QUARTERLY JOURNAL

OF

MICROSCOPICAL SCIENCE:

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VOLUME XXVII.—New Series.

With Lithographic Plates and Engravings on Wood.

LONDON:
J. & A. CHURCHILL, 11, NEW BURLINGTON STREET.
1887.

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The Anatomy of the Madreporaria: II.

By

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With Plate I.

In a previous paper (4), I have described the anatomy of a solitary Imperforate coral, Flabellum; and of a branching Perforate, Rhodopsammia. The present memoir treats of two examples of colonial Perforate forms, Madrepora Durvillei and M. aspera.

Madrepora Durvillei (Milne-Edw. and Haime).

Two fragments of this perforate Madreporarian were kindly entrusted to me for study by Professor H. N. Moseley, who had obtained them during the voyage of H.M.S. "Challenger."

The species was founded by Milne-Edwards (1) from a part of the M. rosea of Esper, but as his account is very incomplete, Mr. J. J. Quelch, of the British Museum, has furnished the following description of the coral. I am glad to be able to take occasion to thank him for this and many other courtesies.

A. "Corallum arborescent, spreading, and remotely ramose, or occasionally sub-prostrate, and almost destitute of branchlets on the under surface. Branches often nearly 2 cm. thick, becoming very thin towards their extremity, subterete, elongated, covered irregularly with crowded capillary polyp-bearing branchlets, which generally give to the branches a sub-cylindrical outline of about 3—5 mm. in diameter.

Branchlets small and short, about 1—2 cm. in length, consisting generally of a few thin and long tubiform calicles; towards the apical parts of the branches they become much less elongated and often quite short. Surface slightly porous, very distinctly costulated throughout, and marked with fine echinulations which are very distinctly arranged on the calicles. Calicles generally tubiform, about 1·5 mm. wide and 1· cm. long, except towards the apical parts of the branches, where they are shorter and smaller, and sometimes tubonariform; a few short tubonariform calicles are generally placed on the surface of the branches between the branchlets. Star distinct, of six more or less lamelli-spiniform septa, two of which, the distal and the proximal, are usually much enlarged, and meet one another, often deep down in the fossa; while occasionally, as in the terminal calicles, the six septa are subequal, and coalesce at the centre."

"This species seems to be distinguishable from the M. echinata (Dana) simply by the costulations of the surface, which in the latter is smooth or finely granulated. It is doubtful, however, whether this character will prove to be sufficiently constant to separate the two species, when a larger number of forms has been examined."

Figs. 1 and 2 represent the dorsal and ventral aspects of a fragment of a branch, and show most of the characteristics mentioned in the above description.

In a transverse section of the corallum (fig. 3), the peripheral ring of polyp cavities is cut somewhat obliquely (*a a.*), owing to the inclination of branchlets and calicles to the branch; while the more central ones, cut at a lower level and more transversely, are approximately circular in outline (*a' a'.*). They lie, roughly speaking, on three sides of the branch, none are apparent on the fourth. The shorter radius of the latter seems to imply that the growth in diameter of the branch depends upon the outward growth of the polyps.

In the axis of the branch is a central cavity (*c. c.*), into which project six septum-like ridges; this probably represents a cavity previously inhabited by the now apical polyp. The

tops of both my specimens having been broken off, I have not been able to prove this; nor again to investigate the method of budding; but in M. aspera is such another central cavity with six septa, which is continuous with that of the apical polyp. All other polyp cavities converge towards, and, by means of canals, eventually open into, this central cavity, but no more definite connection is traceable. Tissues not unlike mesenteries are sometimes visible in it, but the alcohol in which the specimens were killed did not penetrate sufficiently rapidly to preserve the central parts in good histological condition. In some sections the six septa are not recognisable, and the axis of the branch is occupied by a wide-meshed network of coral; this is probably due to reabsorption of part of the skeleton.

In transverse section are also seen concentric series of longitudinal canals (c^2.) permeating the corallum; their arrangement appears to indicate that the radial growth of the branch is effected in the following manner. Directly beneath the external body wall of the colony a series of longitudinal canals runs between the costæ (fig. 4, c^1); and it is probable that, for increase in the diameter of the branch, the costæ grow outwards, and then, bulging laterally, fuse over these canals, so as to enclose them entirely in corallum (*cf.* fig. 10, *x*.). Thus there results a series of internal longitudinal canals, concentrically arranged, with radii of coral between them which represent former costæ. Not only does the appearance of such a transverse section as fig. 3 suggest that this is the mode of growth, but also "dark lines of growth" (fig. 5) run radially from each costa towards the centre, so continuously as to indicate that what was a costa when the diameter of the branch was very small, has continued to grow as such, and to be still such, when the diameter is very much larger. New costæ, when required owing to the increased circumference of the branch, appear to take their origin from the point of fusion of previous costæ.

More minutely, growth is effected, presumably by the activity of calycoblast cells, through the addition to and formation of crystalline ellipsoids, similar to those described

by v. Koch in Stylophora (2). These ellipsoids have a distinct sweep from one "line of growth" to the next.

The calyces are all of approximately the same size, and that so minute as to render investigation of the anatomy difficult.

The septa are very irregular of occurrence; the complete number appears to be six, but three are rarely to be seen in one section, often none at all. They are not constant through the whole depth of the polyp cavity, but occur as discontinuous ridges (fig. 6, *Ab.*). In every polyp, however, either an axial or abaxial sèptum is present, which enables the orientation of the polyp to be effected as in the Alcyonaria. (These terms, axial and abaxial, are used in preference to the ordinary and misleading "dorsal" and "ventral," and were suggested originally by Professor Milnes Marshall, 'Trans. Roy. Soc. Edin.,' 1883.)

There is no columella, but often the axial and abaxial septa fuse, low down in the polyp cavity, so as to divide it into two equal halves (fig. 3, *a'*), in a manner suggestive of the "median plate" in Pocillopora and Seriatopora figured by Professor Moseley (3).

The costæ bear apparently no relation to the septa in the well-grown colony, whatever may have been the case in the founder-polyp. Not only is no connection traceable between them in a transverse section of the branch, but even in a single polyp standing off from the stem, where the number of septa is under the most favorable conditions but six, about twenty costæ surround the calicle.

B. **Anatomy.**—The whole of the corallum is covered externally by a definite body wall of ectoderm, mesoderm, and endoderm (fig. 6, *ext. b. w.*, fig. 4, *ect. me. en.*), immediately beneath which lie, as in Rhodopsammia, external longitudinal canals parallel to the long axis of the corallum (figs. 3, 4, 6, c^1). These, however, are not the result of the same anatomical relations in both cases; in Rhodopsammia, lamellæ of mesoderm with a layer of endoderm on each side are given off from the external body wall, and unite with the endoderm

and mesoderm which clothe the exterior surface of the theca; and into the canals thus formed project the costæ. In M. Durvillei, the layer of endoderm and mesoderm which is immediately apposed to the exterior surface of the corallum, rises in a ridge towards the external body wall; and at the points where these layers meet and fuse are formed the costæ, i.e. in the angle of the mesoderm; and therefore between the costæ lie the canals. A comparison of fig. 4 with (4) fig. 17 will make clear the anatomical difference.

There is thus no trace of any structure resembling the "peripheral continuations of the mesenteries of v. Koch."

These canals appear to open over the lip of the calyces into the polyp cavities; they are connected with each other transversely between the spikes (echinulations) of the costæ (figs. 6, 7); and further, by radial canals (figs. 3, 4, c^3.) they open into the internal longitudinal canals, which I believe, as above stated, to have, at an earlier period in the history of the branch, occupied a position similarly external to the corallum. The whole system which thus perforates the corallum, and allows free current of fluid to even the most remote parts of the colony, is lined by endoderm and mesoderm throughout, and opens into similarly lined polyp cavities.

The general structure of the colony is, therefore, (1) an external body wall, under which and between the costæ lies (2) a series of external longitudinal canals opening into each other, and also through the corallum, into (3) the internal canals, mainly longitudinal, with radial and transverse connections, communicating in their turn with (4) the cœlentera of the polyps. Into the last the external longitudinal canals also open directly, through the theca. The whole system is of course merely a complication of the primitive cœlenteron.

Of the polyps there are at least two distinct types, which are full of interest as constituting the first record of marked dimorphism among the Madreporaria. Both are Actinian in structure.

Type A has in the highest sections twelve perfectly normal

mesenteries, and a stomatodæum which is a simple invagination of the external body wall. A little way down in the polyp, six of the mesenteries, in every case the same six, assume a curious modification of structure, which will be described first as seen in a series of transverse sections. Fig. 8 represents the characteristic features of a polyp of this type; the mesenteries numbered 2, 4, 6, 7, 9, 11 are those which undergo modification, and are diagrams of a series of drawings made from the same mesentery with camera lucida at different heights.

There appears first (fig. 8.2) an involution of the stomatodæum directed towards the mesentery, on the floor of which the ectodermic cells are long, but shorter at the sides. By fusion of the mesoderm and obliteration of the ectoderm on each side of this involution, a small canal with a definite lumen is found to be pinched off, and to lie enclosed in the mesoderm lamella of the mesentery (fig. 8.4). In the neighbourhood of this involution, the endodermic cells lining the mesenterial chamber become enormously lengthened and vacuolated, though the layer is still apparently only one cell deep.

Some sections lower down in the polyp (fig. 8), another similar involution appears in the stomatodæum, in which the ectodermic cells are short on the floor, but pass into deeper ones at the sides; this similarly results in the enclosure of what appears to be a second canal in the centre of the mesentery (fig. 8.7). In the first canal, as is shown in the diagram, the longer ectoderm cells face towards the stomatodæum; in the second away from it.

Further down yet, where the stomatodæum ceases, the free edge of the mesentery is enlarged into a perfectly normal filament (fig. 8.9); and finally (fig. 8.11), the whole modification disappears suddenly, the two canals meeting below; the mesentery then presents a perfectly normal appearance, namely, a mesoderm lamella with a layer of small endodermic cubical cells on each side of it, and bearing the usual filament.

The compilation of these sections, which I have attempted to express in fig. 6 M, shows that on an ordinary mesentery

occurs a swelling due to elongation of the endoderm cells, through which runs, in the mesoderm, a canal lined by ectoderm, doubled back on itself, and opening at both ends into the stomatodæum, with the ectoderm of which its lining is continuous.

Of twenty-one polyps examined, seven present this modification of six (and in all cases of the same six) mesenteries, namely, those numbered 2, 4, 6, 7, 9, 11, according to the method employed in the diagram; the other six mesenteries, 1, 3, 5, 8, 10, 12, and all the twelve mesenteries of the other polyps, are perfectly normal, and show no tendency to such a modification. Were it possible to explain the sectional appearances by a contortion of the mesentery, the regularity with which it occurs would be sufficient proof that it is a definite modification of structure, the parallel of which has yet to be sought in the Anthozoa.

The unmodified mesenteries in Type A, generally die out before the plane of the opening of stomatodæum into cœlenteron is reached, in transverse sections. If they present a filament, which is seldom the case, it is of the same character as that figured (fig. 8.11), i.e. identical with that of a modified mesentery; more frequently none is present, or at most a slight endodermal swelling on the free edge.

The mesenteries 4, 9, run very much deeper into the corallum than the others.

Type B, of about the same diameter as A, is of the normal Actinian structure. The twelve mesenteries are simple, and exactly like those unmodified in Type A. Most of them die out after a very short course, but those numbered 2, 4, 6, 7, 9, 11, on the same notation as in fig. 8, present a more developed filament than the other six, and extend further down into the corallum, and of these 4, 9, have by far the longest course, and are the only ones that bear ova.

We have thus two distinct types of polyp, the one distinguished only for entire normality; the other with a hitherto undescribed form of mesentery. In both is observable a differentiation affecting the same six mesenteries, exhibited in the

one case as a tendency to a longer course, and to the more complete development of the filament; in the other as the peculiar modification described above; and in both types two of these six have of all the longest course, and are, so far as I have observed, the only ones that bear reproductive organs.

Neither type is confined to certain areas of the branch, but both appear to be irregularly distributed.

Tentacles are not recognisable in my specimens, but it is probable that in the living animal they occur as slight evaginations of the chambers, and have shrunk under the action of the alcohol in which the polyps were killed.

Muscles are obviously present on the mesoderm lamella of the mesenteries, but owing to their minute size it is impossible to detect how they are arranged. I see no reason to doubt that they agree with Actinia. So far as it is possible to judge without this clue, the septa are entocœlic.

c. **Histology.**—There is but little to be said under this head, except as regards the modified mesentery, an almost transverse section of which is represented in fig. 9. The state of the specimens did not allow of an exhaustive study of cell structure, but those cells, the elongation of which causes the peculiar swelling on both surfaces of the mesentery, are apparently simply lengthened, much vacuolated, and amœboid at their free ends. No food particles were detected in them, or indeed in any other part, but many zooxanthellæ are embedded amongst them. These cells pass gradually into the ordinary endoderm, and their appearance suggests strongly that their condition is merely an exaggeration of that of the "Flimmerstreifen" of the brothers Hertwig, i. e. of the two lateral lobes of the mesenterial filament.

In a recent paper (5) Dr. Wilson has suggested that these lateral lobes are ectodermic in origin, circulatory in function, and homologous with the "ectodermic bands" described by him on the axial mesenteries of certain Alcyonaria. I may here state that, so far as histological evidence from the adult is valuable, it points, in all the Madreporaria that I have yet examined, distinctly in the other direction. The central

"Nesseldrüsenstreifen" have precisely the same microscopic appearance as the stomatodæal ectoderm; while the "Flimmerstreifen," in the unbroken gradation by which they pass into the endoderm, and by their characteristic staining, seem to be much more nearly connected with that layer than with the ectoderm, and to exhibit an intermediate condition between the ordinary cubical or pavement cells of the endoderm and the enormously lengthened cells of M. Durvillei. v. Heider (6), on the same grounds, had previously come to the same conclusion with regard to Cerianthus.

The ova, which in my specimens were few in number, are surrounded by a mesodermal capsule, and possess the ordinary structure. In the one case, in which an ovum was observed on a modified mesentery, it was borne on the neck between the endodermic swelling and the mesenterial filament.

D. General Conclusions. — This form has four interesting features in common with the Alcyonaria (Octactiniæ):

1. The marked tendency to an absence of polyps on one (the ventral) side of the branch and branchlets.

2. The very definite orientation of the polyps by a stronger development of axial and abaxial septa; and the concomitant bilateral symmetry, the plane of bisection being at right angles to the long axis of the branch or branchlet.

3. The differentiation of mesenteries, which, confined in the Alcyonaria to two, is here extended to six, and more particularly to two of these, though not the same two as in the other group.

4. The distinct dimorphism.

Of the true significance of this dimorphism no certain explanation can be gathered from this form studied merely by itself; it can only be resolved by a comparative study of allied species. Differentiation of function appears to be incomplete; both forms are reproductive, both apparently digestive. The most that can be said is that A is, perhaps, more digestive and less reproductive than B, for the filaments are more developed than in the latter form, and I have only once observed an ovum on a modified mesentery. Should the modification

be digestive in function, as is probably the case, A might certainly be termed a "gastrozooid."

But at present any explanation of the function of the structure above described, cannot be other than a mere speculation. It cannot be regarded as a necessary result of the colonial habit, since nothing similar occurs in the next species to be described—M. aspera. It can hardly be connected with reproduction, as ova are of rarer occurrence in the modified than in the unmodified polyps; and an excretory apparatus is not required by an organism whose cells are capable of amœboid activity, egestion as well as ingestion.

The only evidence on the point is derived from the distribution of the zooxanthellæ. These are most plentiful, firstly, in the external canals just under the body wall; and secondly, among the elongated cells of the mesentery. Assuming, as we may fairly do, that nutriment and aëration were the determining factors of such distribution, it would seem that, in the first case, there must be a strong current of nutritive "chyle-aqueous fluid" (to use a word of the older zoologists) in these external canals, and that aëration was effected by diffusion of oxygen through the body wall from the surrounding medium; and in the second place, that the elongated vacuolated cells of the mesentery were in some way assimilative, while oxygenation of the tissues for these special digestive processes (and therefore secondarily and accidentally to the benefit of these symbiotic algæ), resulted from a constant stream of water flowing through the central ectodermal canal of the mesentery.

That such a stream does pass through this canal is extremely probable, for the longer ectodermic cells are all morphologically on the same side of the canal; a wave of ciliary action must therefore result in a current through the canal from one of the apertures into the stomatodæum towards the other. A comparison of fig. 8.7 with fig. 6 M will explain this arrangement of the cells.

It is interesting to note that in M. Durvillei, as in Alcyonaria and Antipatharia, two mesenteries are distinguished

from the rest by running far deeper into the corallum or rachis. This may be a specialisation for circulatory purposes, as has been shown by Dr. Wilson to be true for certain Alcyonaria, or connected with production of the generative elements, as is the case in Antipatharia; in M. Durvillei certainly the latter, perhaps also the former, holds good.

Madrepora aspera (Dana).

For a fragment of this coral, fortunately the upper part of a branch, I am again indebted to Professor Moseley.

The species was founded by Dana (7), who gives a good figure of the colony.

A. Corallum.—A transvers esection of the corallum (fig. 10) shows that the polyp cavities (*a a'*) are arranged in a definite ring, and not merely confined to three sides as in M. Durvillei, round a central cavity into which project six septa, more or less fused together at their free edges. This central cavity (*c. c.*) is continuous with that of the apical polyp of the branch. The arrangement of the internal longitudinal canals is not so definitely concentric as in M. Durvillei, but the method of circumferential growth of the corallum appears to be similar in both species, since the costæ appear to fuse over the external longitudinal canals (*v.* fig. 10, *x*, and p. 3).

In the apical polyps are found six distinct entocœlic septa, and six smaller exocœlic, of which all are not always present; in the others generally only an axial or abaxial septum. A similar difference between them was observed by v. Koch (8) in M. variabilis, where both exosepta and entosepta were present in the apical polyps, but entosepta only in the rest.

In this form, as in the former species, there appears to be no relation in number and position between costæ and septa, the former being by far the most numerous.

The costæ are apparently formed as in M. Durvillei, that is, at the points where the endoderm and mesoderm apposed to the exterior surface of the corallum touch the external body wall (*v.* p. 5 and fig. 4), but in both species, owing to alcoholic contraction, the latter has so shrunk on to

the corallum that the costæ project through it, and the exact conditions are difficult to determine with certainty.

