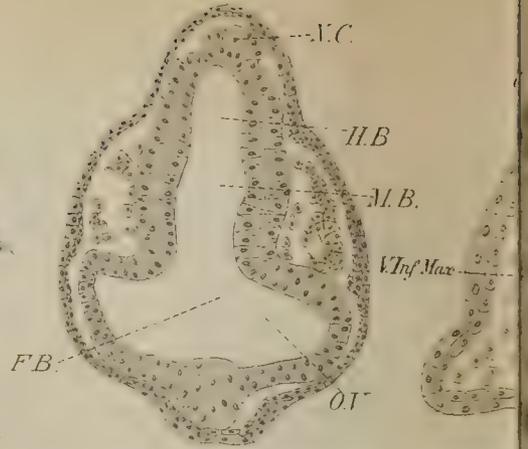


Fig. 16.

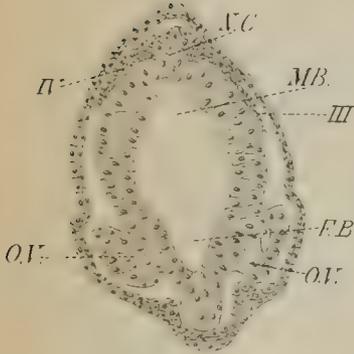


Fig. 17.

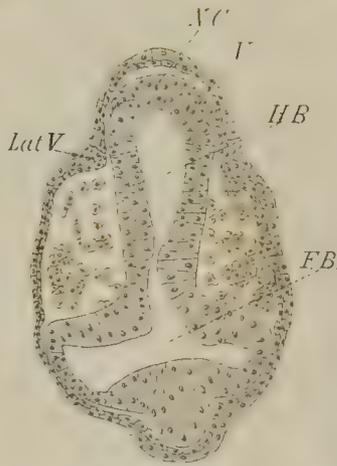


Fig. 18.

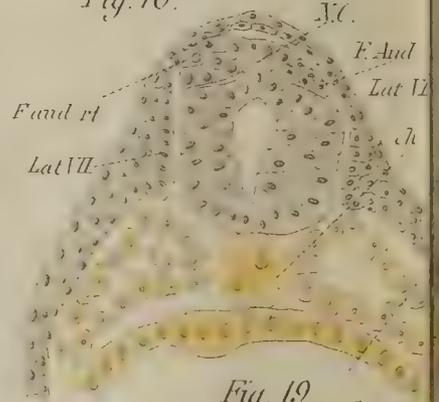


Fig. 19.

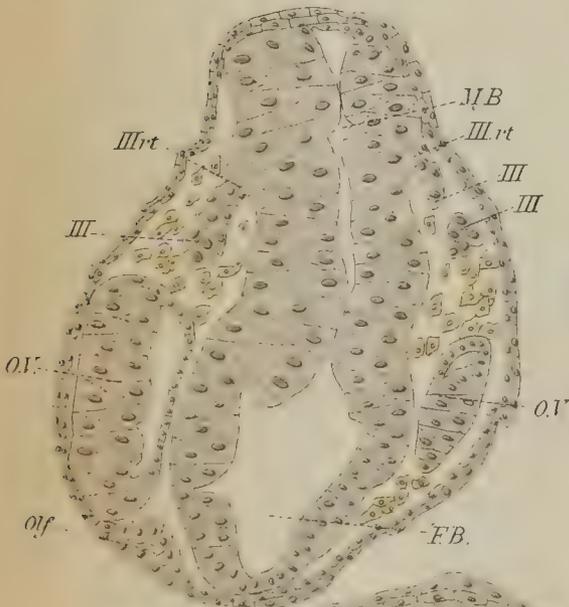


Fig. 20.

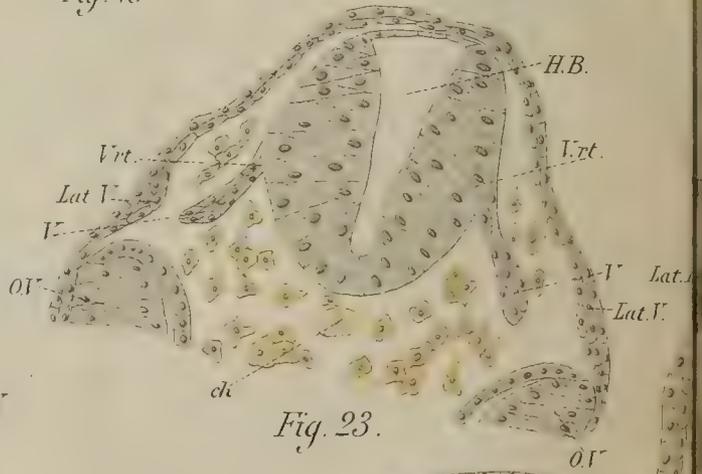


Fig. 23.

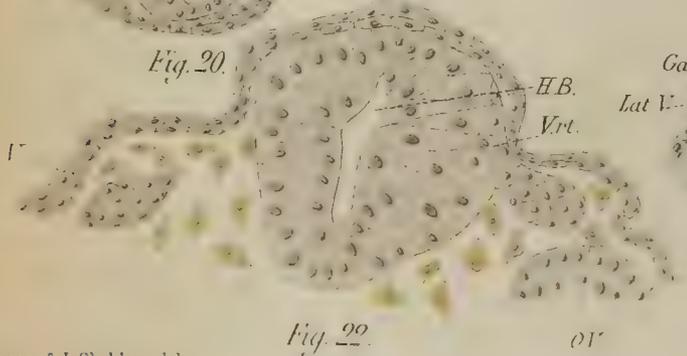


Fig. 22.

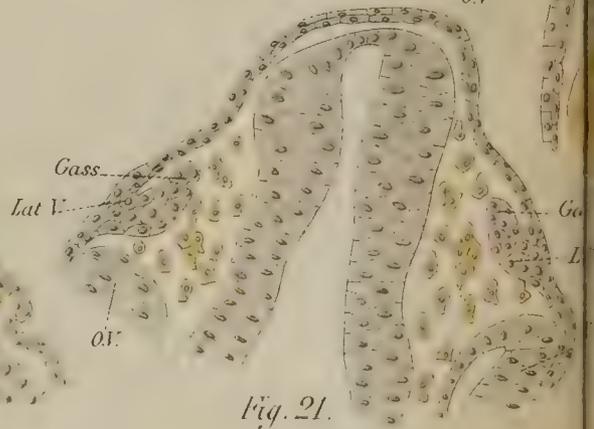


Fig. 21.