B. **Anatomy.**—The general anatomy of the colony, as regards the relations of canals, body wall, polyp cavities, &c., agrees with that of M. Durvillei. Beyond the fact that in M. aspera the polyp cavities are placed closer together, and that therefore there are fewer canals in the corallum, there is little or no difference between them. As regards the polyps, however, there is no dimorphism; all the polyps, except those which are obviously immature buds, are identical in structure.

A typical polyp possesses twelve perfectly normal mesenteries, and a stomatodæum which is a simple invagination of the external body wall. When numbered on the same system as in M. Durvillei, it is found that those mesenteries marked 1, 2, 4, 6, 7, 9, 11, 12, are the ones which develop mesenterial filaments, that is, the same mesenteries as in M. Durvillei, with the addition of the abaxial "directives;" while the others, 3, 5, 8, 10, generally have no filament, and do not extend to the bottom of the stomatodæum.

The apical polyps are about twice the size of the others, but, except for their possession of more septa, are identical in structure with them.

The muscles in both apical and lateral polyps are arranged on the mesenteries just as in Actinia, and present nothing unusual in structure.

Tentacles I was unable to recognise, macroscopically or by sections, but a figure by Dana shows that they are present, and twelve in number. In this, as in the species last described, they have shrunk into insignificance, owing to the action of the spirit in which the specimens were preserved. They agree with M. variabilis, in which, according to v. Koch, they are also exocœlic and entocœlic.

The histology calls for no remark, agreeing with that of forms already described. Calycoblasts were very distinctly present in the growing parts of the colony.

C. **Method of Budding.**—With regard to this, I have been able to glean but little information; since the immature polyps

are so crowded with zooxanthellæ, owing presumably to the amount of nutriment supplied to them, that the tissues are much obscured.

The stomatodæum is invaginated to a considerable depth into the future polyp cavity before it is perforated for communication between the cœlenteron and the exterior, and also apparently before any mesenteries are formed. The cavity into which it is invaginated is already of considerable diameter, and larger than the ordinary canals of the colony; though smaller than that of a fully formed polyp, at that point it is probably never enlarged by reabsorption of coral, but its continuation upwards by future growth of the polyp possesses a gradually increasing diameter.

In a young polyp in which the stomatodæum was invaginated, but not yet perforated below, the latter appeared to be supported by tissue surrounding the future septa, just as the external body wall is supported by tissue enclosing the costæ. In sections below the stomatodæum, and unconnected with it, were seen two small mesenteries with filaments, which appeared to be growing upwards towards the stomatodæum, and to have not yet joined it. It is therefore possible that these grow upwards from the canal system, and are formed quite independently of the rest of the polyp. This view is further supported by the observation that, in sections quite at the top of a branch, above the plane of any lateral polyps, occur in the canals one, sometimes two, little mesenteries with filaments, which I believe to be growing upwards towards the sites of future polyps. They appear to take rise, near the cavity of the apical polyp, from the wall of the canals.

In the only other stage of development from which any observations could be made six mesenteries had appeared; of these the two furthest from the axis carried muscles on the outer faces, though it does not necessarily follow that they were the abaxial "directives" of the adult. The muscles of the other two pairs were not sufficiently developed to allow of their arrangement being recognised.

Conclusion.—From M. Durvillei, the present species is

widely separated by a strong morphological distinction, the absence of dimorphism; since the difference between the apical and lateral polyps in M. aspera is hardly strong enough to be reckoned as such. That such a distinction should exist between two species of a genus is very remarkable; but, considering the great antiquity of these forms, the similar structure of the colony in both, and the fact that they exhibit a similar differentiation of certain mesenteries, it is not to be inferred that their systematic relations are unsound.

Note.

For microscopic sections through both hard and soft parts of the coral, such as are figured in (4) Pl. XLI, figs. 14, 15, I have found the method, originally applied by v. Koch to these forms, extremely useful. The coral, having been left in borax carmine for three days, and treated with acidulated alcohol for six hours, is transferred to absolute alcohol, and from this to ether; into the ether is dropped absolutely dry powdered Canada balsam in small quantities at a time, till enough is dissolved to make a block, rather larger when dry than the specimen. The ether is driven off by a gentle heat, leaving the coral permeated throughout by balsam. About a week should be devoted to this part of the process.

Sections are then cut with a lapidary wheel, or, if this is not procurable, with a fret saw; and ground like geological sections on a slate, then polished on a water of Ayr stone. Oil and emery powder should be avoided, water alone being used for the stones.

One surface of the section having been ground and polished, it should be affixed permanently by that surface to a glass slide, on to which some dry Canada balsam has been melted, and not again be moved. When the other surface has been similarly ground and polished to the required thinness, it should be brushed lightly, first with absolute alcohol, then immediately with oil of cloves; this removes all dirt from the surface. A drop of balsam in benzole is then placed on the section, and the cover glass lightly dropped on it.

Erratum.

In my previous paper (4) Pl. XL, fig. 1^2, the septa were wrongly numbered; they should have been marked 1, 4, 3, 4, 2, 4, 3, 4, 1, reckoning on each side from the central "directive" septum, D.

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DESCRIPTION OF PLATE I,

Illustrating Mr. G. Herbert Fowler's Paper on "The Anatomy of the Madreporaria."

a. Polyp cavities, cut obliquely. *a'*. Polyp cavities, cut transversely. *Ab*. Abaxial (ventral) septum. *Ax*. Axial (dorsal) septum. *C*. Costæ. *c. c*. Central cavity, continuous with the apical polyp. c^1. External longitudinal canals between the costæ. c^2. Internal longitudinal canals. c^3. Radial and transverse connecting canals. *Co*. Corallum of the main branch. *d*. Cut edges of the endoderm and mesoderm lining the cœlenteron. *ect*. Ectoderm. *en*. Endoderm. *ext. b. w*. External body wall of ectoderm, mesoderm, and endoderm. *M*. Mesentery, showing the endodermal swelling. *me*. Mesoderm lamella. *S*. Septum. *S. C*. Septal Columella-plate. *St*. Stomatodæum. *Th*. Theca of polyp. *Z*. Zooxanthellæ. *x*. Fusion of costæ over ext. long. canals. All except Fig. 10 are from Madrepora Durvillei.

Fig. 1.—Dorsal view of the corallum of two fragments of a branch, bearing calicles, and branchlets formed of other calicles.

Fig. 2.—Ventral view of the same specimens; one of which is entirely bare of calicles on this side, and on the other only a few are present.

Fig. 3.—Transverse section of a branch, showing the polyp cavities, the central cavity, and the canals running in various directions. The concentric

arrangement of the latter is well shown. Into the central cavity project the six septa. In two of the innermost ring of polyps, the axial and abaxial septa have fused into the septal columella-plate.

Fig. 4.—Diagram of a transverse section of a polyp and of part of the branch. The external body wall is shown to be supported on the costæ, as its mesoderm and endoderm are continuous with those lying on the outer face of the corallum. The polyp cavity shows at this point twelve mesenteries supporting the stomatodæum. (In nature the mesoderm lies closely apposed to the surface of the corallum, and there is no space between them, such as is introduced into the diagram for clearness.)

Fig. 5.—Transverse section of a portion of the branch, to show the lines of growth, running between the canals radially and terminating each in a costa.

Fig. 6.—Diagram of a longitudinal section of a polyp along the dotted line in Fig. 8. The tentacles are omitted, as they were not recognisable in my specimens; the canal system in the corallum is also omitted. On the left the section passes between the axial septum and mesentery No. 7, and above the polyp down an external longitudinal canal; on the right, through the abaxial septum and down a costa, of which the echinulations and the canals between them are shown. The numbers indicate the same mesenteries as in Fig. 8. On the mesentery 7 is figured the endodermal swelling, with the bent canal indicated by dotted lines. In the stomatodæum are shown the two openings of the canals of mesenteries 7, 9, 11; and below the stomatodæum the free edges of these three mesenteries alone appear, the others dying out before this plane is reached. The dotted line indicates the junction of theca and septa, and the discontinuous character of the septum (*Ab.*) is clearly shown.

Fig. 7.—The external body wall viewed from the exterior; the lighter spots are the places where the echinulations of the costæ have pierced the body wall on account of its shrinkage. This drawing shows the arrangement of the external longitudinal canals, and their connections between the spikes of the costæ. (Camera lucida.)

Fig. 8.—Diagram of the various forms and conditions of the mesenteries in a polyp of Type A. Those numbered 1, 3, 5, 8, 10, 12 are unmodified and normal. The others, 2, 4, 6, 7, 9, 11, are modified in all the polyps of this type; they are from camera lucida drawings of the same mesentery at different heights. The arrows and Roman numerals in Fig. 8 show the planes in which the successive sections are taken.—2 shows the endodermal swelling, and the upper opening of the canal; 6 shows the lower opening; 9 is below the stomatodæum, and bears a filament; and in 11 no trace of the modification remains, the mesentery being normal, and similar to those of Type B.

Fig. 9.—Transverse section of a modified mesentery, passing through both arms of the canal.

Fig. 10.—Transverse section of the corallum of a branch of M. aspera.

On the Formation of the Germinal Layers in Chelonia.

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With Plates II, III, IV, and V.

In the spring of 1884 we made the acquaintance of Mr. Hattori, the proprietor of a large fish-hatching establishment in Honjō, a suburb of Tokyō. His father before him, and he, had succeeded in making the snapping turtle—Trionyx Japonicus, Schlegel—breed freely and naturally in captivity, and thus in furnishing the market with a constant and large supply of its delicate flesh. In his farm hundreds of these turtles are annually hatched, and if the eggs are marked as they are laid the exact age of any given deposit can be determined with great precision, even to minutes in many cases. Such an opportunity for the investigation of Reptilian development seemed to us too good to be thrown away, especially as nobody had, so far as we were aware at the time, worked on the embryology of Chelonia since the days of Agassiz and Clark, and therefore with modern methods of investigation. Mr. Hattori kindly consenting, we went to his farm daily during the breeding season of 1884 and of 1885, and succeeded in collecting a fairly complete series of the Tryonix embryos, beginning

with the time when the eggs are deposited, and ending with their hatching out. The present paper gives the results of our study on the formation of the germinal layers. Papers on other points and later stages of development will follow from time to time since the investigation is being continued, as the pressure of other duties permit us.

We wish to return our warmest thanks to Mr. Hattori for cheerfully acceding to our numerous demands on his good nature, and for furthering greatly our work with his intelligent assistance. Thanks are also due to the authorities of the University of Tokyō for the payment of necessary expenses attending the investigation, and for the use of instruments, reagents, &c. Finally, we wish to express our deep obligations to Dr. Isao Jijima for valuable suggestions in regard to the methods of investigation.

We made many intercsting observations on the breeding habits of Trionyx, but we reserve these for some other occasion, as foreign to the purpose of this paper. We simply mention that the Trionyx eggs are nearly spherical in shape, and have a hard brittle shell like that of the fowl, and not leathery, as in some Chelonia. Their size is very variable, the smallest we measured being 10 mm., the largest 23 mm., the most usual size about 21—22 mm. in diameter. This difference in size seems to be due mostly to the size of the parent. With this we pass on at once to the consideration of the subject proper of the present communication.

The earliest stage of which we will give a detailed description is taken from an egg opened directly after its deposition. Our attempts to obtain still earlier stages by opening pregnant females have proved but partially successful. In almost every case, with only some doubtful exceptions, the eggs we found in the oviduct were unfortunately fully as much advanced as those just laid.

On opening an egg directly after its deposition the blastoderm is always found at the pole turned above. The embryonic shield, with the pellucid area around it, stands out conspicuously as a small, nearly circular spot, on the yellow surface of the

yolk. The general appearance of the embryonic shield at this stage is represented in fig. 1 *a* and *b*, enlarged about thirty diameters. Fig. 1 *a*, shows it as seen from the dorsal side, and fig. 1 *b*, as seen from the ventral side after the removal of the shield from the egg. The embryonic shield does not lie in the centre of the area pellucida (*a. p.*), but is placed excentrically nearer its hind end, so that here it is continuous with the area opaca (*a. o.*). The ectoblast has already spread itself over a large part of the egg, although we did not determine its exact limits (see fig. 16). On the dorsal view the blastopore (*bl.*, fig. 1 *a*) forms the most conspicuous feature; it is seen as a wide transverse slit across the posterior part of the embryonic shield, occupying considerably more than one third of the breadth across. From the blastopore a passage leads obliquely forward and ventralward, and opens about in the centre of the ventral surface with a circular opening (*v. o.*). The walls of the ventral opening are posteriorly quite high, but become gradually lower and lower toward the front, until they sink to the general level of the ventral surface. For the sake of brevity this passage, leading from the blastopore dorsally and opening below, we shall hereafter call the blastoporic passage. It becomes eventually the neurenteric canal. Returning to the dorsal surface, the shield in front of the blastopore presents a broad flat expanse, in which are seen indistinctly three opaque lines radiating from behind forward, like the prongs of a trident. On referring to the ventral side we see that the two lateral opaque lines correspond to the thickenings which form the walls of the inferior opening of the blastoporic passage. Accordingly they are thickest posteriorly, and gradually thin out toward the front. The middle prong of the trident corresponds to the roof of the blastoporic passage, and its continuation to the front edge of the embryonic shield. It is, in fact, the chorda entoblast, which is still in the process of formation in front, as will be made clear by sections. The remaining parts of the ventral surface not taken up by these three thickenings present the appearance of a honeycomb. Of this we shall speak later on. Coming back to the dorsal

surface again, the area behind the blastopore, especially the median longitudinal space, is on a lower level than the parts in front. This, the sections show us, is the line of the primitive streak. At the part where the embryonic shield posteriorly joins the area opaca there is a considerable transverse thickening (*sl.*), shown both in the dorsal and ventral views—in the latter covered with yolk matter. This undoubtedly corresponds to the "sichel" or "sickle" which Kupffer describes in a similar Lacerta embryo (No. 5, Taf. i, fig. 1, *sl.*). We should add that these differences in level become much more conspicuous after the embryonic shield has been removed and treated in reagents than when it is stretched over the yolk, and also that the embryos of this stage vary considerably in their surface views, especially when they are hardened.

Figs. 7—15 are selected from the series of transverse sections obtained from the embryo represented in figs. 1 *a* and *b*. The figures are arranged in order from behind forward. Figs. 7—9 pass through the part behind the blastopore, figs. 10, 11 through the blastoporic passage, and figs. 12—15 through the part in front of the blastopore.

In fig. 7, the most posterior section represented, the ectoblast extends over the whole, being two or three layers of cells thick in the embryonic shield, but gradually thinning out to a single layer of flat cells toward both sides. The yolk occupies the entire lower stratum. Nuclei (*n. n.*) are visible in it. The space between the ectoblast and the yolk is occupied by a mass of mesoblast cells which is here distinctly separate from both the ectoblast and the yolk.

In fig. 8 (which by the way is taken from another embryo of the same deposit, as the section corresponding to this in the first series is unfortunately injured) the ectoblast is continuous in the median line with the mesoblast, i. e. it is very actively proliferating and giving off cells abundantly to the mesoblast.

Fig. 9 passes through the region directly behind the blastopore. The ectoblast is distinct laterally, but toward the median line, and at some distance from it, passes gradually into a mass of cells in which no layers can be distinguished. Dif-

ferent from fig. 8 where the ectoblast cells, although continuous in the median line with the mass below, still maintain their columnar shape over the whole dorsal surface and thus give an impression of the ectoblast extending entirely across; the ectoblast is in this section fused into the median mass without retaining the slightest trace of the columnar arrangement, and the median mass of cells thus expose their surface to the exterior for a short space in the axial line (*yk. p.*). We wish to emphasise the fact that this part directly behind the blastopore is neither at this nor at any subsequent time until considerably later (if ever at all), covered by the ectoblast of the general surface of the body. This area we consider to be the remnant of the yolk-plug of Rusconi found in the Amphibian embryos. This will become clear in the later stages. From the axial mass, where the layers are indistinguishable, there extends toward each side a thick mesoblastic wing under the ectoblast. The yolk seems to be distinct from the mass above, although, throughout this region, protoplasmic threads seem to connect the two.

Fig. 10 passes just in front of the dorsal lip of the blastopore where the ectoblast reflects downward and forward to become continuous with the axial strip of the entoblast or chorda-entoblast (compare fig. 16). The blastoporic passage (*bl. p.*) seen as a transverse space is still open on the left to the exterior. The floor of the passage is formed by a mass of cells continuous with the yolk-plug; in fact we may consider this a part of the plug. At a lower level the mesoblast (*mes.*) stretches out laterally as two wings from the median mass. The relations of the yolk are the same as in fig. 9.

So far the sections seem to have passed through the part known as the "sickle."

The next section represented (fig. 11) evidently passes through what may be called the neck or isthmus, i. e. the point from which the three prongs of the trident referred to in the surface view radiate (compare fig. 1 *b*). Accordingly, the entoblast is found only in the median line as a thickening constituting the walls of the blastoporic passage (*bl. p.*), which is

now irregularly circular in section. The roof and the sides of the passage are formed by a columnar epithelium two or three cells thick (*enc.*). This is continuous with the ectoblast at the dorsal lip of the blastopore (compare figs. 16 and 10). It is the chorda-entoblast of Hertwig (No. 6). The floor of the passage and the lower part in general is made up of irregularly scattered cells. This is not only the continuation of the yolk-plug but also of the yolk itself, which occupied the lowest stratum in figs. 7—10, and which has been in the last two or three sections gradually merging itself into the floor of the blastoporic passage. Thus, although it does not appear in any single transverse section, the three germinal layers are fused in the region behind the blastopore. Laterally the entoblast is very thin and passes gradually into the yolk. The section is out of the region of the "sickle," and there is no longer a mesoblastic wing on each side.

Fig. 12 passes through the posterior part of the lower opening of the blastoporic passage. The thickenings which form the lateral walls of the opening are therefore still quite thick (compare fig. 1 *b*). The columnar chorda-entoblast is found as before forming the roof and the sides of the passage, which is now open below. Towards the lower part of the side walls the columnar arrangement is lost and the cells are irregularly scattered. Further out at the sides the cells form a loose network, and then at the edge of the embryonic shield passes into the yolk.