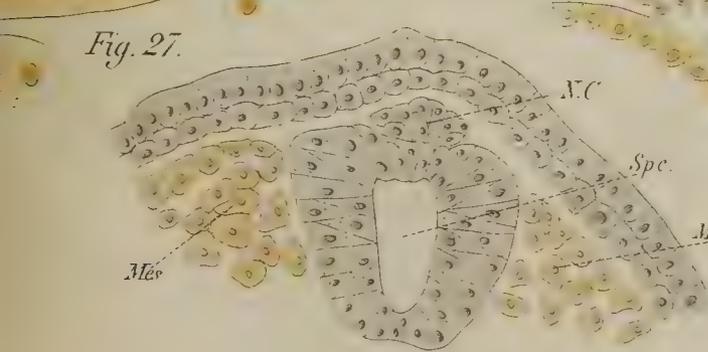
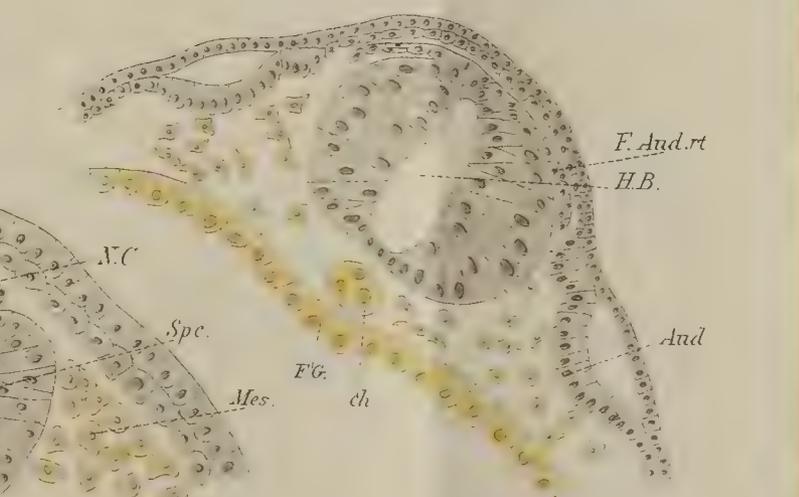
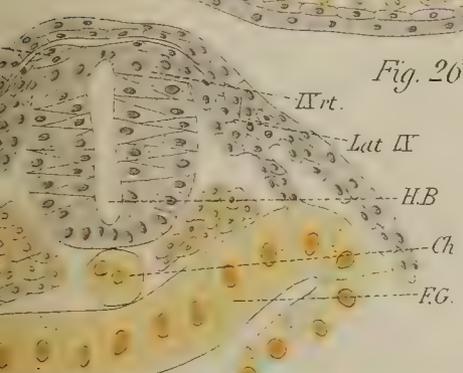
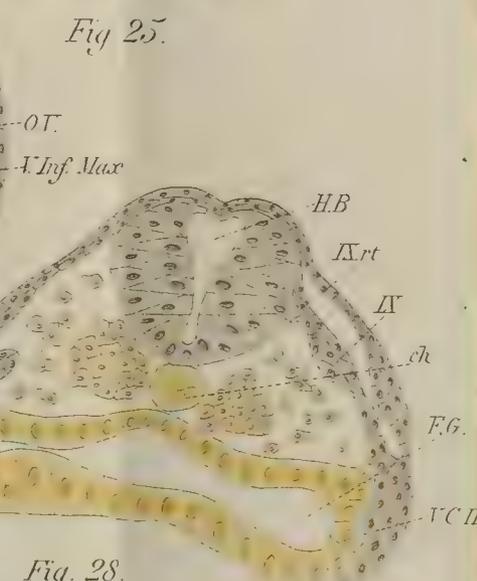
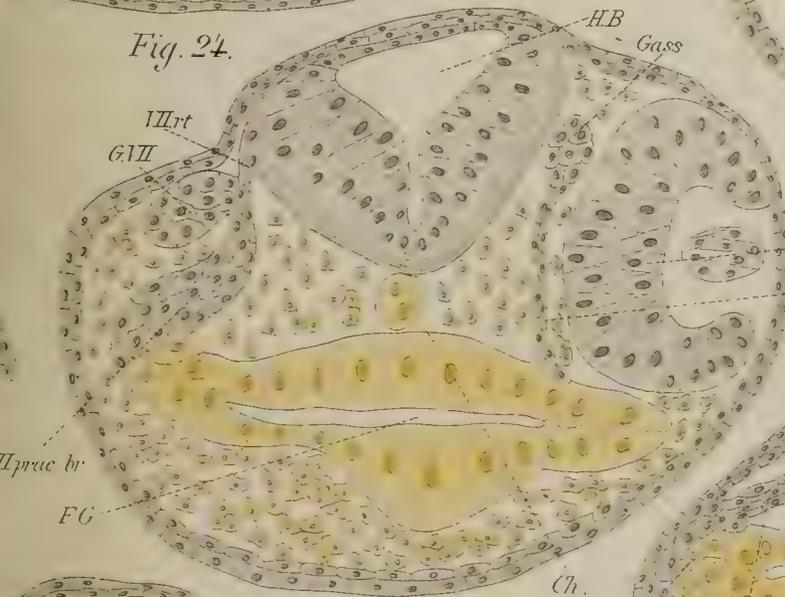
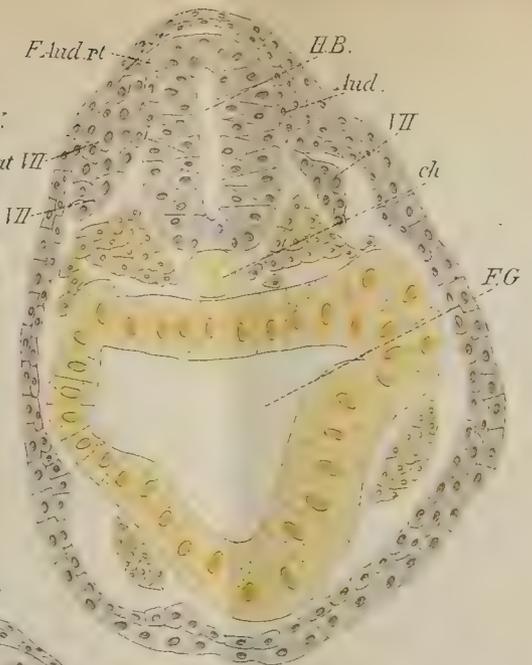
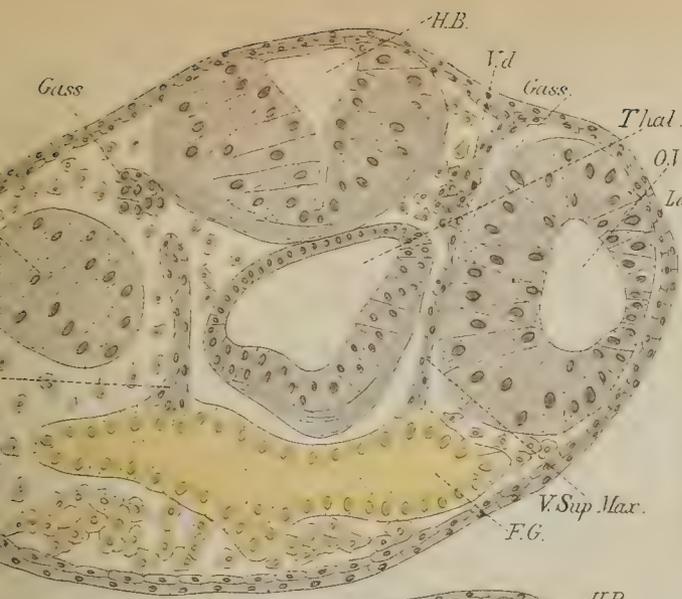


Fig. 30.

Fig. 29.



Fig. 1



Fig. 20



H.B.



Fig. 31. FG Ch



Fig. 36.

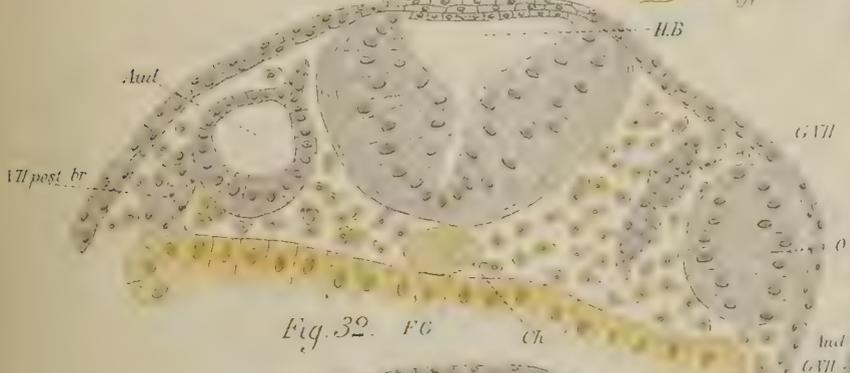


Fig. 32. FG Ch

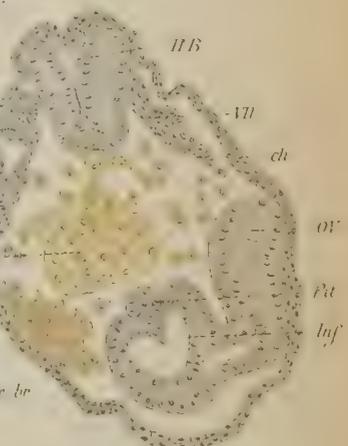


Fig. 37.

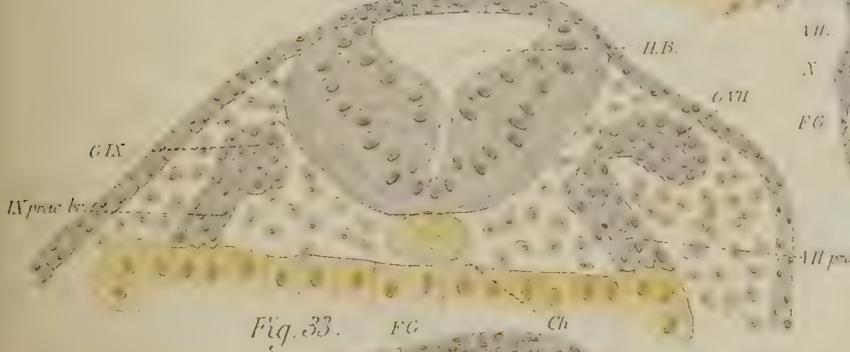


Fig. 33. FG Ch

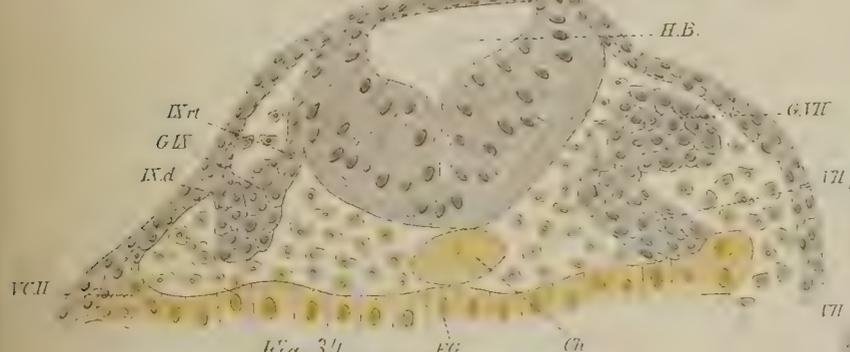


Fig. 34. FG Ch

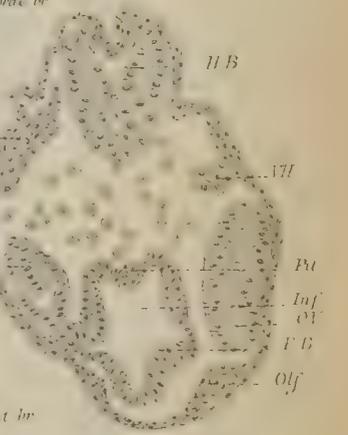


Fig. 38.

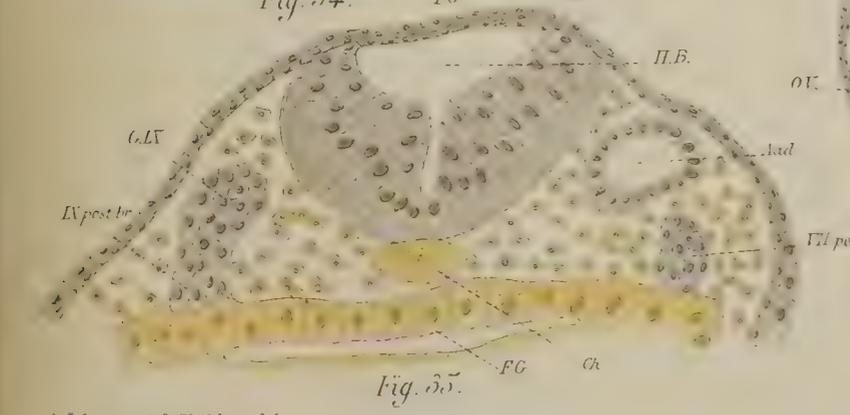


Fig. 35. FG Ch

FIG. 29.—Transverse section through the head end of a Frog embryo, showing the origin of the facio-auditory nerve as an outgrowth from the dorsal surface of the hind-brain. The thickening of the nervous layer of epiblast to form the ear is also shown.