Fig. 13 passes through the anterior part of the ventral opening of the blastoporic passage, which has now flattened itself out into a shallow groove in the median line. Its roof is still formed by the distinctly columnar chorda-entoblast. Laterally, the chorda-entoblast gradually passes into a mass of cells arranged in an irregular loose network, which in its turn is replaced by the yolk at the edge of the embryonic shield.

Passing forward, the chorda-entoblast begins gradually to confine itself more and more to the ventral median surface, until in the seventh section from fig. 13 it has the appearance presented in fig. 14. Here the columnar shape is confined to a few cells

in the ventral median line. They pass above gradually into the loose network of cells which has now extended itself entirely across. The meshes of the network have also become larger than in the previous sections. It is evidently this loose network that produced the appearance of a honeycomb in fig. 1 *b*.

Fig. 14 passes in front of the ventral opening of the blastoporic passage, and indicates that the loosely scattered lower layer cells are here arranging themselves into the chorda-entoblast in the ventral median line of this region, i. e. along the front part of the middle prong of the trident apparent in the surface views (figs. 1 *a* and *b*).

Fig. 15 passes near the front end of the embryonic shield. There is no longer any trace of the chorda-entoblast; the entire entoblast is an irregular stratum of stellate cells not thick enough to form a network. It passes into the yolk at the sides.

We may here call attention to the appearances which are seen in some embryos of this stage. Round the edge of the lower opening of the blastoporic passage, especially toward the front, there is a shelf-like extension of the entoblast into the archenteric cavity somewhat like the velum of a hydromedusa. Fig. 14 *a*, Pl. III, represents such an appearance. The section is well in front, so that the shelf-like extension is continuous across and divides a small space above from the main digestive cavity below. In sections posterior to this, the small space opens below. We do not know what the significance of this is, unless we suppose that the embryo is younger than that given in fig. 1, and therefore the ventral opening of the blastoporic passage is not yet entirely clear.

Fig. 16 is the median longitudinal section of an embryo taken from the same lot as that represented in fig. 1. The blastoporic passage is very distinct. On its dorsal lip, the ectoblast is reflected forwards and downwards and becomes continuous with the chorda-entoblast which passes in front into a loose network of cells with wide meshes, and finally, into the yolk at the edge of the embryonic shield. At the posterior wall of the blastoporic passage, the three layers, the ectoblast,

the mesoblast, and the yolk (i.e. the entoblast) are merged into one another; in other words, the ectoblast and the entoblast are here fused and from the fused place a mass of mesoblast cells extends posteriorly. The three layers are independent a short distance behind the blastopore. As the cross-sections of this region (figs. 8, 9, and 10) show that the mesoblastic mass is similarly extending to each side, we may conclude that, in addition to the primitive streak (fig. 8), the mesoblast is being given off from the posterior wall of the blastoporic passage, or at least from its upper part in all posterior directions for an arc of 180°, somewhat in the shape of an open fan; and this posterior unpaired mesoblastic mass causes the swelling known as the "sickle." Examining the ectoblast of the posterior part more in detail, we find it gradually losing its columnar character as we approach the blastopore from behind, but the space where the fused median mass of cells is dorsally exposed to the exterior, viz. the yolk-plug (compare fig. 9) is not as conspicuous in the longitudinal section as in the later stages. The entoblastic part of this fused mass extends quite forward. This corresponds to the cells seen in the floor of the blastoporic passage in fig. 11. A slight projection from its extreme tip is, we imagine, the remnant of the shelf-like structure mentioned in reference to fig. 14 *a*.

The principal facts brought out by the study of this stage may be summed up as follows:

1. There is a passage which, beginning with the blastopore on the posterior part of the dorsal surface, takes a forward and downward course to the ventral surface, opening in about the middle part of the latter by a circular opening.

2. At the dorsal lip of the blastopore the ectoblast is reflected and becomes continuous with the chorda-entoblast.

3. In front of the blastopore there are as yet only two primary layers, the ectoblast and the entoblast.

4. The entoblast is having its axial part arranged into a columnar epithelium to form the chorda-entoblast. This process proceeds from behind forward.

5. At the posterior wall (i.e. floor) of the blastoporic passage the ectoblast and the entoblast are fused, and from the point of fusion the mesoblast is being given off posteriorly in all directions for the space of 180°.

6. Also, behind the place where the two primary layers are thus fused, the ectoblast is giving off cells to the mesoblast along the median line (fig. 8). This is the line of the primitive streak. It is very short and is present in only two or three sections.

7. The mesoblastic mass derived from the two sources mentioned in (5) and (6) is unpaired and constitutes the transverse swelling in the posterior part of the embryonic shield, "the sickle." This is the only place where the mesoblast is present at this stage.

8. The median mass formed by the fusion of the three layers at the posterior wall of the blastoporic passage appears for a short space on the dorsal surface (fig. 9)—the remnant of the yolk-plug of Rusconi.

Formation of the Mesoblast and of the Chorda Dorsalis.

In the previous stage, the mesoblast was found only in the region behind the blastopore. We may now proceed to describe its formation in front of the blastopore. We call attention first to the embryo represented in figs. 2 *a* and *b*. It was taken out exactly forty-eight hours after the deposition, but as the weather was unusually cold for the season during the interval, it has made very little progress in development, and is not as far advanced as many thirty-six hours old. As before, a dorsal and a ventral view of the embryonic shield is given, although these are not taken in this case from the same embryo. The shape of the shield has not changed materially from the previous stage. In the dorsal view (fig. 2 *a*) the blastopore has assumed a horseshoe shape, and is more of a slit than before. Occupying the concavity of the horseshoe is the rudimentary yolk-plug. Round the blastopore, and

along the median line in front of it, there is an opacity. This seems to be due simply to the fact that the cell layers are thicker in this region than elsewhere. In the middle of this opacity in front of the blastopore and apparently starting from the latter there is a shallow median groove, which probably corresponds to the "Primitivrinne" or "Rückenrinne" described by Hertwig in the Triton embryo (No. 6). On the ventral side (fig. 2 *b*), we wish to call especial attention to the ventral opening of the blastoporic passage (*v. o.*). In the previous stage, it was a circular opening without definite limits. In this stage it has acquired well-defined limits on all sides except towards the front, where it is only faintly bounded. Along the median line of the roof of the recess thus formed, a wide low ridge is visible and is continued in some specimens in front of this area. This is undoubtedly the chorda-entoblast. In the ventral view, the posterior part is concealed by a mass of yolk which has accumulated here in the process of removing the shield from the egg.

As we are going to describe somewhat in detail the next stage, we may omit the description of the sections of this, except one through the ventral opening of the blastoporic passage. Fig. 17 is such a section. It passes through the front part of the lower opening. There is in the median line a slight notch in the ectoblast which corresponds to the groove seen in the surface view. In the entoblast we see the axial chorda-entoblast formed as usual of columnar cells. Laterally, it passes on each side into a mass of polygonal cells—the darm-entoblast of Hertwig (No. 6)—which becomes in its turn continuous with the yolk at the edge of the embryonic shield. At the point where the chorda-entoblast and the darm-entoblast meet each other, the darm-entoblast projects as a ridge into the digestive cavity and thus constitutes one of the lateral edges which bound the ventral opening of the blastoporic passage (compare fig. 2 *b*). Conforming to the groove in the ectoblast, the chorda-entoblast projects downwards in the median line. This corresponds no doubt to the ridge seen in the surface view within the lower

opening of the blastoporic passage. This section also shows that the roof of the well-defined area which forms the lower opening of the blastoporic passage is formed by the chorda-entoblast and that the latter thus occupies by itself a special recess of the digestive cavity. From just where the chorda-entoblast and the darm-entoblast join each other, there goes out laterally on each side a string of cells (*mes.*) placed dorsally to the darm-entoblast and ventrally to the ectoblast and distinct from both. This is the commencing mesoblast. The mesoblast is therefore not continuous from the first across the median line.

The surface view of the next stage is represented in fig. 3. The embryonic shield has become pear-shaped, the broader end being the front end. The blastopore is horseshoe shaped, as in figs. 2 *a* and *b*, and occupying its concavity is the yolk-plug. The head-fold has just begun, and is seen as the posterior of the two semilunar curves found near the front end of the embryonic shield. The anterior curve is probably the commencing amniotic fold. Between the head-fold and the blastopore there is seen in the median line an opaque streak, which is narrowest in the middle, and becomes broader anteriorly and posteriorly. This is the chorda, which is nearly completed in the middle, but still unfinished toward each end. The area pellucida is, as before, found only toward the front and the sides. The pear-shape of the embryonic shield seems to have been produced mainly by its posterior part having lengthened.

Figs. 18—23 are selected from a series of cross-sections obtained from an embryo of this stage, and are arranged from behind forwards.

Fig. 18, the most posterior section represented, goes through the lateral limbs of the horseshoe-shaped blastopore and the yolk-plug occupying its concavity. The ectoblast, which consists of only a single layer of cells at the sides, becomes gradually thicker towards the median line, which it does not, however, reach. At a short distance from the latter, and at

the lips of the blastopore, the ectoblast turns ventralward, and becomes lost in the mass of cells found in the axial line. It retains, however, its columnar character for some distance downwards. The considerable space between the two lateral lips of the blastopore is filled almost entirely by a plug (*yk. p.*) of considerable size, which projects upwards from the axial mass of cells as far as the level of the general surface of the embryo. The difference between the ectoblast and this plug is at once unmistakeable and striking. While the cells in the ectoblast are columnar and always arranged perpendicularly to the surface, the cells in the plug are polygonal and without any definite arrangement. We shall return to the discussion of this structure directly.

As just stated, the ectoblast turns downwards near the median line, and loses itself in the axial mass. All the germinal layers are, in fact, fused here, for the entoblast, although it has some appearances of being differentiated, is not entirely distinct, and the mesoblast also stretches away from this mass on each side. Toward the sides the entoblast is yet undifferentiated; it consists of an abundant protoplasmic network with numerous nuclei, and is full of yolk-spheres and granules. There is no question whatever that laterally the mesoblast receives cells from the entoblast or yolk. Especially along one line (*a*, figs. 18 and 20) nuclei are heaped in a special mass, from which cells are being given off to the mesoblast. This contribution to the mesoblast from the germinal wall is only in the posterior part, as it is no longer observable in fig. 23, and as the germinal wall itself, even in a more advanced stage, is found only round the posterior part as a horseshoe-shaped ridge (fig. 5).

Having gone over the description of the various parts of this section, let us return to the discussion of the plug (*yk. p.*) which sticks out to the external surface between the lateral lips of the blastopore. When we compare our figure 18 with the frontal section through the yolk-plug of a Triton embryo, which Hertwig (No. 6) gives in his fig. 9, Taf. ii, we think nobody will hesitate long before concluding that the plug in our figure is homologous with the yolk-plug of

Rusconi found in the Amphibian eggs. Allowing for the differences between a holoblastic and a meroblastic egg, the relations in the two figures are almost exactly alike, part for part. If the slits between the plug and the lateral lips of the blastopore extended in our figure a little more into the midst of each mesoblastic mass the resemblance would be complete; but even for the Amphibian eggs the slits do not always extend as far as represented in fig. 9, as Hertwig himself mentions (No. 6, p. 14). At any rate, in each case there is an axial mass of cells, (1) into which the ectoblast turns down at the lateral lips of the blastopore, (2) in which the entoblast is not to be distinguished, (3) from which the mesoblastic masses start away toward each side, and (4) which sends a plug upwards between the lips of the blastopore. If we compare the longitudinal section of the plug in Trionyx (fig. 24, *yk. p.*) with the sagittal section of the Amphibian yolk-plug (Hertwig, No. 6, Taf. ii, fig. 4), we see again that the relations of different parts are alike. It is true that the plug in Trionyx is not bounded posteriorly by a groove, and passes directly into the ectoblast of the primitive streak, but when we consider that the plug in Trionyx is only rudimentary this is not to be wondered at, and is of little significance.

We think we are justified, on these grounds, in concluding that we have in the mentioned structure of Trionyx the remnant of the yolk-plug, which appears conspicuously in the Amphibian egg. Strahl describes the same structure (compare No. 13, ser. iii, figs. 0, 0.1, 0.2; ser. iv, figs. 0, 0.1; ser. v, figs. 0, 0.1, 0.2, 0.3; ser. vi, figs. 0, 0.1; ser. vii, fig. 0.1, also No. 9, Taf. i, figs. 6, 7, 14, and 15; and No. 10, figs. 2 and 3), but, so far as known to us, has never explained its nature. Kupffer describes the "Zapfen" occupying the horseshoe-shaped blastopore of Lacerta (No. 5, Taf. i, figs. 2 and 3, *z*), but does not state its homology. He mentions that in Coluber Aesculapii the plug is sometimes divided into two parts by a median fissure (No. 5, Taf. iv, fig. 40, *f* and *g*). We have also observed a similar appearance in some of the earlier embryos of Trionyx, but we are satisfied that there is no true

median fissure. What appears to be such is the optical expression of the primitive streak, along which the ectoblast is proliferating, and giving cells to the mesoblast below. Even in the earliest embryos with this appearance it is doubtful if it ever extends to the extreme tip of the plug. As far as we are aware, the only author who mentions what seems to be the yolk-plug in an amniotic Vertebrate is Gasser, who observed it in an abnormal fowl embryo (No. 4, Taf. x, figs. 4—7). The reason why the yolk-plug in Trionyx is more conspicuous at this stage than earlier stands, we think, in close connection with the fact that the blastopore has become a much better defined horseshoe-shaped slit.

We return now from this long digression to the description of the embryo before us. The sections behind fig. 18 show that immediately behind the yolk-plug, which persists distinctly in only one more section after fig. 18, the ectoblast extends over the whole surface as shown by the characteristic columnar cells. For a short space, however, the ectoblast is proliferating in the median line and is continuous with the mesoblast below. This is seen in only three sections after which the ectoblast becomes independent. The entoblast seems, however, to be connected with the mesoblast for a greater length and to be actively contributing cells to the latter. This is the region where the germinal wall makes a horseshoe-shaped bend round the posterior part of the embryo (fig. 5). Except in this last detail, the relations of the various parts behind the blastopore exactly as in the stage represented in fig. 1.

Going forward, fig. 19 passes through the blastoporic passage. As it is directly in front of the dorsal lip of the blastopore, the ectoblast is still continuous for a little space with the chorda-entoblast, which as usual vaults over the passage. The columnar cells extend to the sides also, but on the floor of the passage the cells are polygonal, so that this part which is the continuation of the yolk-plug differs in its appearance from the roof and the sides. To this part, too, the darm-entoblast (*end.*) is attached. From the entire side of the axial mass the mesoblastic sheet goes out on each side.

Fig. 20 passes slightly in front of the ventral opening of the blastoporic passage. In the median line the chorda-entoblast (*enc.*) forms directly the roof of the digestive cavity, without the intervention of the darm-entoblast (*end.*) which stops at a short distance from the axial line. On the left side of the section, more clearly than on the right, the darm-entoblast is seen to make a fold at its innermost point where it abuts against the chorda-entoblast and then to turn outside again to be lost in the mesoblast. The mesoblast is therefore partly continuous with the chorda-entoblast and partly with the darm-entoblast. In other words, it starts from the point where the chorda- and the darm-entoblast meet each other. The mesoblast cells in this region show a peculiar arrangement. Those cells next the ectoblast are columnar and look like the continuation of the chorda-entoblast. The cells placed ventrally to these are polygonal and without any definite arrangement. Laterally cells are being added to the mesoblast in the whole region of the germinal wall, but especially at *a ;* proliferation seems to take place in the posterior region even from the outer part of the darm-entoblast, as in this section. This is, however, confined to the part which still consists of two or three layers of cells, and never extends to the inner part which has only a single layer of cells, and constitutes the well differentiated darm-entoblast.

We pass over for the present figs. 21 and 22, and come to the most anterior section represented (fig. 23). It is in the region of the head-fold as shown by a notch (*h.f.*) on one side in the ectoblast. The darm-entoblast, which is laterally quite thick and consists of columnar cells, is internally very thin and becomes continuous with the chorda-entoblast near the median line. From the point of junction as well as from the sides of the chorda-entoblast mesoblastic cells are budding off on each side. There is in this section a small mass of mesoblast cells outside of the head-fold which is distinct from the main mass. This isolation has been brought about by the ectoblast folding downward as the head-fold; more posteriorly the lateral mass fuses with the main mass. In this section the germinal wall

is absent, and thus no additions are made laterally to the mesoblast from the entoblast.

Returning to the middle region of the body, figs. 21 and 22 serve to show the first steps in the formation of the notochord. The chorda-entoblast which in fig. 20 passed laterally without any interruption into the mesoblast, is in fig. 21 marked off from the mesoblast, at least in the upper part. The cells at the border between the two are turned away from one another; thus the cells of the chorda are directed inwards and downwards, while the contiguous cells of the mesoblast are directed outwards and downwards. The mesoblast is still united with the darm-entoblast. As yet, the chorda is only a mass of columnar cells. In fig. 22, five sections in front of fig. 21, the chorda has become rounded in outline and considerably smaller in section. The most dorsal and median cells alone are columnar, and the remaining cells are arranged as if the more lateral cells have folded inwards and downwards from the two sides and met in the median line. The mesoblast is now distinctly separated from both the chorda and the darm-entoblast. The last abuts against the chorda, but seems separate from it. This is as far as the formation of the chorda has advanced in this stage. In front of fig. 22 the chorda becomes wider again, until in the region of the head-fold it is as represented in fig. 23; a similar arrangement is found at the posterior end of the embryo.

Fig. 24 is a longitudinal section of another embryo from the same deposit of eggs as the one represented in fig. 3. It passes very nearly in the median line. The blastoporic passage is considerably narrower than in fig. 16. Its angle of inclination to the surface of the ectoblast is now greater, approaching more nearly a right angle; hence it has become also much shorter than before. At the dorsal lip of the blastopore the ectoblast is reflected and becomes continuous with the chorda-entoblast. Owing to the fact that the chorda is most developed and therefore narrower in the middle region of the embryo than in front or behind, and perhaps also to the fact that the section is slightly oblique, the mesoblast (*mes.*)

appears for a short space ($*$—*c.*) in this section. The entoblast, which is very thick in front, especially in the head-fold (*h. f.* marked by a notch in the ectoblast), becomes suddenly reduced at the point *c* into a thin ventral layer (*end.*) which stretches posteriorly as far as the point marked with a $*$, where it seems to unite with the chorda-entoblast. From the point of junction and also continued forward from the chorda-entoblast, the mesoblast sheet stretches forwards as far as *c*, above the darm-entoblast (*end.*) and beneath the ectoblast. Behind the blastoporic passage there is a large mass of cells projecting downward (the Endwulst). On the dorsal surface, directly behind the passage, columnar cells are absent for a short space. This is the longitudinal section of the yolk-plug. Following it, the ectoblast cells appear, but cannot at first be separated from the large mesoblastic mass, for this is the region of the primitive streak where the ectoblast is giving off cells below. Very soon, however, it becomes an independent sheet. The continuation downwards of the yolk-plug forms the whole posterior wall of the blastoporic passage, and is therefore seen as its floor in cross-sections. The entoblast is continuous with it at the extreme front of the "Endwulst," but becomes a distinct layer on the ventral surface. The mesoblast, utterly indistinguishable from the yolk-plug, stretches away posteriorly. Behind the blastopore the three germinal layers are thus fused. The mesoblast, which is separate from the entoblast on the ventral surface of the "Endwulst," is receiving more posteriorly additions from the yolk or germinal wall.

In the next stage which we figure (figs. 4 *a* and *b*), the head-fold has considerably advanced, and the amnion (*am.*) covers it already so that it is not visible from the dorsal side. The medullary folds have touched each other. At the posterior end the yolk plug is included between the diverging medullary folds.

The sections through the head region of this stage show beautifully, and in an unmistakeable and conclusive manner, the mode of the formation of the mesoblast and of the chorda

dorsalis. Figs. 25 — 28 are selected to illustrate these points.

Fig. 25 is the most anterior section represented. It goes through the posterior part of the head. The amnion is closed over it, but the digestive cavity is still widely open below. The darm-entoblast formed by columnar cells does not reach the chorda-entoblast, but is separated from it by an interval where cells are most actively proliferating and giving rise to the mesoblastic mass. Fig. 29 is a similar section from another embryo of the same stage. Here also the chorda-entoblast, instead of passing directly into the darm-entoblast, is separated from it on each side by a space where cells are actively dividing and giving rise to the mesoblast. This figure shows also more naturally than fig. 25 that the mesoblastic mass consists of spindle-shaped and stellate cells arranged in such a way as to give an impression of having radiated from their origin.

Figs. 26—28 show clearly the mode of the formation of the notochord. Fig. 26 is two sections behind fig. 25. The mesoblastic masses have separated from the chorda- and darm-entoblast. The chorda-entoblast is arcuate. The darm-entoblast abuts against it but is distinctly separate from it. In fig. 27, the third section behind fig. 26, the chorda-entoblast has become a cord-like mass, against the more ventral side of which the darm-entoblast of both sides is applied. This corresponds to fig. 22 of the previous stage. In fig. 28, the third section behind fig. 27, the darm-entoblast has passed under the notochord from both sides, and united so as to form a continuous sheet across. The formation of the notochord is thus completed.

As in the previous stage, the notochord is finished only in the middle region of the embryo. Toward the posterior region, in front of the ventral opening of the blastoporic passage, the chorda is in the process of formation. The mode of formation is exactly as at the front end. Figs. 30—34 from an embryo of nearly the same stage as that represented in figs. 4 *a* and *b*, are introduced to illustrate this process.

Fig. 30 is the most posterior section given. It is slightly in front of the ventral opening of the blastoporic passage, which is still visible as a groove in the median line. The darm-entoblast (*end.*), which is distinct laterally, does not reach the chorda-entoblast, but passes into a zone from which the meso-blastic sheet spreads away laterally, and which in its turn becomes continuous with the chorda-entoblast. This corresponds to fig. 25 or fig. 29 of the anterior region, or to fig. 20 of the previous stage.

In fig. 31 the chorda-entoblast is beginning to be marked off from the mesoblast, which is, however, still united with the darm-entoblast, at least on the left side. This corresponds to fig. 21 of the previous stage.

In fig. 32 the mesoblast has become entirely separated from both the chorda- and the darm-entoblast (excepting a little spot on the left). The chorda-entoblast is now a compact mass by itself, against the sides of which the darm-entoblast is applied. This is more clearly shown on the right side than on the left. This corresponds to fig. 26 of the anterior region, and to fig. 22 of the previous stage.

In fig. 33 the darm-entoblast has passed some way under the chorda which has almost the appearance of the finished structure. This corresponds to fig. 27.

In fig. 34 the darm-entoblast has passed completely under the chorda and forms a continuous sheet across, and the formation of the notochord is finished. This corresponds to fig. 28.

The formation of the chorda at the anterior region comes to an end much earlier than in the posterior region, where it is continued on until considerably later, and where the growth in length of the embryo seems mainly to take place.

It remains now to state the fate of the blastoporic passage. In an embryo taken out two days later than that given in figs. 4 *a* and *b*, from the same deposit of eggs, in an embryo, therefore, five days old with five or six mesoblastic somites, the passage is no longer dorsally open. The medullary canal has completely closed over it and the blastoporic

passage has been changed to the neurenteric canal. Figs. 35 *a*—*d*, will show the relations of the germinal layers round the passage. In *a*, the most anterior section given, the darm-entoblast, the notochord, the mesoblast, and the medullary canal are all separate. In *b* the chorda has fused above with the walls of the medullary canal, appears for a little space in the median line on the roof of the digestive cavity, and divides the darm-entoblast of the two sides which seem to rest against it. In *c* the canal opens below into the digestive cavity. The mesoblast is now continuous with the darm-entoblast and the walls of the neurenteric canal at the junction of the two. In *d* the posterior part of the neurenteric canal has been cut. In the next section (not figured), the cells in the axial region are only more compact than elsewhere, and show that the posterior wall of the canal is reached. Thus from the mass behind the blastoporic passage (i. e. the "Endwulst"), the posterior wall of the neurenteric canal seems to have been developed in sitû. From this mass the mesoblast is extending laterally on each side. It is not possible for us to state exactly how the yolk-plug disappears. A part of it which formed the posterior wall of the blastoporic passage is no doubt changed into the posterior wall of the neurenteric canal. A part placed more dorsally is perhaps changed directly to the ectoblast of the general surface of the embryo.

In an embryo six days old, i. e. one day older than that of fig. 35, the neurenteric canal still persists. In an embryo seven days old it is no longer found. We are not in a position to state how its disappearance is brought about.

To state briefly the principal facts brought out by our observations on the formation of the mesoblast and of the notochord:

In the embryo represented in figs. 1 *a* and *b*, the mesoblast was found only in the region behind the blastoporic passage, radiating in the shape of an open fan from the posterior wall of the passage, as well as from the ectoblast along the primitive steak, and constituting the structure called the "sickle." In further course of development the mesoblast becomes extended into the region in front of the blastoporic passage.

Here it arises as a paired mass, and its point of origin is invariably at the junction of the chorda-entoblast with the darm-entoblast. In other words, one part of the mesoblast is always continuous with the chorda-entoblast, while the other part passes into the darm-entoblast.

Besides this source the mesoblast receives large contributions of cells from the germinal wall, and even from the outermost part of the darm-entoblast contiguous with the germinal wall.

The notochord is formed out of the chorda-entoblast. It is completed first in the middle, and then extends both backward and forward. Its mode of formation is the same, both in front and behind. First, at the point of the origin of the mesoblast the connection of the three structures that meet there, viz. the mesoblast, the chorda-, and the darm-entoblast, is loosened. The mesoblast is then found as two separate masses, one on each side of the median line. The darm-entoblast rests with its free edges against the sides of the chorda-entoblast; it, however, passes gradually under the chorda-entoblast, until finally the darm-entoblast of two sides fuses in the median line, and forms a continuous sheet over the digestive cavity. In the meantime the chorda-entoblast has arranged itself into the finished chorda-dorsalis.

The formation of the Blastoporic Passage.

There are differences of opinion among previous writers on the subject in regard to the formation of the blastoporic passage in Reptilia. Balfour (No. 2, p. 424-5) says: "After the segmentation and the formation of the embryonic shield (area pellucida) the blastoderm becomes distinctly divided into epiblast and hypoblast. At the hind end of the shield a somewhat triangular primitive streak is formed by the fusion of the epiblast and hypoblast, with a number of cells between them, which are probably derived from the lower rows of the segmentation cells. At the front end of the streak a passage arises, open at both extremities, leading obliquely forwards

through the epiblast to the space below the hypoblast." Here Balfour does not say how this passage arises. In his 'Comparative Embryology' (vol. ii, p. 168) he says: "At the front end of the primitive streak an epiblastic involution appears, which soon becomes extended into a passage open at both extremities, leading obliquely forwards through the epiblast to the space below the hypoblast." Kupffer (No. 5) is of substantially the same view. Weldon (No. 14, p. 136) says: "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. . . . . 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. 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. . . . . This process is evidently an invagination comparable to that which takes place in an Elasmobranch. It especially resembles the process described by Scott and Osborne in the newt." Strahl gave his views first in an article published in 1882 (No. 8), and again in a later writing (No. 13, p. 55). His views, as expressed in the latter, are briefly as follows:—Before the neurenteric canal is present the germinal disc consists throughout only of ectoblast and entoblast, except in the region of the primitive streak, which is oval or pear-shaped, or nearly triangular in form. In such a disc three processes, which may be independent of one another, now take place.

1. Under the primitive streak the entoblast is differentiated, so far as it has not done so already.

2. In the middle of the primitive streak the canalis neurentericus is sunk, at first perpendicularly below and then horizontally forward.

3. In the region of the primitive streak the ectoblast differentiates from the mesoblast. This differs in the regions before and behind the neurenteric canal. In front of the canal the whole mass is differentiated into the ectoblast and the mesoblast (i. e. mesoblast according to his views: we would call it the chorda-entoblast). In the region behind the canal, only the epidermal layer of the ectoblast is differentiated, the differentiation of the remaining cells into the structures for which they are destined: viz. the extreme end of the medullary canal, of the chorda, &c., takes place at a much later date.

As we stated before, we did not succeed in obtaining the stages earlier than fig. 1. We will try, however, to reason back from our earliest stages and to deduce what processes have given rise to such a form. Of course, such à priori reasoning is liable to mistakes, and we offer the following remarks merely as suggestions which need verification by future investigations. If the blastoporic passage really commences as an epiblastic invagination, it seems to us that Kupffer is quite right in considering the invaginated sac as the gastrula cavity much reduced in size (No. 5, p. 2). But apart from the inherent improbability that the bottom of the archenteron should afterward give way and the archenteron should become connected with some cavity beyond itself, we think we have another sufficient reason in rejecting the view of an epiblastic invagination in this fact that directly behind the passage, when it is established, there is an area which is not covered by the ectoblast, i. e. the yolk-plug. We think then that what really takes place must be very much as Weldon and Strahl describe it, for these two writers differ after all, when we leave out minor points, only in this, that the former thinks the passage arises at the front end, and the latter at the middle of the primitive streak. Our views, then, on these earliest stages are as follows:

At the end of the segmentation the blastoderm becomes divided into two primary layers, the ectoblast above consisting of columnar cells, and the entoblast below consisting of irregularly shaped cells without any definite arrangement. At the region of the future blastopore and primitive streak, this process of differentiation is somewhat modified from what takes place elsewhere. When the differentiation of the ectoblast has proceeded backward and come to the future dorsal lip of the blastopore, it does not extend further in the median line over the blastodermic surface, but becomes reflected downward and continuous with the axial strip of the lower layer cells which acquire the columnar character from this point forward in the median line of the future embryo, and arranged themselves into the chorda-entoblast. This process has proceeded to the front end of the embryonic shield in the embryo represented in fig. 1. Whether there is any actual invagination of cells from the dorsal lip of the blastopore we cannot tell, but this is of no moment so long as the ectoblast becomes continuous with the axial strip of the entoblast at the dorsal lip, and the arrangement of the lower layer cells into the chorda-entoblast proceeds from here towards the front. We can conceive the blastoporic passage itself arising in this way. As the cells arrange themselves into the chorda-entoblast, these columnar cells separate from the cells directly behind them and thus a fissure or canal is produced just at the same rate as the cells arrange themselves into the chorda-entoblast. The posterior wall of this canal would thus be composed of undifferentiated cells, as it actually is.

While the differentiation of the ectoblast thus stops, in the median line, at the dorsal lip of the blastopore, and the above-mentioned changes leading to the formation of the blastoporic passage are going on, we can suppose that the differentiation of the ectoblast is at the same time proceeding actively in the more lateral parts and is extending backwards and meeting in the median line again slightly behind the blastopore (see fig. 6). There would thus be left behind the blastopore a small space not covered by the ectoblast. This is the yolk-plug, which is of course

continuous with the undifferentiated cells forming the posterior wall of the blastoporic passage. From the ectoblast in the median line behind the yolk-plug, cells begin to proliferate and constitute the primitive streak. This may happen before the blastoporic passage is completed (see Strahl, No. 8, Taf. xiv, fig. 11). Proliferation begins also from the posterior wall of the blastoporic passage. We shall then have a stage exactly like that given in figs. 1 *a* and *b*. When we make a careful study of the latter embryo, some such series of changes as we have sketched out will become an absolute necessity. Our views are in the main like those of Weldon and of Strahl, but we think we have filled in more details. Strahl, it is true, says that the passage begins in the middle of the primitive streak. We are inclined to think that in his figs. 8 and 9, Taf. xiv (No. 8), he has stages in which the differentiation of the ectoblast from the entoblast has not proceeded as far as the dorsal lip of the blastopore. In our view, the 2nd and 3rd processes given in his account have the closest relations to each other. Our hypothesis also makes what takes place in Reptilia harmonise well with the development of lower forms, especially of the Amphibia.

Discussion of the Results of our Observations.

In an article published as early as 1875 Balfour (No. 1, p. 208) states that "Amphioxus is the Vertebrate whose mode of development in its earliest stages is the simplest, and the modes of development of other Vertebrates are to be looked upon as modifications of this, due to the presence of food material in their ova." In the same article, as well as in several subsequent publications (Nos. 2 and 3), he endeavoured to work out the comparison of the vertebrate development with the idea given in the above quotation for its foundation. Above all, he has insisted that the mesoblast always arises as paired masses, one on each side of the median line, and that these two masses are to be regarded as paired diverticula of the alimentary canal. Recently O. Hertwig (No. 6), in connection with the "Cœlomtheorie" of himself and his brother, has worked

out this idea very completely in Amphibia, and has also shown, from the investigations of other workers, how the same idea could be carried out through other classes of Vertebrata. We need hardly say that our investigations most completely bear out Balfour's and Hertwig's view. In fact, the agreement between the development of Amphioxus and Amphibia on one side, and of Reptilia on the other, as shown by our work, is as complete as could be desired, when we make due allowance for the fact that on one side is a holoblastic and on the other a meroblastic egg. Let us examine more in detail.

When we compare our fig. 16 with Hertwig's fig. 4 (Taf. ii, No. 6) of Triton, we are at once struck with the close similarity between the two, allowing for the fact that the latter represents a whole egg, and the former only a small part of it. There is in both a passage connecting the cavity which becomes the future alimentary canal with the exterior. This is, according to Hertwig's nomenclature, "die enger Theil der Darmhöhle (*dh.*)," according to ours "the blastoporic passage." At the dorsal lip of this passage the ectoblast in both is reflected, and becomes continuous with the chorda-entoblast. In the region in front of the passage the embryo consists of only the ectoblast and entoblast. In both there is the yolk-plug behind the passage, and contiguous with it the two primary layers are fused, and from the fused point there stretches backward an unpaired mesoblastic mass. Hertwig's fig. 11, Taf. v, and figs. 7 and 10, Taf. vi, of Rana, are essentially alike.

Hertwig's fig. 17 (Taf. iv) is the frontal section through the line *a*—*b* of fig. 4, Taf. ii. It passes through the beginning of the unpaired mass of mesoblast. It presents an appearance very similar to our fig. 8 of the corresponding region. The ectoblast is proliferating in the median line, and giving cells to the mesoblast.

In our figure the entoblast and mesoblast are separate, but we have shown already that they become continuous further forward. Hence exactly the same relations hold in this region in Triton and Trionyx. Compare also fig. 2, Taf. vi, and fig. 5, Taf. viii, given by Hertwig of the corresponding region in Rana.

Hertwig's fig. 9, Taf. ii, is the frontal section through the line *c*—*d* of fig. 4, Taf. ii. It is substantially the same as our fig. 9, although there is a closer resemblance between it and our fig. 18, as we have already shown.

Unfortunately Hertwig does not give a cross section of the front region of an embryo which has not yet developed the mesoblast; but we are sure it will be essentially like our figs. 13 and 14, although we cannot expect to find the lateral parts composed of a network of cells.

Now, as to the origin of the mesoblast, our results agree with Hertwig's account as completely as could be desired. In the region behind the blastopore he says the mesoblast arises as an unpaired mass in the Amphibia. Such is the case with Trionyx, as shown in our figs. 7, 8,16, and 24. In front of the blastopore the mesoblast arises as paired masses separated from each other in the median line by the chorda-entoblast. For this point compare our figs. 17 and 20, or, best of all, figs. 25 and 29, with Hertwig's figs. 1 and 2 (Taf. iii) of Triton. In the latter the chorda-entoblast passes into the parietal layer of the mesoblast, while the darm-entoblast is reflected just where it abuts against the chorda-entoblast, and passes into the visceral layer of the mesoblast, thus constituting what amounts to a pair of diverticula from the alimentary canal, one on each side of the chorda, repeating what is seen in Amphioxus. Hertwig has marked the entrance to these rudimentary diverticula with a star (*) in his figures. We have also marked in our figures what we consider to be the corresponding spots with the same mark (*). We think that morphologists will not find any difficulty in recognising in Trionyx the relations closely similar to those in Amphibia. In Trionyx the mesoblastic mass becomes continuous on each side with the entoblast, just at the point where the chorda- and the darm-entoblast meet each other. The cells being much smaller in Trionyx than in Triton, it is not possible to distinguish the parietal from the visceral layer of the mesoblast; but if both the chorda- and the darm-entoblast pass into the mesoblastic mass, the relations found here amount to the same thing as

found in Triton and in Amphioxus. We think our figs. 25 and 29 ought to convince the most sceptical on this point. It is significant that at one time (fig. 17) the chorda-entoblast occupies a recess of the alimentary canal by itself, and from the two sides of this recess the mesoblastic masses stretch out—a relation which recalls vividly the development of Amphioxus.