FIG. 30.—Transverse section through the posterior part of the trunk of the same Frog embryo shortly after the closure of the medullary canal, to show the neural ridge.

FIGS. 31—35.—Transverse sections through the same embryo as that from which Figs. 24 and 26 were taken, but posterior to them.

FIG. 31. Showing on the right the ganglion and the dorsal and præ-branchial branches of the 7th nerve; on the left the ear and the root of the 8th nerve, and the 1st visceral cleft.

FIG. 32. Showing on the right the ganglion of the 7th nerve; on the left the ear and the post-branchial branch of the 7th nerve.

FIG. 33. Showing on the right the ganglion and præ-branchial branch of the 7th nerve; on the left the ganglion and præ-branchial branch of the 9th nerve.

FIG. 34. Showing on the right the ganglion and præ-branchial branch of the 7th nerve; on the left the root, ganglion, and dorsal branch of the 9th nerve, and also the 2nd visceral cleft.

FIG. 35. Showing on the right the ear and post-branchial branch of the 7th nerve; on the left the ganglion and post-branchial branch of the 9th nerve.

FIGS. 36—38.—Transverse sections through the head end of an embryo, to show the relation of the pituitary body to the fore-gut and infundibulum.

FIG. 36. Showing the fusion of the posterior face of the pituitary body with the wall of the fore-gut. It also shows the ear and the ventral fusion of the 7th nerve with the epiblast of the dorsal wall of the 1st visceral cleft.

FIG. 37. Slightly anterior to the preceding, showing the pituitary body in close contact with the wall of the infundibulum. It also shows on the left side the ear, the ganglion of the 7th nerve, and the ventral fusion of the nerve with the epiblast.

FIG. 38. Showing the free tip of the pituitary body in close contact with the wall of the infundibulum.

On *Dinophilus Gigas*.

By

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Fellow of St. John's College, Cambridge; Lecturer on Invertebrate
Morphology to the University.

With Plate XXVII.

IN the spring of last year Mr. Shipley brought to Cambridge a few specimens of a *Dinophilus*, which he had found in Mount's Bay, near Penzance. These he was kind enough to place at my disposal; and in April last I was able myself to procure a larger number of specimens from the same locality.

The animals were found in considerable numbers on red seaweeds, &c., in pools, near spring-tide low water mark, on the rocks to the west of St. Michael's Mount. The weeds were placed in shallow white basins, with plenty of sea-water, for from twelve to twenty-four hours, when the *Dinophilus* left the weed, and were easily seen against the white wall of the vessel, on the side turned towards the light.

The length of the body varied greatly, the smallest specimens found being about 0.75 mm., while the largest were nearly two millimetres in length. The colour was a brilliant orange, uniformly distributed in granules through the skin, and more intensely developed in the stomach.

The body consists of a head or præ-oral lobe, seven post-oral segments, and a ventral unsegmented tail.

The head is somewhat broader than the segment immediately behind it; its form is that of a truncated cone, and it is covered

with fine cilia, and with stiff sense hairs, the latter being especially prominent in a pair of patches at the anterior end (fig. 1, *s. h.*). On the dorsal aspect of the head are two bright red, kidney-shaped eyes. A small pair of ciliated pits, such as are described by Korschelt, McIntosh, and Hallez was observed (fig. 1, *c. p.*).

The second segment bears on its ventral surface the mouth, which is an elongated slit, bounded by a number of slight folds, which are richly ciliated.

The six following segments are tolerably uniform in diameter, each in the extended condition being slightly dilated in the centre, and separated from its neighbour by an exceedingly shallow constriction.

Behind the last segment the body narrows suddenly, forming the tail.

The "segmentation" of the body is only conspicuous in the fully extended condition. By contraction the whole of the dorsal and ventral surfaces become uniform, and the very slightest indication on the sides alone remains to indicate the series of swellings and constrictions referred to. Fig. 2, drawn from a specimen which had contracted under the influence of corrosive sublimate, but which was not in any way otherwise distorted, shows this.¹

The præ-oral lobe, the ventral surface of the body, and the tail are uniformly covered with short vibratile cilia, and in each segment the cilia are continued into a band which surrounds the animal, while behind the cilia of each segmental ring is a circlet of fine sense hairs (fig. 1, *s. h.*). Sensory hairs were also specially conspicuous on the tail.

The pigment granules and numerous oil-globules in the skin rendered the creature so opaque that little could be made out in the living state, except the outline of the highly-coloured stomach (fig. 1, *st.*) and the mouth (*m.*).

The three species of *Dinophilus*, possessing a brilliant yellow pigment, which have hitherto been described, are *D. vorti-*

¹ Only six post-oral ciliated rings are visible in this figure. I have noticed the absence of the seventh in one or two preserved specimens.

coides (= *D. capitata*), *D. metameroides*, and *D. caudata*. From each of these the Cornish species differs in some respect. From *D. vorticoides* it is distinguished by the absence of a general coating of cilia on the dorsal surface, and by the presence of definite "segmental" ciliated bands; from *D. metameroides* it differs in the entirely superficial nature of the apparent "segmentation," adjacent segments not being separated by infoldings of the body wall.

I have not been able to consult Levinsen's recent description of *D. caudata*,¹ but, so far as I can gather, it resembles *D. vorticoides* rather than the present form.

It will be seen from what follows that a further character is presented by the present species, which has not been recognised in others—the possession of a well-marked nervous system. In the absence of any detailed information as to the structure of other forms it would be premature to regard this as a specific character, but even without it there seems to be sufficient warrant for establishing a new species, which I propose to call *D. gigas*, from the large size of the sexually mature individuals.

II.—ANATOMY.

In its general structure *D. gigas* agrees closely with the *D. atris* of Korschelt, differing from it chiefly in the presence of a nervous system and in the histological structure of the ectoderm. The paper of Korschelt² is so complete, and contains so full an account of the previous observations on the genus, that it is unnecessary to do more than refer the reader to it before passing on to a detailed description of the present species.