Our fig. 19 may prove a stumbling block to some in the way of comparison with Amphibia. But we think this figure is soon reduced to the general rule. We have already pointed out that the cells forming the floor of the blastoporic passage in this figure are different from those of the roof and the sides. If we consider the chorda-entoblast as extending on each side to the spot marked with the star, and this spot as corresponding with the similarly marked spot in fig. 15, Taf. iv, of Hertwig, which passes through the corresponding part of Triton, the comparison will become easy. The apparent difficulty is brought about by the cells of the floor being many layered in Trionyx.

There is another point on which we wish to touch. Although there is no doubt that the mesoblastic masses arise as what morphologically amount to diverticula of the alimentary canal, the development in Trionyx has so far changed from the primitive method that the masses no longer form an epithelium as in Amphioxus or Triton or even compact masses throughout, but at places only loose masses of spindle and stellate cells (figs. 25 and 29). This fact will, we think, answer Kölliker's objection, based upon the shape of cells in the mesoblast, against the epithelial origin of the mesoblast. (We have not access to Kölliker's original paper but take his views as given in Hertwig's paper, No. 6, p. 105). Kölliker is no doubt correct in supposing that such forms are due to very rapid proliferation.

As to the formation of the chorda, it is only necessary to compare our figs. 25—28 with Hertwig's figs. 3—6 (Taf. iii) of Triton, and figs. 8—11 (Taf. viii) of Rana, in order to be convinced of the similarity of the process in Reptilia and Amphibia. Our figs. 25 and 29 correspond with figs. 1 or 2

(Taf. iii, Hertwig) of Triton. Our fig. 26 with fig. 4 (Taf. iii) of Triton, our fig. 27 with fig. 5 (Triton), and, finally, our fig. 28 with fig. 6 (Triton).

As to the contribution to the mesoblast from the germinal wall, there is of course no equivalent in the holoblastic egg of Amphioxus or Amphibia. It seems to us that phylogenetically this source is not of much significance and is brought about wholly by adaptation. Sarasin's (No. 15) researches on the Reptilian egg have brought out the fact that new cells are added on from the yolk to the blastoderm by a process very similar to budding. Why could we not suppose that this process goes on until considerably later, and that the addition of cells to the mesoblast from the germinal wall is but the continuation of this process?

We should like to add another suggestion. In Trionyx the primitive streak is continuous with the lateral edges of the blastopore, enclosing the yolk-plug (see fig. 6). Have we not here a case where a part of the original blastopore lips has met in the median line and formed the primitive streak, while the rest of the edge of the blastopore has retained its original condition?

We think we have succeeded in showing that the development of Reptilia harmonises completely with that of Amphibia. Our observations confirm the conclusions which Hertwig formed in regard to the Reptilian development, basing his judgment on the observations of other workers (No. 6, Theil ii), but we hope we have filled in many details not before noticed. We dissent strongly from Strahl, who in two separate publications (Nos. 11 and 13) oppose Hertwig's views. We think Strahl is singularly unfortunate in the interpretation of his sections.

We think it hardly necessary to go over other papers on the germinal layers of Reptilia (Strahl, Nos. 7, 8, 9, 10, 11, 12, 13; Kupffer, No. 5; Weldon, No. 14; Hoffmann, No. 16), and point out the points of similarity and dissimilarity between those workers and ourselves. The reader must refer to the original papers themselves.

We conclude, expressing the hope that our investigations will furnish a necessary intermediate step in establishing firmly the views of Balfour and Hertwig in higher Vertebrates.

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EXPLANATION OF PLATES II, III, IV, and V,

Illustrating Mr. K. Mitsukuri's Paper on "The Formation of the Germinal Layers in Chelonia."

List of Reference Letters.

a. o. Area opaca. *a. p.* Area pellucida. *am.* Amnion. *a.* Line along which nuclei are specially heaped up in the germinal wall. Figs. 18 and 20.—*bl.* Blastopore. *bl. p.* Blastoporic passage. *ch.* Notochord. *ect.* Ectoblast. *en.* Entoblast. *enc.* Chorda entoblast. *end.* Darm entoblast. *g. w.* Germinal wall. *h.f.* Head-fold. *mes.* Mesoblast. *n.* Nuclei in the yolk. *sl.* "Sickle." *v. o.* Ventral opening of the blastoporic passage. *yk.* Yolk. *yk. c.* Yolk-corpuscles. *yk. p.* Yolk-plug. *z.* Shelf-like extension into the archenteron.

All the figures, excepting Figs. 5, 6, 14*a*, 16, and 35, have been drawn by C. Ishikawa. Figs. 1—6 and 35 have been re-drawn by M. Indō.

Figs. 1—4 have been drawn with Zeiss's A A, × 2; Figs. 7—17 with Zeiss's C C, × 2; Figs. 18—34 with Zeiss's D D, × 2; Fig. 35 with Zeiss's B B, × 2; Fig. 5 not drawn to scale; Fig. 6 is a diagram.

Fig. 1*a*.—Dorsal view of the embryonic shield from an egg just deposited.

Fig. 1*b*.—Ventral view of the same.

Fig. 2*a*.—Dorsal view of the embryonic shield from an egg laid forty-eight hours.

Fig. 2*b*.—Ventral view of another embryonic shield of the same age from the same deposit.

Fig. 3.—Dorsal view of the embryonic shield from an egg laid thirty-six hours.

Fig. 4*a*.—Dorsal view of an embryo from an egg laid three days.

Fig. 4*b*.—Ventral view of the same.

Fig. 5.—Ventral view of an embryo from an egg laid five days.

Fig. 6.—Diagram of the embryonic shield.

Figs. 7—15.—Series of transverse sections of the embryonic shield given in Figs. 1*a* and *b*, arranged from behind forward.

Fig. 7. Section of the region where the three germinal layers are free from one another.

Fig. 8. Section of the primitive streak.

Fig. 9. Section passing directly behind the blastopore.

Fig. 10. Section passing just in front of the dorsal lip of the blastopore

Fig. 11. Section through the blastoporic passage.

Fig. 12. Section passing through the posterior part of the ventral opening of the blastoporic passage.

Fig. 13. Section passing through the anterior part of the ventral opening of the blastoporic passage.

Figs. 14 and 15. Sections passing in front of the ventral opening of the blastoporic passage.

Fig. 14*a*. Section showing the shelf-like extension into the archenteron.

Fig. 16. Median longitudinal section of an embryo closely similar to Fig. 1*a* and *b*, and from the same deposit.

Fig. 17. Section passing through the ventral opening of the blastoporic passage of the embryonic shield, similar to that given in Fig. 2*a* and *b*, and from the same deposit.

Figs. 18—23.—Series of transverse sections of an embryonic shield, closely like that given in Fig. 3, and from the same deposit. Arranged from behind forward.

Fig. 18. Section passing through the lateral limbs of the horseshoe shaped blastopore.

Fig. 19. Section through the blastoporic passage.

Fig. 20. Section passing slightly in front of the ventral opening of the blastoporic passage.

Figs. 21 and 22. Sections passing through the middle region of the shield.

Fig. 23. Section passing through the region of the head-fold.

Fig. 24.—Median longitudinal section of an embryonic shield, closely like Fig. 3, and from the same deposit.

Figs. 25—28.—Series of transverse sections through the head region of the embryo represented in Figs. 4*a* and *b*, illustrating the mode of the formation of the notochord. Arranged from before backward.

Fig. 29.—Transverse section through the head region of another embryo, closely like Figs. 4*a* and *b*, from the same deposit.

Figs. 30—34.—Series of transverse sections from the posterior region of an embryo, very much like that given in Fig. 4*a* and *b*, illustrating the mode of the formation of the notochord in that region. Arranged from behind forward.

Fig. 35.—Series of transverse sections from the posterior region of an embryo, with five or six mesoblastic somites, showing the neurenteric canal. Arranged from before backward.

On the Structure and Development of the Reproductive Elements in Myxine glutinosa, L.

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With Plates VI and VII.

Historical.—The first zoologist who investigated the minute structure of the generative organs and their products in the Myxinoida was Johannes Müller. Between the years 1835 and 1845 the great Berlin naturalist published a monograph on the 'Comparative Anatomy of the Myxinoids,' the parts of which were first read before the Academy of Sciences of Berlin, and subsequently published separately in three folio volumes. The description of the reproductive system occurs in the last volume, published in 1845; the contents of this volume were communicated to the Academy in 1842. The description is brief, but as far as it goes I have found it in most respects correct. I shall here quote it almost completely.

"The sexual organs hang in a long peritoneal fold on the right side of the mesentery. The structure in Bdellostoma and Myxine is exactly the same. The structure in the two sexes is also completely similar, and it is very difficult to distinguish testis and ovary.

"The testes consist of a number of round and roundish long grains (körner), which resemble the eggs; each has an external skin like the egg skin, and a content somewhat similar

to the yolk of the egg; the substance within the testicular vesicles consists of granules of various sizes, but all smaller than the yolk granules. Spermatozoa at the time when the Myxine were examined in the fresh condition (August) were not present; they are apparently only to be observed at the breeding season. The most important difference between the testicular vesicles (hodenbläschen = körner, mentioned above) and the eggs seems to lie in the fact that in the former the germinal vesicle is wanting.

"The eggs are, when small, round; later they become much elongated, and the ripe ones are very large. I have seen them in specimens preserved in spirit as long as 6‴ (13 mm.). At the time when I examined the Myxine alive the eggs were not large. In all young eggs, besides the yolk granules, is seen the germinal vesicle, which is very distinct; it contains, besides smaller granula, two or three cells with nuclei which form the germinal spot. When the eggs have become elongated the germinal vesicle lies always at one of the thin ends of the eggs. The yolk granules are differently constructed to the granula of the testicular vesicles; the granula of the latter are much smaller and round; the yolk granules are, on the contrary, ellipsoidal, and quite similar to the yolk granules of the Elasmobranchs, i.e. they have transverse lines on the surface which indicate a differentiation of substance and recall the amylon bodies. These lines are present in the fresh condition, as in Sharks and Rays."

The following figures are given:

Taf. i, fig. 1.—The right side of the abdominal cavity of a male Myxine; the testis exposed; natural size.

Taf. ii, fig. 3.—Testicular vesicles in the mesorchium of Bdellostoma Forsteri, natural size; the vesicles are here large and distinct, and project from the edge of the mesorchium; in the former figure the separate vesicles cannot be distinguished.

Taf. ii, fig. 6.—Young round egg of Myxine fresh magnified.

Taf. ii, fig. 7.—Young egg slightly elongated.

Taf. ii, fig. 8.—Yolk granules from ripe egg of Myxine; some are free, but some contained in a spherical capsule.

Taf. ii, fig. 5.—Ripe egg from ovary of Bdellostoma Forsteri, natural size; it is 3·1 cm. in length.

The description of Müller is the only one which has ever been given of the male generative organ of Myxine, and it agrees in the main with the structure of the young testis, which I shall describe in this paper; but Müller did not understand completely the more minute structure of the organ, nor recognise the significance of what he saw. At the time when Müller's account of the female organ was written only the ovarian egg was known, and his description of it is correct except that part which refers to the structure of the germinal vesicle. He says nothing of the development of the egg or of its relation to the ovary. His figures have the same qualities as his description; they represent correctly what can be seen by ordinary dissection without minute investigation.

The next addition to our knowledge on this subject was made by Dr. Allen Thomson, in 1859. In the article "Ovum," in 'Todd's Cyclopædia of Anatomy and Physiology,' that author gives the following very scanty account of the mature ovum of Myxine:—"I have found that in the Myxine glutinosa the globular yolk is enclosed in a horny capsule of similar consistence and structure (he has been describing the ovum of Elasmobranchs), but of a simple elongated ellipsoidal shape; and in place of four terminal angular tubes there are a number of trumpet-shaped tubular processes projecting from the middle of the two ends, which probably serve the same purpose as the differently shaped appendages of the ova of the shark and skate."

He gives a figure of the egg which has been copied in most of the recent text-books and works on Ichthyology. It is correct in shape, but it represents in outline the globular ovum contained within the capsule, the former being much smaller than the latter. It is evident, from both description and figure, that Dr. Thomson was under the impression that the capsule of the ovum of Myxine, with its polar processes,

bore the same relation to the ovum as the egg capsule of oviparous Elasmobranchs, and that the two protective structures were homologous. This view has been adopted on Thomson's authority by recent authors; Balfour, for example ('Comp. Emb.'), simply refers to Thomson's description. I have not been able to ascertain whence Thomson obtained the specimen on which his description is founded. It is evident, as will be seen later, that he only examined the egg externally.

In 1862, the Academy of Sciences of Copenhagen offered a prize for an investigation which should solve the problem of the reproduction and development of Myxine. The prize was never awarded, no one having undertaken the work, but in 1863 Professor J. Steenstrup published, as a guide to any who might attempt the research, an account of a specimen of Myxine which contained ripe eggs. Professor Steenstrup began by remarking that no one seemed to have seen males, and of females only those which had young or slightly developed eggs, and that the very young and undeveloped females were as unknown as the males; that in the literature there seemed to be no record that naturalists had seen individuals of less than 8 or 9 inches long, whilst those large egg-bearing females which had previously been investigated were generally 10 to 13 inches in length. The work of Müller and Allen Thomson had apparently not come under Steenstrup's notice. The rest of Steenstrup's short paper is in substance as follows:

The females of 10 to 13 inches in length have generally been regarded as females with fully developed sexual products, not without some reason. They have had not only a large number of eggs which were larger than the ripe egg of Petromyzon, but most of them have had in addition from twelve to twenty eggs which were $\frac{1}{2}$ to $\frac{2}{3}$ inch long, and 2 to $2\frac{1}{2}$ lines broad, and these eggs, which have been situated in moniliform fashion along the margin of the ovary, have been so loosely embedded in its folds that they easily fell out into the body cavity. On account of the interest attaching to the question I made a point of collecting as far as possible all the specimens of Myxine which I could obtain, for the Zoological Museum,

and the number was not inconsiderable. In most of the female specimens which came to hand the large eggs were like cucumbers in shape, tapering to both ends, but in a few specimens the eggs were much thicker in proportion to their length, and not pointed at the ends; these, like the other kind, were arranged in a long series enclosed in the mesoarium, and easily fell out into the body cavity. Lastly, in September, 1862, a specimen was found amongst a number sent to the museum, in which some of the eggs not only had the same great thickness and ellipsoidal form, but were surrounded externally by a firmer, almost horny shell which at the ends was provided with a number of slightly curved or S-shaped horny threads. Each thread ends in a head with three or four projecting lobes or hooks, and thus has a certain resemblance to a ship's anchor. The threads remind one of those which project from the eggs of Sharks and Rays, just as the shell itself reminds one of the egg capsule in those forms. The figure here given shows both the appearance of the capsules and also the manner in which they hang in the mesoarium, together with large unripe eggs and a large number of small ones. The eggs provided with threads were entangled by means of these in the edge of the mesoarium, and with one another. Two conclusions may be drawn from this specimen; one, that the eggs must be destined to be attached by means of their threads to foreign objects or to one another; and second, that the females hitherto obtained have not been in the last stage of sexual activity. It follows from the last conclusion that the fish's known mode of life as a devourer of carcasses must be short and temporary even for the females, and is perhaps only needful until the eggs have obtained a certain stage of development, when the animals probably pass into another mode of existence.

Steenstrup's account agrees with Thomson's except in two features: first, that the former does not describe or represent a globular ovum inside the capsule, and second, that he figures one end of the capsule, forming about one fifth of the whole, detached as a kind of operculum. With reference to the

concluding part of the above account, I have to point out here that Myxine is not nearly so completely parasitic in its habits as has generally been believed. I have found that it lives for the most part concealed in soft mud, and is found in very large numbers on muddy areas of the sea-bottom. There is no direct evidence that it penetrates the bodies of living fish, and although it is often brought to the surface in the bodies of cod and haddocks which have been hooked, it is far more frequently taken on the hooks themselves. It frequently happens, as I have myself witnessed, that when a long line set for haddocks, and baited with mussel or herring, is hauled up near the mouth of the Firth of Forth, as many Myxine as haddocks are hooked, sometimes fifty specimens of the former being taken at one haul. I am in the habit of taking large numbers of Myxine in eel-pots set on muddy ground at a depth of thirty to forty fathoms off the coast of Haddingtonshire, and baited with dead herrings, cod, or haddock. Thus, whatever the reason may be why so few perfectly ripe females are taken, it is not because the animals in this condition no longer bore into the bodies of fish; though the fact might be explained by the ripe females ceasing to feed altogether.

The accounts of the egg of Myxine given by Dr. Günther in his "Study of Fishes," in the 'British Museum Catalogue,' and in his article "Ichthyology" in the 'Encyclopædia Britannica,' 9th edition, are derived from the paper of Steenstrup above quoted.

In Robert Collett's 'Norges Fiske,' which forms the supplementary volume to the 'Vidensk. Selsk. Forh.' of Christiania for 1874, and was itself published in 1875, mention was made of the distribution of Myxine, and the occurrence of its eggs. Of the latter the author says that they have been obtained by Professor Esmarck in the Christiania Fjord, and that they are often taken on soft ground off the coast of Finmark, or found in the stomachs of cod. Thinking that the eggs here referred to were eggs naturally deposited, I wrote to Mr. Collett on the subject, and in a very courteous reply he informed me that all the eggs he had seen, and to which he referred, were destitute

of the characteristic polar threads; those obtained by Professor Esmarck having been taken from the ovary of the female, and those he himself obtained from Finmark having been given to him by Mr. Buck, of Oxfjord, and also probably coming from immature females. On applying to Mr. Buck himself I was informed by him that he had only obtained the eggs from the ovary of the female.

The only specimens of the ripe egg in the hands of naturalists are those obtained by Professor Steenstrup, as above described, which are now in the Copenhagen Museum, and a single specimen, which is in the Anatomical Museum of Edinburgh University. By the kindness of Professor Sir William Turner I have had the privilege of examining the latter, but as it is a unique specimen I was not able to cut it so as to examine its structure. The specimen is represented in Plate VI, fig 1, of the natural size. Externally it agrees with the figures of Thomson and Steenstrup, except that it has no indication of any separation of a part of the capsule to form such an operculum as shown in the figure given by the latter author. The length of the ovum is 25 mm., of the threads about 5 mm. The capsule or membrane enclosing the ovum is thicker at the poles than elsewhere, and the thickened portion can be seen to be transparent, as shown in the figure. At each pole of the ovum there is a slight conical projection, to which the polar threads are attached. One of these projections, the upper in the figure, is larger than the other, and it is beneath this larger projection that the protoplasmic disc is situated—a fact which I infer from my study of the unripe eggs. I was allowed to cut off a couple of threads for microscopic examination. One of these is represented in fig. 1, *a*. The thread is solid, and not tubular; its structure, as seen under a low power, appears homogeneous, and in my investigation of the development of the threads I have found no indication that they are tubular in any stage of growth. The statement of Allen Thomson on this point is therefore erroneous, and doubtless due to his preconceived opinion that the threads were homologous with the processes of the egg capsule in Elasmobranchs.

The history of the specimen of the ripe ovum in Professor Turner's museum cannot, unfortunately, now be traced. It is described in the catalogue as "egg of Myxine, enclosed in its horny capsule, with its terminal openings surrounded by processes." This entry was made by Dr. Spencer Cobbold, but that gentleman informed me that he received no account from Professor Goodsir of the history of the specimen. The terminal openings mentioned do not exist. Though there is a micropyle at the protoplasmic pole it is doubtful if this had been seen by Professor Goodsir. Tradition says that the specimen was obtained by Mr. Shirley, formerly assistant in the Anatomical Museum, and was by him dredged up from the sea-bottom. It is certain that it was brought to the museum in the time of Professor John Goodsir, but no record of its origin is now to be discovered. It is possible that Dr. Allen Thomson's account was founded on this Edinburgh specimen, as I have not been able to find any indication that mature ova of Myxine ever existed in Glasgow, where Thomson was professor.

It is much to be deplored that Dr. Thomson did not give a complete account of the sources of his knowledge of the Myxine ovum. I am inclined to think that the Edinburgh specimen was taken from a mature female, like Steenstrup's, as I have vainly dredged for the deposited eggs with much perseverance in places where the animals were extremely abundant.

The portions of the literature on the subject in Danish and Norwegian were translated for me by my friend Mr. W. E. Hoyle, M.A., of the "Challenger" Commission Office, and to him, as well as to Professor Turner and the several zoologists who have given most courteous attention to inquiries concerning Myxine which I addressed to them, I have much pleasure in expressing here my heartiest thanks.

Course of the Research.—Since last June I have made systematic efforts to obtain the ripe generative products of Myxine, and as the animals were obtained with considerable ease in large numbers in the neighbourhood of the Firth of Forth I had some reason to hope for success. I have conveyed a number of living and well-grown specimens to the aquarium of

the Scottish Marine Station, and some of these have lived there for six months; but they refused to feed, and probably in consequence of this their sexual organs have not developed to the mature condition. I was able to observe the normal mode of life of the creatures when at rest and not seeking food. They lie with their bodies entirely buried in soft mud, with the exception of the extreme tip of the snout, and in this condition respiration is carried on by means of a current of water, which enters at the nostril, passes into the pharynx, and after traversing the gill-sacs escapes by the two branchial apertures situated immediately in front of the liver. This current is rendered evident by the movement of the particles of mud caused on the escape of the water from the latter openings. I have also obtained a large number of specimens of Myxine from fishermen and by means of my own excursions, and as I failed to obtain ripe eggs or ripe spermatozoa I set myself to try and elucidate the nature and development of the ova and spermatozoa by the minute investigation of the immature organs.

Female Organs.—To deal with the ovaries first, careful examination shows that the largest ovarian eggs are situated nearest to the attached border of the mesoarium. The ovary of Myxine agrees in structure and relations with that of other fishes very closely; its chief peculiarities are two in number. Firstly, it is extremely thin from side to side, the edge where the eggs are produced, forming a border only slightly thicker than the mesoarium with which it is continuous: there is no distinct boundary between ovary and mesoarium. Secondly, the mesoarium is attached, not to the back of the body cavity, but along the line of attachment of the mesentery with the straight intestine. The eggs are produced at the free edge of the ovary, which is covered by a thin epithelium; and the eggs are produced from this germinal epithelium in the same way as in other Vertebrates, and are surrounded after their separation by a follicle consisting of a connective-tissue capsule, and a follicular epithelium. I have not attempted to ascertain whether the cells of the follicular epithelium are derived from

the germinal epithelium or produced otherwise; the former method is that believed to obtain by the best authorities in all Vertebrates, and I have no evidence against its occurrence in Myxine. As the eggs grow larger by the accumulation of yolk they pass inwards towards the attached border of the mesoarium, the largest and oldest being always the most internal. These large eggs appear when a specimen is first opened to hang from the edge of the ovary, but examination of the organ in liquid shows at once their true relations. The mesoarium is continuous with the connective-tissue sheath of the follicle in the largest ovarian egg, as well as in the small ones, along a line which passes round the longest circumference of the ellipsoid formed by the follicle, and the transition between the two structures is abrupt; the mesoarium in the immediate neighbourhood of its attachment to the follicle is as thin as elsewhere and is easily torn, so that the larger eggs are easily separated, follicle and all, from the ovary when the animal is roughly handled. The weight of the large egg causes the mesoarium to be stretched, and each egg hangs down beyond the edge of the ovary, seeming at first sight to be enclosed in a bag formed by part of the mesoarium. But the relation of the two is always as I have described above; and it does not differ from the relation between egg follicle and ovarian stroma in Elasmobranchs and other Vertebrates except in the contrast in thickness between the ovarian egg and the surrounding portion of the ovary. In order to ascertain the structure of the follicle and egg membranes I cut series of sections through the polar portions of the largest ovarian eggs I could find. Fig. 2 represents the appearance of one of these sections passing exactly through the pole of the egg. The egg from which the section was taken was 16 mm. in length, and neither by external examination nor from the sections could any trace of the polar threads be discovered. In the section the thickest and most external layer is the connective-tissue capsule (*a*), composed of very thin interlaced fibrils with numerous small nuclei. This layer is disposed in laminæ parallel to the surface of the follicle, and in it are numerous small elongated spaces, some of which

are blood-vessels. The connective-tissue layer passes off into the thin flat mesoarium (*me.*). On the surfaces of the latter, as well as on the outer surface of the follicle, there is doubtless a thin flat epithelium, but this is so indistinctly differentiated from the connective tissue that it does not show itself in sections except by its nuclei. Within the connective-tissue layer is the epithelium of the follicle (*b*). This epithelium is composed of elongated cells disposed with their axes perpendicular to the surface of the epithelium. There are several layers of these cells as shown in the figure, but the layers are not regularly arranged, in some places three, in others four or five nuclei succeeding one another in a radial direction. At the exact pole of the egg there is a differentiated portion of epithelium, where a proliferation of the latter has taken place. This portion is composed of polygonal cells which are little or not at all elongated, and towards the egg it runs out into a thin cylindrical process which penetrates the next layer, as shown at *e.p.* The next layer (*c*) is thin and membranous. In the living egg it is doubtless in contact with the epithelium, and the separation between the two shown in the figure has been produced by the action of the hardening reagents employed in the preparation of the egg. This layer as shown in the figure appears under a low power single and homogeneous, and it is in immediate contact with the substance of the ovum proper, or, as it is sometimes called, the vitellus. The polar portion of the vitellus which is in immediate contact with the membrane (*c*) is granular in structure, stains well, and is protoplasmic in nature. In this protoplasmic cap is found the germinal vesicle, shown in some of the other figures. Beneath the membrane (*c*) at other parts of the ovum there is no separate protoplasmic layer, the yolk-discs extending to the inner surface of the membrane. The protoplasmic cap with its germinal vesicle forms thus a germinal disc similar to that found in the bird's ovum, and other meroblastic vertebrate ova. The rest of the ovum is composed principally of yolk elements, the elliptical vitelline discs show in the figure. The nature of the membrane (*c*) must here be particularly consi-

dered; we can obtain some probable conclusions concerning it by referring to what is known concerning the egg membranes in other Vertebrata. The account which Balfour gives in his 'Comp. Emb.,' vol. i, of the egg membranes in Craniata, is as follows. There are three membranes which may all coexist, or one or two only may be present. These are:

1. An outermost homogeneous membrane without striæ or fine pores, by most authors regarded as a chorion (i. e. as produced by the follicular epithelium), but by Balfour as a vitelline membrane (i. e. as produced by the ovum itself).

2. A radiately striated membrane, internal to the former when the two coexist, which can be broken up into a series of separate columns. These give to the membrane its radiate striation, but it is probable that there are fine pores between the columns. This membrane is the zona radiata of most authors. It is a differentiation of the outermost layer of the yolk.

3. Within the zona radiata a third and delicate membrane is occasionally found, especially when the ovum is approaching maturity.

According to Balfour, the first membrane to be formed in Elasmobranchs is the vitelline membrane, the first of the three above defined; this appears in some instances before the formation of the follicle, a fact which appears to show that it is really formed as a differentiation of the protoplasm of the egg. In Elasmobranchs this membrane attains a very considerable development. A zona radiata is generally if not always present in Elasmobranchii, but arises later than the vitelline membrane. The zona radiata always disappears long before the ovum is ripe. The vitelline membrane also gradually atrophies though it lasts much longer than the zona radiata. When the egg is taken up by the oviduct, all trace of both membranes has disappeared.

Is there any evidence to show whether the membrane (*c*) in the Myxine ovum owes its existence to the ovum itself or to the follicular epithelium? The only evidence to which I will point at present is that the follicular epithelium is very much thicker

at the poles of the follicle than in the equatorial region, and the membrane in question varies in thickness with the epithelium. I shall recur to the question of the nature and origin of the membrane further on. In this paper I shall call the membrane the vitelline membrane, using that term to include the whole of the primary egg membranes produced within the follicle. The term chorion will not be used, as its application in the case of mammals to a membrane which is partly derived from the blastoderm renders it unsuitable in connection with primary egg membranes. The term vitelline membrane, as used in this paper, implies no assertion as to derivation from follicular epithelium on the one hand, or ovum on the other. In Myxine, as will be conclusively shown in the course of what follows, the vitelline membrane forms the sole protective covering of the deposited ovum.

It is now necessary to trace the destiny and elucidate the significance of the process from the follicular epithelium above described. In the sections succeeding the one shown in fig. 2 this cylindrical process is seen to penetrate the vitelline membrane, occupying a tubular cavity in the latter, and passing through it to form a hemispherical projection on its inner surface. This tubular aperture in the vitelline membrane, with its contained epithelial cells, is shown in fig. 3, as seen under a high power. The section lies almost in the plane of the canal, and so exposes nearly the whole of its cellular contents, including the hemispherical projection surrounded by the protoplasm of the germinal disc. This cellular projection is covered by a thin membrane continuous with the vitelline membrane, and is not in immediate contact with the germinal disc. In fig. 3 the outer end of the canal, that towards the follicular epithelium, is closed, owing to the direction of the plane of the section, but in previous sections, as stated above, the cellular cylinder filling up the canal is seen to be continuous with the process projecting from the surface of the follicular epithelium. The structures now described, as I have convinced myself by a series of sections from more than one egg, exist only at one pole of the ovarian ovum. There is thus at one pole of the

nearly ripe ovum a tubular canal extending through the chorion, but not open internally, filled up by a cylinder of cells projecting from the follicular epithelium. It is evident, on consideration of the above facts, that this aperture is to form the micropyle in the ripe ovum, and we have here, as will be explained more fully below, the explanation of the process of fertilisation in the ovum of Myxine. It is very improbable that spermatozoa could penetrate such a thick dense capsule as is formed by the vitelline membrane in the ripe ovum, and thus the presence of a micropyle is necessary. Another point of some interest in this connection is that we have here for the first time the complete history of the origin and formation of the micropyle in a vertebrate ovum. A micropyle is known to exist in many Teleostean ova, but little investigation has been made as to how the structure is produced in the course of the ovarian development of the ovum.

In a paper which has recently come into my hands ("Studien über das Ei," 'Mémoires de l'Acad. Imp. St. Peters.,' 1885), Ph. Owsjannikow describes some observations on the micropyle in the ovum of Osmerus eperlanus, the comparison of which with my description and figures is very interesting. The eggs studied by Owsjannikow were not naturally shed, but taken when almost ripe from the ovary. In this condition the layer of follicular epithelium, or granulosa, as the Russian investigator calls it, is frequently found attached to the egg membrane. The egg membrane consists of two layers, called zona radiata externa and zona radiata interna. A micropyle pierces both of these layers, and is expanded like a funnel externally. A conical projection from the granulosa was seen to extend into the micropyle. This conical projection was not solid as in the case of Myxine, but hollow, forming a lining to the micropyle. A thin thread was seen to extend from the end of the funnel-shaped opening in the external zona, through the internal micropyle, which was a narrow canal in the internal zona. A row of granules was also seen to extend from the inner end of the internal micropyle into the vitellus. Concerning the thread and row of granules Owsjannikow gives no

conclusion as to their nature; he describes the projection from the granulosa as extending only through the external micropyle as far as the inner surface of the external zona. But it seems to me extremely probable that the thin thread and row of granules above mentioned belong to the follicular epithelium, and are originally continuous with the conical projection of the granulosa. Thus the differences between the condition in the egg of the smelt and in that of Myxine, are two: first the projection from the follicular epithelium into the micropyle in the smelt is a hollow cone, not a solid one as in Myxine; secondly, the inner end of the micropyle in the smelt is open. With regard to the latter point, the micropyle is completely open in the later stages of the egg of Myxine, and whether the micropyle is at first closed internally in Osmerus is not known. It is probable that careful investigation would show that in all Teleosteans whose ova possess a micropyle that structure is produced by a projection of cells from the follicular epithelium.

It is at least possible that in all Vertebrates the micropyle will be found on investigation to be produced in the same way as in Myxine, namely, by the growth of a cellular process from the follicular epithelium towards the vitellus while the vitelline membrane is being formed.

Amongst some specimens of Myxine sent to me in December last I found some in which the ovarian eggs appeared older than any I had examined before, the poles being more obtuse and the transverse diameter greater than usual. These eggs were 20 mm. in length. I cut series of sections through the polar regions of some of these eggs, and one of these sections passing through the protoplasmic pole is shown in fig. 4. Several differences are seen from this figure to exist between the follicle of these older eggs and that shown in fig. 2. The connective-tissue envelope is thinner; the follicular epithelium has its cells arranged somewhat differently, the nuclei being crowded towards the deeper surface. But the greatest difference of all is the presence in the epithelium of deep pits expanded at the bottom, and corresponding to these, processes from the

vitelline membrane. In the figure a wide interval is shown between the vitelline membrane with its processes and the epithelium with its pits, but as in the former case, there can be no doubt that this is merely a result of the process of preparation; in the natural condition the membrane was in contact with the epithelium, and its processes filled up completely the pits in the latter. It is rather curious that the epithelium thins out very much over the tops of the processes, and this would seem to be in opposition to the belief that the vitelline membrane, processes and all, is due to the secretory activity of the epithelium; but it is probable that the formation of the processes takes place at the sides where the epithelium is very thick, and that they are pushed up by growth at the base. In these processes, rudimentary as they are, it is not difficult to recognise the early stages of the polar threads which distinguish the ripe deposited egg of Myxine. Here, then, at last we have the explanation of these processes, which have hitherto received no interpretation except the erroneous one of Allen Thomson. The capsule enclosing the ripe ovum is undoubtedly the vitelline membrane, and the polar threads are processes from this. I have already pointed out that I have ascertained by actual examination that these processes are not tubular, as Allen Thomson supposed, but solid. Thus, there is no homology or comparison possible between the capsule of the egg of Myxine with its polar threads, and the horny capsule in which the eggs of oviparous Elasmobranchs are enclosed. The Elasmobranch capsule is produced by a special gland in the oviduct, and the fact that Myxine has no oviduct might alone have prevented the comparison which was instituted by Dr. Thomson, and which so many authors have repeated. Referring, again, to fig. 4, it is seen that the vitelline membrane has much increased in thickness since the stage shown in fig. 2. The proliferation and differentiation of cells at the pole in the follicular epithelium has disappeared, but the cylinder of cells, though reduced in size, still remains in the micropyle, and is evidently destined to keep the latter open until the maturation of the ovum is complete. Close beneath the

micropyle, in the section passing actually through the pole, is seen the germinal vesicle, which is large and spherical in shape, and therefore circular in section. It is composed of a thin membrane containing fibrils and granules, but no conspicuous germinal spot. Its position and appearance are seen in the figure.

On January 29th of the present year I obtained specimens of Myxine in which the development of the eggs had proceeded so far that the growth of the polar threads produced an effect on the external appearance of the follicle. At each end of the follicle was a slight protuberance, which was considerably larger at one end than the other. On the surface of the protuberance were minute rounded elevations, evidently due to the presence of the threads beneath. One of these eggs contained in its follicle is represented of the natural size in fig. 11. Its length is about 21 mm., breadth 7 mm. Fig. 12 shows a section through the pole of that end of the ovum distinguished by the larger protuberance. The protuberance is seen to consist of the connective-tissue layer, the processes, and the thickened follicular epithelium and vitelline membrane in their neighbourhood. The thickness of the membrane close to the base of one of the threads is ·29 mm., of the follicular epithelium at its thickest parts ·30 mm. The connective-tissue layer is thinner than at the stages already described, and is especially reduced above the terminations of the polar processes; at the point marked *x* this layer was only ·02 mm. in thickness; at the pole *y* it was ·5 mm. thick. The germinal vesicle and germinal disc are not altered in structure. The micropyle is somewhat narrower, and the cells present in it at previous stages have disappeared almost completely, only a little débris remaining. The micropyle seemed also in these ova to be open internally, though of this point I am not absolutely certain. If there is a membrane closing the inner end it is an extremely thin one. The vitelline membrane and the follicular epithelium are both much increased in thickness, as will be evident from a comparison between figs. 12 and 4; the latter is magnified fifty times, the former only thirty-five

This increased thickness is more pronounced at the polar region of the egg than elsewhere, but the two layers are thickened to some degree over the whole surface of the ovum. The structure of the prominence at the opposite pole of the ovum is similar except that the growth of the threads is not so far advanced, and of course micropyle, germinal vesicle, and germinal disc are absent. At the germinal pole a circular depression is present in the external surface of the vitelline membrane, above the micropyle, as shown in fig. 12. For the sake of absolute accuracy of statement I must mention that in the section from which fig. 12 was drawn the same separation between chorion and follicular epithelium, due to the action of the hardening reagents, was present, as is shown in figs. 2 and 4. The length of the specimen from which the eggs I have described were taken was about 15 inches. It was taken with several others on haddock lines twenty-four miles south-south-east of the Isle of May. There were a few other specimens in the same lot in which the largest ovarian eggs showed polar protuberances due to the presence of the chorionic processes, but the description I have given is taken from the specimen in which the protuberances were most developed.