The ectoderm, as has already been seen, varies in character in different parts of the body. In the head a transverse section (fig. 3) shows a well-marked difference between the dorsal and ventral portions. On the ventral side are seen cells

¹ 'Viddensk. Meddel. fra den naturh. Foren. in Kjöbenhavn,' 1879-80.

² 'Zeitschr. f. w. Zoologie,' Bd. xxxvii, Hft. 3.

of three kinds; the most numerous (fig. 3, *gr.*) are columnar, staining moderately deeply, and crowded with granules; wedged in between these are certain cells, the peripheral extremities of which are conical (*m. ep.*), but which send inwards fine processes, some of which are probably muscular, while others are nervous. The cells of the third kind (fig. 3, *x*) are pale, with deeply staining nuclei. Immediately below the ectoderm, on the ventral side, is a delicate layer of transverse muscles (*r. m.*), the fibres of which are, I believe, continuous with many of the processes of the cells marked *m. ep.*, though this connection is not so easily seen in the head as it is in the trunk (cf. fig. 10).

The dorsal ectoderm of the head is composed of an indifferent epithelium several cells thick (cf. fig. 3, where, however, the curvature of the head has caused this portion to be cut tangentially, so that the thickness of the ectoderm appears too great).

Passing backwards, the dorsal and lateral surfaces of the body are uniformly covered, between the head and the anus, with a more or less cylindrical epithelium, one cell thick (cf. figs. 4, 5, 6, 8), which is ciliated only in the region of the transverse rings already referred to.

The ventral ectoderm, in the region of the mouth and lips, is a simple columnar epithelium with narrow, elongated cells (figs. 4—6), but behind the mouth, on the whole ventral surface of the trunk, it has much the same structure as on the corresponding side of the head. The myo-epithelial cells, with their processes, are, however, much better marked (figs. 8 and 10, *m. ep.*), and their connection with the circular muscles is more easily seen (fig. 10), while the cells lying between them are all of one kind—large, finely granular, and paler, with rounded nuclei (figs. 8 and 10, *gr.*). In the figures the whole of the conical ectoderm elements with processes are labelled *m. ep.*; it is, however, obviously probable that many are nervous in nature.

In the tail the ectoderm is throughout of the same character as that on the ventral side of the trunk, except that the

granular interstitial cells are replaced by elements secreting a more or less sticky mucus. By means of this secretion the animal can attach itself with some degree of firmness to foreign objects.

Closely attached to the ectoderm is the central nervous system, which consists of a brain and a pair of lateral ventral nerve-cords.

The brain (fig. 3, *n. f.* + *n. c.*) entirely fills the præ-oral lobe. It consists of a central mass of nerve-fibres (*n. f.*) surrounded by ganglion cells (*n. c.*). Embedded in its substance are the two eyes (E), each consisting of one or two cells loaded with granules of deep red pigment, surmounted by a small cuticular lens.

The lateral nerve-cords (figs. 4, 5, 6, 8) are everywhere in close contact with the skin. Large anteriorly, they grow gradually smaller in passing backwards (cf. figs. 4 and 8) till in the last segment they altogether disappear. Each cord consists of a mass of fibres (fig. 4, *n. f.*), which is in the anterior region more or less completely separated from the skin by nerve-cells (*n. c.*); in passing backwards, however, the nerve-cells almost entirely disappear, and it is to this that the diminution in size of the cord is chiefly due.

No trace of commissures between the cords, nor of any branches, could be found, though the presence of well-developed regions of sense hairs, already referred to, makes it certain that some kind of peripheral nervous plexus exists.

Just above the nerve-cords, throughout the whole length of the trunk, runs a small bundle of longitudinal muscle-fibres (*l. m.*). These, and the ventral circular fibres already mentioned, are the only traces of a muscular system which could be found. The walls of the alimentary canal, except a small part of the pharynx, and apparently the whole dorsal region of the body, are entirely destitute of muscles.

The space between the body wall and the alimentary canal is everywhere traversed by strands of connective tissue, which forms a network with large spaces between the meshes. There is no trace of an epithelial boundary to the spaces thus formed,

neither is there any sign of a division of the cavity by transverse septa.

In certain of the connective-tissue cells which thus traverse the body cavity are "flame cells" belonging to an excretory system of the ordinary platyelmith type. The granular and opaque character of the ectoderm made it extremely difficult to observe these organs in the living animal, and I did not succeed in finding them in sections. I can only say that there is certainly a group of "flame cells" at the points marked *ne.* in fig. 1.

The alimentary canal presents all the well-known characters distinctive of the genus. The mouth (fig. 1, *m*) is an elongated slit bounded by curved, ciliated lips. It leads into an upwardly-directed pharynx, which communicates anteriorly by a narrow opening with the œsophagus. The œsophagus itself passes horizontally backwards. The section represented in fig. 4 is taken immediately behind the point of communication between these two structures, so that the œsophagus (*œ.*) is here entirely shut off from the pharynx (*v. ph.*). The pharynx itself is seen to be a bounded vertical wall, composed of pale, columnar, ciliated cells; outside these lie masses of gland-cells (*m. g.*), which are in places closely attached to the pharyngeal epithelium; other similar gland-cells (*e. gl.*) lie at the base of the ectoderm of the lip.

A section or two further backwards (fig. 5) the pharynx is seen to be composed of two portions—a main vertical portion, the same as that seen in front, and a horizontal portion (*h. ph.*), in the form of a lateral pouch on each side. In this, as in the preceding section, groups of gland-cells are seen, attached both to the pharynx and to the œsophagus.

Passing on to the region behind the mouth, the epithelium of the vertical portion of the pharynx becomes darker and streaked with bands of mucus thrown into it by the glands, which still surround it (fig. 6). The ventral pouches have now united to form a horizontal limb below the main body of the organ, so that its lumen becomes **⊥**-shaped. Finally, still further backwards, the vertical portion ends in a large muscular

bulb (fig. 7, *m. ph.*), lying ventral to the commencing stomach, while the horizontal portion closes and in section disappears.

From a consideration of these sections, and from the diagrammatic longitudinal section given in fig. 11, it is obvious that the pharynx of this *Dinophilus* has the same structure as that described by Korschelt, Hallez, and others, in the better known species of the genus.

I have, however, been unable to make the animal evert its pharynx, as some species are said to do. Irritation with fresh water, acetic acid, &c., or stimulation by pressing the cover-slip, were equally useless in this respect. Further, in no case did my preserved specimens evert the pharynx in dying.