It is necessary now to consider more minutely the structure of the vitelline membrane, and to determine, if possible, its mode of formation and its homologies. As seen under a low power it appears, as I have said, homogeneous and structureless. In the preparations from ova hardened with chromic acid and stained with borax carmine I was unable to find any structure, even with the highest powers; but in sections from ova hardened with Perennyi's fluid (chromic and nitric acid mixed) under a high power striæ perpendicular to the surface of the membrane are plainly seen. The structure thus brought to view is shown in fig. 4, *a*, taken from a preparation of the same series as fig. 4. The striæ are more deeply stained than the rest of the membrane; they are for the most part parallel to one another, but sometimes they branch into two. Several of them are moniliform. In some cases I have traced them at the external surface of the membrane, where the latter was

separated from the epithelium, into continuity with protoplasmic fibrils outside the membrane. These fibrils I believe to be in the natural condition continuous with the cells of the epithelium, and I conclude that the striæ are really pores in the membrane, which are filled up by protoplasmic processes from the cells of the follicular epithelium. A similar condition of things exists, and has been described by many authors in Teleostean ova, and has been very completely elucidated by Owsyannikow in the paper already cited. Owsyannikow obtained his most satisfactory results from examination of the egg of Perca fluviatilis. In this species the vitelline membrane consists of two layers, both of which contain pores, and are therefore called by him zona radiata externa and interna. The zona externa is in the ripe ovum gelatinous, and causes the adhesiveness of the egg membrane. I have myself examined sections of the vitelline membrane of the ovum of the cod after deposition. The pores here are quite straight and never branched, and they are somewhat further apart than those in the egg of Myxine. In the cod I could discover no separation of the membrane into two layers. It seems evident to me that the vitelline membrane in the ovum of Myxine, which is a zona radiata in the usual sense of the term, is homologous with the zona radiata in Teleostean ova. When there are two zonæ radiatæ, as in Perca fluviatilis and Osmerus eperlanus, according to Owsgannikow, these seem to be simply parts of one membrane differentiated in physical properties, but essentially similar in structure. Balfour regards the zona radiata in vertebrate ova as a differentiation of the outermost layer of the yolk. It seems to me much more probable that it is produced by the follicular epithelium. It is concluded by most authorities that the pores in the zona radiata are occupied by processes from the cells of the follicular epithelium, and that the function of these processes is to convey nutriment to the ovum. In Myxine such processes exist. The manner in which the membrane arises I conclude is as follows :—The deeper part of the elongated epithelium cells is gradually changed into the zona radiata, the

substance of the cells being partly transformed into the substance of the membrane, while threads of protoplasm, at more or less regular intervals, remain unchanged, and thus give rise to the pores of the membrane. On this hypothesis—for it is little more—any membrane which may be formed by the ovum itself is to be sought on the inner side of the zona radiata. In Myxine I have found no such membrane. I have not hitherto had an opportunity of comparing the structure of the egg follicle in Petromyzon with that in Myxine. According to Calberla the ovum in Petromyzon is surrounded by a zona radiata in two layers, both radiately striated. According to Kupffer and Benecke the outer layer is not striated. Calberla asserts the existence of a micropyle, while Kupffer and Benecke have thrown doubts on the presence of such an aperture. Judging from what I have seen in Myxine I am inclined to think that Calberla is correct, and that the condition of things in Petromyzon is exactly similar to that in Myxine, except that the zona radiata in the former is, as in Perca, divided into two layers. In the ovum of Myxine I have observed in some sections a very faint trace of a separation into two layers, and have indicated this in fig. 12, but I do not think this (in the absence of any difference in character in the two layers, and in view of the fact that the line of separation can never be traced continuously for any distance) indicates difference of origin of the two strata of the membrane. The membrane is not developed till a late stage in the growth of the ovum.

In fig. 13 is shown a section through the pole of an ovarian ovum 11 mm. in length. The germinal disc is seen to have the same characters as it has in the more mature ova, except that the germinal vesicle is elliptical in section. This latter peculiarity is probably an accident caused by the action of reagents. Under a system magnifying 50 diameters, the follicular epithelium at this stage can scarcely be distinguished, while the connective-tissue layer is very thick. On applying a higher magnifying power it is seen that no membrane of any kind exists at this stage between the follicular epithelium and the vitellus. The epithelium itself is only one cell thick, and the

cells vary in size, so that the deeper surface of the epithelium is irregular (*vide* fig. 13*a*). Amongst the specimens obtained on January 29th, I recognised for the first time a female which had recently discharged its ova. In place of the 19—25 large ova which are usually present there were a corresponding number of collapsed follicles; each of these had a slit-like aperture at one end, through which the ovum had been expelled. I found afterwards that similar "spent" specimens were present among a lot obtained on December 24th, 1885, and I have obtained them on several occasions since. Thus it is proved that the deposition of ova occurs in Myxine in the neighbourhood of the Firth of Forth during the months of December, January, February, and March. In spite of this discovery, and many zealous attempts, I have hitherto completely failed to obtain a single deposited and fertilized ovum.

Before recognising the specimens which had quite recently discharged their ova, I had frequently found corpora lutea in the ovaries of old females; and I have since observed various intermediate stages in the process of absorption of the follicles from which the ova have escaped; the last stage being that of minute yellow nodules in the mesoarium.

It is obvious from the above that the eggs described by Steenstrup had escaped from the follicles and remained in the body cavity entangled by their polar processes. The eggs may have been expelled from the follicles naturally, and not yet discharged from the body cavity; but it is more probable that the eggs were very near perfect maturity in the follicles when the animal was captured, and that the follicles were ruptured by the rough handling the specimen received from the fishermen.

The yolk-elements are of two kinds; flat, elliptical, transparent and highly refringent discs such as were described by Johannes Müller, which are doubtless albuminous, and clear spherical-lobed which are probably of a fatty nature (fig. 5). In the very young eggs these yolk-elements are not present, but only fine granules which make the ovum when viewed by transmitted light black and opaque. In still younger eggs,

such as are found in very small females, the eggs in the fresh state are spherical and quite clear, showing the germinal vesicle somewhat eccentric in position, and clear protoplasm all round. Fig. 6 represents a stained preparation from the ovary of a specimen only six inches in length, which was taken in an eel-trap, July, 1885. The free edge is marked *x*, the attached *y*; the eggs extend almost up to the attached border, the mesoarium not being yet developed.

Male Organs.—We pass now to the consideration of the male sex. It is a curious and hitherto unexplained fact that the male Myxine is very seldom taken. Among the hundreds of specimens which have passed through my hands I have only succeeded in identifying eight males, and these are all very immature. It is a matter of some difficulty to recognise the immature testes in these young males, and some few young male specimens may have escaped my recognition, as the matter requires careful examination with the microscope. But it is certain that the vast majority of the specimens which I have obtained have been females with large eggs in the ovary, eggs varying from 1 to 20 mm. in length. The same experience has been recorded by others who have occupied themselves with the subject, and until I engaged in the research no one had seen the ripe spermatozoa of Myxine. My examination of the young male convinced me that Johannes Müller was dealing in his description with specimens in a similar condition to my own, but his account of the minute structure of the testes is inadequate. His figure of the male reproductive organ in its natural size and *in situ* is perfectly correct, and on this point I must be content with a reference to his figure. The organ is similar in general arrangement to the ovary: it lies along the right side of the body, the organ of the left side not being developed, and it consists of an extremely thin flat mesorchium, with a slightly thickened free border. In this border the male elements of reproduction are produced. When a piece of the border is cut out and examined under a low power of the microscope in the fresh state the thickened border is seen to consist of connective tissue containing a number of

more or less spherical capsules, varying very much in size. By careful compression, the contents of these capsules can be brought into view, but the structure is not very distinct. When a capsule is burst by compression spherical cells are seen to escape from it. These cells have a very delicate outline; they are very transparent, and the nucleus is only to be distinguished by careful focussing. These cells vary slightly in size, the average diameter being 17 μ; their appearance in the fresh state is represented in fig. H. 7

The interior of the capsules is completely filled up by these cells, which in the fresh state are spherical while still within the capsule, but become more or less polygonal after hardening. The structure is brought out more distinctly by preparing a piece of the tissue with hardening reagents, e.g. corrosive sublimate or chromic acid, staining it, and then mounting in a transparent medium. Fig. 7 shows the appearance of such a preparation when magnified 50 diameters. The capsules in the thickened border of the testis are seen to be crowded with nuclei, but nothing further can be distinguished. Under a higher power, if the preparation has been compressed, the separate nucleated cells in the capsules can be distinguished. By cutting sections transverse to the thickened border the structure is brought clearly into view. Fig. 8 shows the appearance of such a prepared and stained section magnified 70 times. The outlines of the cells are not distinct, but they can be followed under a higher power. I concluded from the above facts that the cells in the capsules, after subdivision, were converted directly into spermatozoa, which by rupture of the capsules escaped into the body cavity of the animal. There is every reason to believe that the fertilisation in Myxine takes place outside the body.

After identifying the male organ and investigating its structure, I was surprised to find that in nearly all specimens with very immature eggs the posterior portion of the sexual organ had the same structure as the testis. This testicular portion occupies about 2 inches of the posterior end of the

sexual organ, and I have only found it in specimens in which the eggs were very small, that is, less than 4 mm. in length.

Fig. 10 shows a prepared and stained section of the male portion of the organ in such a hermaphrodite specimen, under a magnifying power of 100, and it will be seen that it agrees in all respects with the figure of a section of the testis.

In one of the hermaphrodite specimens obtained on January 29th last, I discovered, on teasing up a portion of the testicular portion of the generative organ, a number of spermatozoa, and stages of spermatogenesis. The various structures as seen in the fresh state are shown in fig. 14. The spermatozoa possess a pear-shaped head, which is very highly refringent, and has a distinct outline; round the posterior thicker end of the head is a translucent protoplasmic body, which is produced into a long tail. These spermatozoa were not moving, and were not present in vast numbers as is usually the case. The specimen from which they were taken had been dead a few hours. I have not been able to obtain a sufficient supply of material to make a complete study of the spermatogenesis; the few stages I could make out are shown in fig. 14. In some cases two spermatozoa were connected by their tails, and on the connecting thread thus produced were slight dilatations composed of clear protoplasm. In other cases a cell somewhat spherical in shape gave off two processes, one of which was the tail of a spermatozoon, while the other terminated in a point, the head of the spermatozoon belonging to the process having probably become detached in the operation of teasing. There were also seen cells in which were present one or more structures resembling the heads of the spermatozoa. These structures and the heads of the spermatozoa stain deeply in carmine; they doubtless consist of nuclear substance, but I have not been able to trace their origin from the nuclei of the cells in which they occur. It is evident that the cells and spermatozoa described were derived from the spherical cells of the testicular capsules. These cells apparently develope the heads of the spermatozoa which then grow out from the cells, trailing a thread of protoplasm which forms the tail. The curious thing about the spermatogenesis

observed in Myxine is that the spermatozoa are attached to the spermatoblast by the tails, and not by their heads, as usually occurs. I found no cases in which large numbers of spermatozoa were united in bundles such as occur in most animals; there were never more than two spermatozoa connected with one another or with a spermatoblast. The spermatozoa were contained in the capsules of the testicular tissue, and it is thus proved conclusively that the spherical cells usually found in these capsules are really spermatoblasts. In the fresh preparation from which fig. 15 was drawn, although none of the spermatozoa showed any motion, the protoplasm of the cell represented at A was slowly vibrating. In two other hermaphrodite specimens, one obtained in March, I have found a few spermatozoa, which in one case exhibited a slow vibratory movement of the tail. But I have found no more satisfactory evidence of the process of spermatogenesis. In all the cases the number of spermatozoa was not large, and it is difficult to believe that fertilisation is effected by such a meagre production of spermatozoa as was seen in the specimens I have mentioned. In no case have I discovered any trace of spermatogenesis in a specimen which was completely masculine.

In all specimens of Myxine with well-developed ovarian eggs which I have examined, with one exception, no testicular portion was present in the sexual organ. The only conclusion I can draw is that in the young state the females are nearly, but not quite always hermaphrodite, and that the testicular portion normally disappears as the eggs become more mature. It seems probable that fertilization is normally effected by hermaphrodites, since true males are so rare.

In the exceptional instance above mentioned, I found, together with large ovarian eggs in the anterior part of the sexual organ, the posterior portion, two inches in length, much thicker at the free border than either the true testis or the male part of the immature hermaphrodites. But in this portion, when examined by means of prepared and stained sections, I found, instead of the structure already described in the testis, a general cellular structure in which no definite

capsules were visible. There was a stroma of connective tissue in which were large irregular spaces containing nucleated cells, and I concluded that this was an abnormal case in which cellular hypertrophy of the testicular portion had taken place. It was a case probably in which the testicular portion present normally in the young state, had, instead of being absorbed, undergone a kind of cellular degeneration accompanied by hypertrophy.

Summary.

The firm membrane enclosing the ripe deposited ovum of Myxine is a primary egg membrane produced within the follicle.

The polar threads are processes from this membrane.

The membrane is single and not differentiated into layers: it possesses minute pores perpendicular to its surface, and is therefore a zona radiata.

The zona radiata in Myxine is homologous with the single or double zona radiata of Teleosteans and of Petromyzon.

The membrane at one pole of the ovum, that at which the protoplasmic germinal disc is situated, is perforated by a micropyle.

The micropyle is produced by a process from the follicular epithelium.

The immature testis in Myxine consists of a thickened border of the mesorchium containing more or less spherical capsules, which are filled with hyaline nucleated spermatoblasts.

A large proportion of immature Myxine are hermaphrodite, the posterior portion of the reproductive organ containing testicular capsules which have the same structure as those found in the male.

Spermatozoa have been found in the testicular portion of the hermaphrodite organ, but not in the males.

Catalogue of Literature concerning the Reproductive Organs of Myxine.

1845. Johannes Müller.—"Untersuchungen über die Eingeweide der Fische," 'Schluss der Vergleichenden Anatomie der Myxinoiden,' Berlin, 1845, pp. 4 and 5, Taf. i, Fig. 1, Taf. ii, Figs. 3, 4, 5, 6, 7, 8.

1859. Dr. Allen Thomson, Professor of Anatomy in the University of Glasgow.—Art. "Ovum," 'Todd's Cyclopædia of Anat. and Phys.,' vol. v, 1859, two figures in woodcut.

1863. Prof. Steenstrup.—"Oversigt. Dansk. Vidensk. Selsk. Forhandl." Kjbenhavn, 1863, p. 233, woodcuts.

1875. Robert Collett.—"Norges Fiske," published as Supplement to the 'Vidensk. Selsk. Forh.' of 1874, Christiania, 1875.

DESCRIPTION OF PLATES VI and VII,

Illustrating Mr. J. T. Cunningham's Paper on the "Reproductive Elements in Myxine glutinosa, L.

Fig. 1.—Specimen of the mature deposited ovum of Myxine in the Anatomical Museum of the University of Edinburgh. Nat. size.

Fig. 1, *a*.—One of the polar threads of the same specimen. Magnified 30 diam.

Fig. 2.—Section through protoplasmic pole of ovarian egg of Myxine. Length of the entire ovum, 16 mm. Plane of the section passes through longer axis of the ovum, and is perpendicular to the mesoarium. *a*. Sheath of vascular connective tissue. *b*. Follicular epithelium. *c*. The vitelline membrane. *d*. The protoplasmic disc. *ep*. The process of the follicular epithelium which passes into the micropyle. *me*. The mesoarium. Magnified 50 diam.

Fig. 3.—The micropyle in section: from an ovum of the same ovary as Fig. 2. *c*, *d*, as in preceding figure. *ep*. point to the hemispherical termination of the cellular process. Magnified 280 diam.

Fig. 4.—Section through protoplasmic pole of ovarian egg 20 mm. in length. Plane of the section as in Fig. 2. *a*, *b*, *c*, *d*. as in Fig. 2. *mi*. The micropyle, with contained cellular thread. *P*. Pits in the follicular epithelium, in which the polar processes are contained. *p*. The polar processes of the

vitelline membrane, corresponding to the pits. *g. v.* The germinal vesicle. Magnified 50 diam.

Fig. 4 *a*.—Follicular epithelium and vitelline membrane, as seen in a section of same series as Fig. 4, to show the canals in the vitelline membrane. Magnified 350 diam.

Fig. 5.—Yolk-elements from ovarian egg in fresh condition. *α*. Vitelline discs contained in spherical capsules. *β*. Similar discs free. *γ*. Spherical bodies, probably composed of fat.

Fig. 6.—Portion of ovary of young Myxine 6 inches long, stained with borax-carmine. *x*. The free, *y*. the attached border. Magnified 100 diam.

Fig. 7.—Stained preparation from testis of immature male Myxine. *ca*. Capsules full of nucleated cells (spermatoblasts). *me*. Mesorchium. *bl*. Blood-vessels. Magnified 50 diam.

Fig. 8.—Section of testis from same specimen as Fig. 7. The plane of the section is perpendicular to the thickened border of the testis. *ca*. Capsules. *me*. Mesorchium. *co*. Connective tissue. Magnified 70 diam.

Fig. 9.—Spermatoblasts of immature male Myxine, as seen in the fresh state after escape from the ruptured testicular capsules. Magnified 280 diam.

Fig. 10.—Section of testicular portion of reproductive organ of immature hermaphrodite Myxine. *ca*. and *co*. as in Fig. 8. Magnified 100 diam.

Fig. 11.—Ovarian ovum of Myxine contained in its follicle, having a projection at each pole caused by the development of the polar processes of the vitelline membrane. Natural size.

Fig. 12.—Section through the protoplasmic pole of an ovum, similar to that shown in Fig. 11. *a, b, c, d, g. v., p.*, as in Fig. 4. Magnified 35 diam.

Fig. 13.—Section through protoplasmic pole of young ovarian ovum of Myxine. Length of ovum, 11 mm. *a, b, c, d, g. v.*, as in Fig. 12. Magnified 50 diam.