The œsophagus has already been seen; it is a narrow tube lined by a ciliated epithelium (figs. 4—6), which opens, at about the beginning of the second segment, into the large, wide stomach (figs. 1, 2, and 8, *st.*), distinguished by its wide lumen and its granular, brilliantly pigmented epithelium. The cilia of the stomach are very long, and during life their action produces a most violent agitation of the contents of the organ.

In the sixth segment the stomach bears on its ventral side a small pyloric opening (fig. 9), leading into an intestine, which is also ciliated. The stomach is prolonged, as a kind of cæcum, for a short distance behind the pylorus. The intestine passes backwards through the seventh segment, diminishing gradually in diameter, till at last it narrows suddenly and opens to the exterior in the dorsal middle line.

The reproductive organs are in both sexes similar to those described by Korschelt¹ in the female of *D. apatris*; that is, they each consist of a Y-shaped mass of cells, the anterior limbs of which lie under the posterior half of the stomach (fig. 8, τ_2), while the posterior unpaired limb lies under the intestine, or else, as is more generally the case (fig. 9, *me.*), pushes this latter organ to one side. The two sexes are similar externally, until the ripening of the reproductive cells renders the ova or spermatozoa distinguishable through the skin. At the time of sexual maturity the gonads enlarge, so as to com-

¹ Loc. cit.

pletely fill the body cavity, the alimentary canal becomes much reduced in size, and it and the ectoderm appear to undergo a kind of fatty degeneration. I could find no ducts of any kind for the generative products, and from the condition of the tissues of ripe individuals, I have no doubt that, when the generative products are mature, the animals rupture their body wall and die. If this be true, it explains the sudden disappearance of *Dinophilus* at the end of spring, which has been noticed by Hallez¹ and others. In the case of *D. gigas*, all the individuals collected at Mount's Bay on April 22nd had undergone so much degeneration that they were quite useless for histological purposes, while the absolute number of individuals collected between the 16th and 23rd of April was so small compared with the number obtained in the same time a fortnight earlier, as to show that the process of disappearance was beginning.

III.—ON THE SYSTEMATIC POSITION OF DINOPHILUS.

It is hardly necessary to indicate the points of resemblance between *Dinophilus* and a fairly late Chætopod larva. The ciliated rings and the ventral plate of ciliated ectoderm, associated with a pair of unsegmented lateral nerve-cords; the ciliated alimentary canal, with its large stomach, its narrow œsophagus with a muscular pharynx, and its intestine; these are features in which all species agree with a late *Polygordius* larva, while in *D. gyrociliatus* Ed. Meyer finds that the excretory system is "almost identical with that of a *Nereis* larva."² The only point of difference between *Dinophilus* and the Archiannelids is the absence of an epithelial body cavity, and this character, in spite of the importance given to it by many observers, seems to be, in this case at least, of secondary importance. For in the first place the body cavity of *Saccocirrus* seems to be devoid of any definite epithelium;³ while in the second place

¹ 'Histoire naturelle des Turbellariés,' Lille, 1879.

² Quoted by Lang, 'Monographie der Polycladen,' p. 679.

³ Compare the figures given by Fraipont, 'Archives de Biologie,' Tome v, Pl. xiv, which are confirmed by sections in the Cambridge Laboratory.

the head cavity of *Criodrilus* and of many Polychœts is, at an early stage,¹ exactly in the condition which is permanent in *Dinophilus*; it is a cavity, not bounded by any definite "cœlomic" epithelium, but traversed by mesodermic fibres, which form a plexus running through it.

From these considerations it may plausibly be argued that we have in *Dinophilus* a form representing in its main features a stage in the evolution of Chætopods which is in the existing members of that group repeated only in the larval condition—a form in which the only archiannelid character which is not developed is the epithelial and segmented character of the body cavity.

That the epithelial character of the body cavity may be acquired within the limits of a group, *Saccocirrus*, as already pointed out, seems to prove; while the acquisition of segmentation is well seen in the various species of *Dinophilus* itself. Thus, in *D. vorticoides*² we find the whole body unsegmented, with a uniform covering of cilia; in *D. apatris*³ we have an external segmentation which is not shared by the excretory system; while in *D. gyrociliatus* we find the nephridia composed of "simple, intracellular, segmental organs, terminating in flame cells;"⁴ and lastly, in *D. metameroïdes* we have the appearance of a commencing segmentation of the body cavity.⁵

But the anatomy of *Dinophilus* seems to show that from its near connection with the Trochozoon⁶ it is related to other forms besides Chætopods. The pharynx seems especially to show this. Comparing the longitudinal section (fig. 11) with a similar section through the pharynx of *Histriobdella* (fig. 13) we see that the pharyngeal apparatus is obviously

¹ Cf. Hatschek, "Stud. üb. Entw. d. Anneliden," 'Arb. a. d. Zool. Inst. Wien,' 1878, and others.

² E. van Beneden, 'Bull. Acad. Roy. Belg.,' Tome xviii.

³ Korschelt, loc. cit.

⁴ Ed. Meyer, quoted by Lang, loc. cit.

⁵ Hallez, loc. cit.

⁶ I use this term to imply simply the type, whatever that may have been, which is now ontogenetically represented by the trochospheres.

homologous in the two cases. But the pharyngeal appendix of *Histriobdella* carries three chitinous teeth, showing that this organ may in some cases develop skeletal structures; and when once this is ascertained the resemblance to the Molluscan odontophore becomes obvious. Further, in *Terebella*, and other Polychæts, the pharyngeal armature is developed from a ventral and posterior diverticulum of the stomodæum (fig. 14), which is apparently homologous with the corresponding diverticulum of the Archiannelid pharynx. The wide distribution which some organ of this kind had among the Trochozoa is evident from its persistence in the larvæ of such creatures as *Sipunculus* and many others.

It seems, therefore, legitimate to conclude that in the pharyngeal appendix of *Dinophilus* and the Archiannelids we have a persistent record of some ancestral organ from which developed the stomodæal armature of least the Molluscs and Chætopods, and probably also of Rotifers and Crustacea.

As for the derivation of *Dinophilus* and the forms which it represents from simpler types, there are, as Korschelt has already pointed out, many features which connect it with the Rhabdocæl Turbellarians. The body cavity and excretory system especially are in exactly the same condition as those of a Rhabdocæl with well-developed cœlomic spaces, such, for example, as *Mesostoma*.

It is commonly stated that myo-epithelial cells are absent from the ectoderm of Rhabdocæls, and that the muscle-fibres are in this group devoid of nuclei. I hope, however, shortly to show that, in *Convoluta* at least, certain of the ectoderm cells have a structure practically identical with that just described in *Dinophilus*.

The only characters of importance which separate *Dinophilus* from the Rhabdocæls are, the possession of an anus, and the metameric repetition of ciliated bands. Of these, the second may very possibly have arisen within the limits of the genus, since *D. vorticoides* is uniformly ciliated; but in any case we have in *Allostoma*¹ a precisely similar formation

¹ Graff, 'Monographie der Turbellarien,' Bd. i, Taf. 19.

of a single ciliated ring in an undoubted Rhabdocœl. The assumption of a pelagic life might easily cause in any Rhabdocœl a hypertrophy of the cilia in certain definite regions and the consequent appearance of ciliated bands; and it seems safe to predict that a more thorough investigation of the pelagic inhabitants of those warm seas which are most favorable to the development of surface faunas will reveal the existence of genera in which this character has been developed.

The researches of Lang on *Oligocladus* and *Cycloporus*¹ have shown that at least in Polyclads there is no difficulty in the temporary establishment of an anus in any region of the body, and when this is once recognised the passage from a temporary to a permanent condition is easy.

The pharynx of *Dinophilus* and of the lower Chætopods offers another strong proof of Turbellarian affinities. On comparing the diagrams given in figs. 11 to 16 we see that the stomodæum of *Dinophilus*, *Polygordius*, and *Histriobdella* possesses a posterior muscular thickening lying in the wall of a lateral outgrowth from the pharynx, which is in all cases conceivably, and in *Dinophilus* certainly, eversible. In the embryo *Terebella* (fig. 14) a similar posterior outgrowth from the stomodæum exists, which subsequently² envelopes the whole circumference of the pharynx, and constitutes the rudiment of the pharyngeal armature. In *Nais* (fig. 15) we have a similar muscular thickening on the anterior wall of the stomodæum.

These facts receive at least a plausible explanation, if we suppose that the various forms of pharyngeal apparatus just mentioned are derived from a structure which primitively surrounded the whole organ, persistence in the posterior region only being in such forms as *Polygordius*, perhaps associated with the filling up of the præ-oral lobe by the brain, while the existence of an elongated probosciform prostomium in *Nais* renders it most convenient to preserve the musculature in front. But such a circumœsophageal apparatus as is here in-

¹ Lang, *op. cit.*, pp. 155, et seq.

² Salensky, 'Archives de Biologie,' t. iv.

dicated is exactly furnished by the Rhabdocel pharynx (fig. 16).

We seem, therefore, to have in *Dinophilus* a form which, related on the one hand to the Archannelids, retains on the other many features characteristic of the ancestor common to those groups (especially Chætopods, Gephyreans, Mollusca, Rotifers, and Crustacea) which possess a more or less modified trochosphere larva; and of these the relations of the body cavity, of the excretory system, and of the pharynx, seem to point unmistakably to a Turbellarian origin.

EXPLANATION OF PLATE XXVII,

Illustrating Mr. W. F. R. Weldon's Paper on a "Species of *Dinophilus Gigas*."

List of Reference Letters.

an. Anus. *c. p.* Cephalic ciliated pits. *ci.* Transverse ciliated bands. *E.* Eye. *e. gl.* Gland cells of lips. *gr.* Granular cells of ectoderm. *h. ph.* Horizontal diverticulum of pharynx. *In.* Intestine. *l. m.* Longitudinal muscle-fibres. *M.* Mouth. *m. ph.* Muscular appendix of pharynx. *m. ep.* Myo-epithelial cells of ectoderm. *Me.* Median lobe of gonad. *ne.* Position of observed nephridia. *n. f.* Nerve-fibres. *n. g.* Nerve-cells. *n. l.* Lateral nerve-cord. *œ.* Œsophagus. *r. m.* Circular muscles. *st.* Stomach. *s. h.* Cephalic sense hairs. *sh¹.* Post-cephalic rings of sense hairs. *x.* Deep cells of cephalic ectoderm. *Br.* Brain. *St.* Stomodæal musculature.

FIGS. 1—10.—*Dinophilus gigas*.

Fig. 1. The live animal extended, seen by transmitted light.

Fig. 2. A specimen contracted by treatment with corrosive sublimate solution, but not otherwise distorted. This figure shows fairly well the shape assumed on irritation by the live creature.

Fig. 3. A transverse section through the præ-oral lobe.

Figs. 4—6. Transverse sections through the pharyngeal region.

Fig. 7. The muscular bulb of the pharynx, in transverse section.

Fig. 8. Section through the middle of the trunk.

Fig. 9. Section through junction of stomach and intestine.

Fig. 10. Section of ventral ectoderm. Zeiss's im., oc. 2.

FIGS. 11—16.—Diagrams of various forms of pharyngeal apparatus, as seen in longitudinal sections of the head.

Fig. 11. *Dinophilus* (original).

Fig. 12. *Polygordius* (schematised from Uhljanin).

Fig. 13. *Histriobdella* (schematised from Foettinger).

Fig. 14. *Terebella* larva (schematised from Saleusky).

Fig. 15. *Navis* (schematised from Vejdovsky).

Fig. 16. *Vortex* (schematised from von Graff).

Fig. 1.

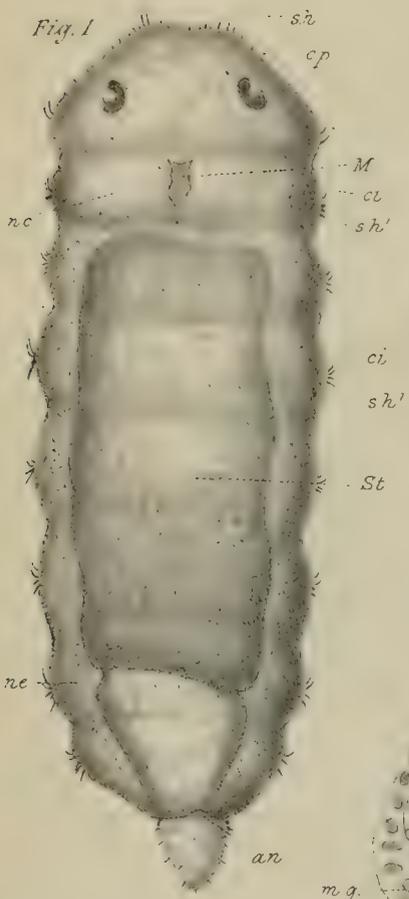


Fig. 2.



Fig. 3.

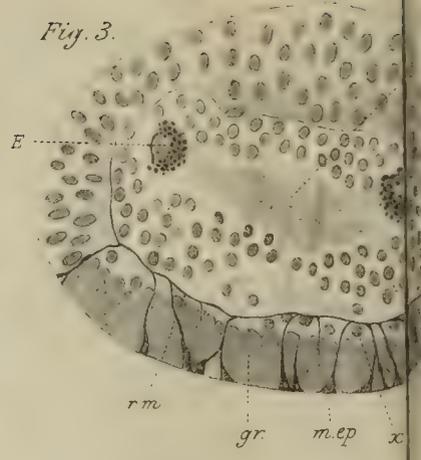


Fig. 6.

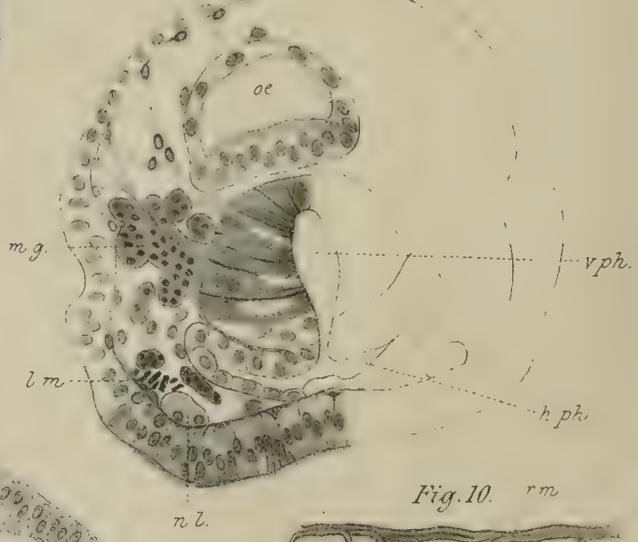


Fig. 9.



Fig. 10.

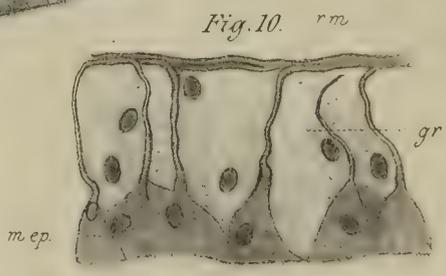


Fig. 11.



Fig. 12.



Fig.

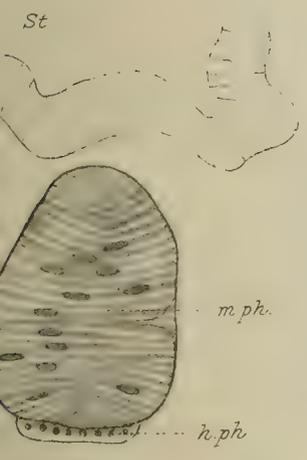


Fig. 8.

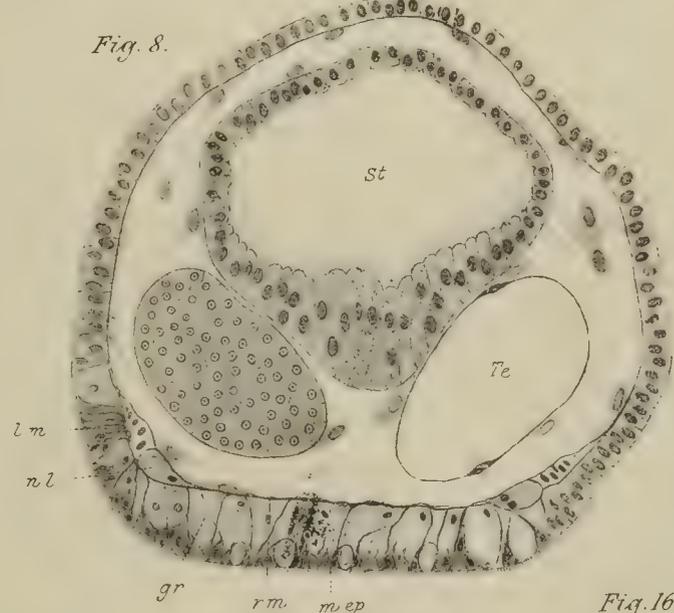


Fig. 16.

Fig. 14.

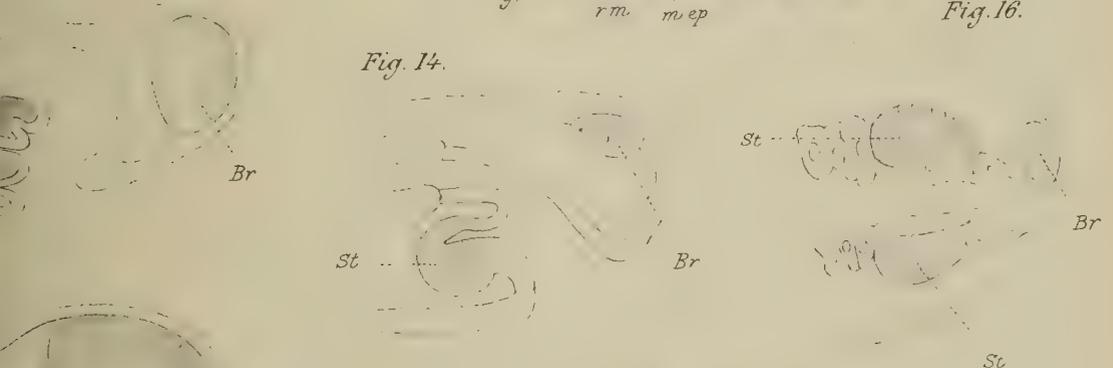
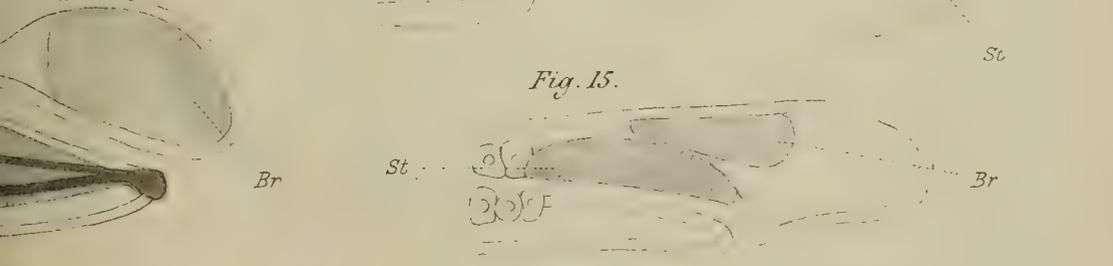


Fig. 15.



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