Fig. 13 *a*.—Follicular epithelium and neighbouring parts from same section as Fig. 13. *a, b, d*, as before. Magnified 200 diam.

Fig. 14.—Spermatozoa, and stages of spermatogenesis, from testicular portion of reproductive organ in a hermaphrodite Myxine. Magnified 250 diam.

Studies on Earthworms. No. II.

By

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With Plates VIII and IX.

In continuation of Part II of my previous paper on this subject,[1] I shall describe three new genera belonging to Perrier's group of Intraclitelliani. Although Beddard has shown[2] that the grouping of Earthworms according to the relative position of the male pore and the clitellum, does not hold good even within the limits of one genus, yet it is a convenient classification, and, I think, may be used at present till a more satisfactory system is introduced. The genus Acanthodrilus to which I refer is post-clitellian in most of its species, but Beddard describes A. novæ-zelandiæ and other species as having the male pore situated in the clitellum.

I have received all the worms which I am about to describe from Prof. Ray Lankester, who has for many years taken every opportunity of procuring exotic specimens. The new genera show interesting combinations in one worm of characters which have been regarded as of generic value for different worms: thus Urobenus possesses intestinal glands known hitherto only in Urochæta, and also cæca similar to those which are known only in Perichæta. Again Trigaster combines an alimentary tract somewhat like that of Digaster, with the four prostates and male pores of Acanthodrilus.

Since writing my previous paper on Microchæta I have

[1] This Journal, February, 1886, p. 213.

[2] 'Proc. Zool. Soc.,' 1885, p. 810.

received some more Earthworms from Professor Lankester which belong to this genus, but differ in several points from the species there described: to the new form I have given the specific name Beddardi, as it was he who gave the name of the genus to a worm described by Rapp[1] as Lumbricus microchetus. These worms were none of them in a good condition for histological study, so that many gaps will appear in the description of their minute structures.

Microchæta Beddardi, nov. sp.

These worms were sent to Professor Lankester from Natal by Mrs. Saunders. In a preserved condition they are dark coloured, with a slightly greenish tinge and with a brown clitellum; they are rather longer but narrower than a large-sized Lumbricus agricola, being 14 or 15 inches in length, and about $\frac{1}{4}$ inch in breadth; the number of somites, 365, is easily counted owing to the soft and extended condition of the worms. They are nearly cylindrical, slightly wider anteriorly than posteriorly, with an obtuse aural region.

The prostomium is represented by a portion separated from somite I by a shallow groove, and with its anterior free edge crenated (figs. 1 and 2).

In the clitellum the intersegmental grooves are less conspicuous than elsewhere, while the nephridiopores are much more evident. The extent of the clitellum varies slightly in the six specimens in my possession, but usually occupies somites X to XXII, and in one case somite XXIII is included. (M. Rappi has a clitellum extending through somites XIII to XXV.)

The somites III to VIII inclusive are biannulated, but the remaining somites of the worm are simple.

The setæ and nephridiopores have the same arrangement as in M. Rappi, Bedd., but the relation between them is more clearly seen than in the previous species, as the setæ are extruded from the body, and the nephridiopores are now seen to be in a line with the lower setæ of the lateral couples.

There are no dorsal pores.

[1] 'Wurtemb. Naturwiss. Jahresb.,' vol. iv, 1848.

I could find no male pores, but, curiously enough, the pores of the oviduct are fairly evident. These are placed in the groove between somites XII and XIII, one on each side of the middle line. By means of a series of transverse sections I found they were the external openings of the funnel-shaped organ described and figured by me ("Studies on Earthworms," No. I, Pl. XVI, fig. 7) for M. Rappi, of the function of which I was then doubtful. I believe now that this organ is the oviduct, and that these pores are their apertures (Pl. VIII, fig. 3, *e*).

The pores of the spermathecæ differ in position from those of the former species. They lie in a line with the nephridiopores, usually one on each side, on the anterior edges of somites XI and XII; these pores I could see by the aid of a lens, after I had found the spermathecæ (fig. 3, *b*).

The Internal Anatomy.—The general anatomy agrees with that of M. Rappi, but one or two noteworthy differences are presented.

In the alimentary tract the only difference is in the shape and extent of the intestinal glands. In M. Rappi, the gland on each side has the appearance of a rounded swelling on the side of the intestine, which it partly covers dorsally, and into the lumen of which the cavity of the gland opens by a wide aperture. The gland occupies the whole length of somite IX, and only a small portion of it lies in the preceding somite (fig. 9). But in this new species the gland is bilobed, being nipped, as it were, by the septum, which divides it into two very nearly equal parts; the larger lobe lies in somite VIII, the smaller lobe in somite IX. Moreover the gland is greatly constricted off from the intestine (fig. 8) so that the communication between the two cavities is reduced to a small aperture.

The genital organs present several important differences. Instead of two pairs of seminal reservoirs and of ciliated rosettes, there is only one pair of each in this new species. The seminal reservoirs lie in somite X, and the ciliated rosettes in somite IX. In the three specimens opened the reservoirs are small, looser in structure, and more irregular than in M. Rappi; in one case only is the ciliated rosette not enclosed in

the reservoir. I think, therefore, that the worms are not quite genitally mature.

The ovaries have the same position as before,—on the posterior face of the anterior septum of somite XIII. On the opposite face of this septum, and lying therefore in the preceding somite, is the funnel of the oviduct; this is the organ marked "*x*" in the figures illustrating my paper on M. Rappi.[1]

It seems at first sight somewhat peculiar that this should be the case; the same sort of arrangement has, however, been mentioned by Beddard for Megascolex (Pleurochæta).[2] And in many sections an appearance is presented which, I think, allows no doubt that this interpretation is correct.

The oviduct has not such a folded appearance, nor is the edge fringed by narrow lobes as in M. Rappi. By following the duct through a continuous series of transverse sections I was able to trace it from its external pore up to its internal expanded funnel, which is closely attached to the septum XII—XIII, which is itself perforated (fig. 10). The appearance represented in the figure was repeated in several sections; the ovary is seen on one side of the septum lying in somite XIII, and the edge of funnel, formed of columnar cells (doubtless ciliated, though the sections do not show this) on the other side of the septum in somite XII. There is a perforation in the septum, or rather the septum is here incomplete, allowing ova to pass through it into the duct.

I was unable to find the structure which is marked "*y*" in my previous paper. Beddard has noticed a structure in a similar position in Ac. dissimilis and other species[3] repeated in the two somites in front of the true ovary, and he suggests that they may be remnants of two pairs of ovaries; indeed in one specimen one of these structures contained ova.

The spermathecæ show a remarkable and interesting difference from the arrangement seen in M. Rappi. In that species they consist of four rows of from one to four small

[1] This Journal, February, 1886, Pl. XV, fig. 4; Pl. XVI, figs. 7 and 14.

[2] 'Trans. Royal Soc. Edinb.,' xxx, 1883, p. 481.

[3] 'Proc. Zool. Soc.,' 1885, p. 827.

horse-shoe shaped bodies in the anterior region of somites XII, XIII, XIV, XV. In the present species, as a rule, only four spermathæcæ are present; a pair in each of the somites XI and XII, and each is an elongated pyriform sac (fig. 6); but in one specimen an asymmetrical condition obtains, for here there are, on one side, two pyriform sacs in each of the somites XI, XII, while on the other side there is only one spermatheca in each somite, one of which is crozier shaped (fig. 7). The wall of the spermatheca is thick and muscular; its internal lining consists of tall columnar cells. In these two species then we have a difference corresponding to that shown in Perichæta aspergillum, E. P.,[1] where there are numerous small sacs (which, however, surround a larger one), and in P. elongata, which has a simple large sac. It may be that as the worm becomes more mature a larger number of spermathecæ will appear, for these specimens are apparently not quite mature. If this were so it would certainly militate against the theory of the homology between the spermathecæ and a portion of the nephridium.

The nephridia closely resemble those of M. Rappi, but their tubular portion is less developed, except in the case of the first pair, where the coiled portion has a glandular appearance, as in so many Earthworms, and may perhaps serve in some way as a salivary gland though it has the usual nephridial structure and an external aperture (see Darwin, 'Vegetable Mould and Earthworms,' p. 42). The external pore of this pair is situated, not on the anterior edge of somite II, to which it belongs, but on a slight prominence in somite I (fig. 2).

In the vascular system of M. Rappi, the dorsal vessel is doubled in somites IV, V, VI, VII, VIII, in the last of which the walls are much thickened, so as to give a heart-shaped appearance to the vessel. In M. Beddardi, there is the same condition, but limited to somites VI, VII, VIII. Beddard has noticed a similar difference in the extent of doubling of the dorsal vessel in specimens of Ac. novæ-zelandiæ,[2] where usually the

[1] 'Nouv. Arch. de Mus. d'hist. nat. de Paris,' viii, 1872.

[2] 'Proc. Zool. Soc.,' 1885, p. 821.

vessel is doubled throughout the body, except at its passage through the septa, but in one specimen it was a single tube throughout.

Urobenus brasiliensis, nov. gen. et sp.

I have named this worm after my friend Dr. A. G. Bourne, of the Presidency College, Madras, the name being formed by transliteration. Its specific name refers to its habitat. It came, together with a species of Titanus, from Pedza açu, and was given to Prof. Lankester by Prof. Edouard van Beneden of Liège.

External Characters.—The worm is 6 inches in length, and about $\frac{1}{3}$rd of an inch in breadth; it consists of ninety-two somites, which are of nearly equal size throughout the body. The worm is cylindrical, and tapers gradually anteriorly, where it ends in a well-marked though narrow prostomium (Pl. VIII, fig. 11) which is embedded in the buccal somite only to a slight extent. The worm was soft and not much contracted, so that the somites have not the annulated appearance so frequently noticeable in Earthworms.

The clitellum is fairly well developed and does not extend completely around the body, in this respect resembling Lumbricus, Microchæta and others. The latero-ventral edge is placed between the ventral and lateral rows of setæ; the clitellum extends through somites xiv to xxv (Pl. VIII, fig. 12). The setæ are arranged in four couples in each somite—a ventral couple on each side, and a more lateral couple on each side (figs. 12 and 20); this condition holds throughout the body. The setæ themselves have the ordinary shape of an elongated *f*; the free extremity, however, is slightly more hooked than in Lumbricus; there is the usual thickening about midway along the setæ (fig 13, *a*). The setæ from the ventral series of the clitellum have their embedded portion more distinctly curved than the ordinary setæ (fig. 13, *b*). The length of the setæ is 65 mm.

The nephridiopores are placed in the anterior region of the somites in a line with the outermost setæ of the lateral couples (fig. 12, *c*).

I was unable to see either the pores of the sperm-ducts or those of the spermathecæ, but on dissection I found that the sperm-duct opened to the exterior in the anterior region of somite xx slightly dorsad of the ventral couple of setæ on each side (fig. 12, *d*).

The spermathecæ open close to the nephridiopores in the anterior region of somites vii, viii, ix, on each side.

Internal Anatomy—All the septa are thin and easily torn; the nephridia are delicate, but the spermathecæ are very prominent (fig. 14).

The alimentary canal (fig. 14) consists of six well-marked regions. The thin-walled buccal region occupies two somites, and leads into the pharynx, which extends very nearly up to the end of somite v. There is nothing specially noticeable about these parts; the muscular wall of the pharynx does not extend so far backwards on the ventral as it does on the dorsal surface. This seems a more or less usual condition in Earthworms. The œsophagus, which follows, is somewhat sacculated and is bent forwards upon itself in somite vi, and after another bend back again, enlarges in somite vii to form a proventriculus, such as is found in Lumbricus, but which is not so well marked in other Earthworms. Owing to the infundibulate septa, the œsophagus appears to extend through more somites than it actually does, but by carefully tracing the nephridia (for the septa are very thin and easily broken and therefore not reliable for the purpose) it is found that the gizzard (*e*) occupies somite viii. This has the ordinary structure with the chitinous lining within and the nacreous muscular appearance without. Behind the gizzard commences the tubular intestine (*g*), which is continued through somites ix to xv; this is narrower than the œsophagus and quite straight; it is hidden by the seminal reservoirs in the somites in which these structures lie. In each of the somites ix, x, and xi is a pair of intestinal glands (fig. 14, *f*), similar in shape to those figured by Perrier for his genus Urochæta,[1] and called by him "glandes de Morren." Each of these six

[1] 'Arch. de Zool. Experim. et gener.,' iii, 1874.

glands is a reddish, ovoid body opening into the intestine by means of a short, narrow stalk, and constricted near its free extremity in such a way that this portion has the appearance of a short cone, inverted on the end of the ovoid portion.

The structure of these glands resembles mainly that of the calciferous œsophageal glands of Lumbricus; they appear in transverse sections to be made up of a number of tubules cut across, lined by an epithelium (the nature of which could not satisfactorily be made out, owing to the condition of the worm), resting upon a basement membrane (Pl. IX, fig. 43). Between and around the tubules are large irregular blood spaces, communicating with an abundant vascular network on the surface of the gland. In the lumina of the tubules I observed oily-looking globules (*a*) of various sizes. Masses of these were found in the alimentary canal itself in the region of these glands. These globules appear to be the secretion of these glands, and from analogy with what is known in Lumbricus and Microchæta one may consider them as carbonate of lime, though I did not use any tests in this case. Similar glands occur also in Acanthodrilus; and Beddard, in his description of them,[1] says that they are made up of lamellæ of connective tissue carrying blood-vessels, which dip into the lumen of the gland. He does not say whether these lamellæ anastomose, so as to give the appearance of tubules, and from his description it would appear that in that genus they do not do so.

In somite XVI the intestine suddenly changes its character; it becomes about three times its previous size, and is deeply constricted as it passes through the septa. These constrictions of the pouched intestine (figs. 14, 16) are not merely caused by the septa, as in the sacculated region, but are due to a series of ten pairs of short and wide-mouthed pouches or cæca from the axial lumen of the intestine. There is a thickening (? glandular) along the vertical wall between two consecutive cæca (fig. 16, *c*). This pouched region extends through somites XVI to XXV.

Behind the last pouch is a rather deeper constriction, after

[1] 'Proc. Zool. Soc.'

which the canal gradually widens out again to form the typhlosolar region or sacculated intestine (fig. 14, *j*). This commences in somite XXVI, and extends for a considerable distance backwards. The wall is much thinner than in the preceding region, and it is only slightly constricted as it passes through the septa. The typhlosole commences and ends concurrently with this division of the intestine. In somite XXVI there are seen two small elongated cæca, the end of one appearing on each side of the intestine (fig. 14, *k*). These spring close together from the ventral wall of the intestine, near the middle line, just at the junction of the pouched intestine with the sacculated region, and pass outwards upwards (fig. 15, *b*). They resemble the characteristic intestinal cæca found in Perichæta, being narrow, cylindrical, with the free end rounded. This is the first time, I believe, that these cæca have been found in Intraclitellian worms, or, indeed, in other genera than Perichæta.

It is interesting to find a worm combining the two sets of intestinal outgrowths—the "glandes de Morren" found in Urochæta and these intestinal cæca of Perichæta. It would be interesting to ascertain the structure of these outgrowths, in order to compare them with that of the already known outgrowths of the alimentary tract of various Earthworms; but my specimens are not well enough preserved to show their structure satisfactorily. The typhlosole is a simple dependent fold, containing the usual blood-vessel, and is not a cylindrical valve, as in Lumbricus, Microchæta, &c.

The Genital Organs.—Of these I was able to find only some of the male organs (not the testes) and the spermathecæ; no ovaries nor oviducts were distinguishable. The seminal reservoirs are four in number, one pair lying in somite XII and XIII, and the second pair in somite XIV (fig. 17, *g*); they have a looser structure than those of Lumbricus, and a much more irregular outline; they are not so intimately connected with the septa as in the latter form, and so are easily displaced and broken.

The ciliated rosettes are, similarly, four in number, are

comparatively large, and lie quite freely in their somites. One pair lies in somite XII, the other pair in somite XIII (fig. 17, *h*, *j*). Apparently, therefore, the posterior ciliated rosette on each side lies in front of its seminal reservoir. Perrier has described a similar condition in Pontodrilus, and Beddard for Acanthodrilus dissimilis, but in these cases the first rosette is in front of the seminal reservoir. It seems to me that, at any rate in Urobenus, this condition is due to the immaturity of the worm; for in Lumbricus agricola the seminal reservoirs appear at first as outgrowths from the septa anteriorly and posteriorly in the case of the first pair, or posteriorly only in the case of the second pair, without any connection between those of the two sides in the somite in which the ciliated rosettes lie, so that if the worm be dissected in an immature condition it would present somewhat the appearance seen in Urobenus.

Similarly, the freedom of the ciliated rosettes seems to have the same explanation, since these also are free in Lumbricus in the above-mentioned condition, but, as is well known, become, in the fully mature worm, completely enclosed in the seminal reservoirs. I have mentioned the same condition in Microchæta Beddardi.

A sperm-duct passes from each of the ciliated rosettes to the body wall; here it turns backwards, and the two sperm-ducts on each side unite in somite XIII, and the common duct thus formed runs along the body wall to somite XX, where it opens to the exterior in the anterior region of the somite, between the ventral and lateral couples of setæ.

There are no accessory glands on the sperm-duct, nor copulatory papillæ on the exterior.

The only female organs which I could find are the three pairs of spermathecæ (fig. 17, *f*). Each spermatheca is a white, elongated, pyriform sac, the free extremity of which is rounded, while the opposite extremity gradually narrows to form a delicate duct; it is bent upon itself once or twice in its course, and opens to the exterior in the anterior region of the somite, quite close to, but distinctly separated from, the

nephridiopore. These spermathecæ are placed in the somites VII, VIII, and IX.

The nephridia occur in every somite behind the second (fig. 17, *n*, *n'*). Those of the seven anterior somites differ slightly from those in the rest of the somites. An ordinary nephridium, taken from a somite behind somite IX, consists of a delicate, loosely-coiled, short tubule, containing two, or in some parts three, parallel lumina; the lumen communicates, on the one hand, with the cœlom by means of the funnel, and on the other with a vesicular diverticulum, which opens to the exterior (fig. 18). This vesicle is a long, thin-walled sac, the free blind extremity of which, directed dorsally, is rounded; while the narrow duct, after receiving the tubule of the nephridium, dips into the body wall and opens to the exterior by the nephridiopore, placed in a line with the lateral setæ (fig. 20, *o*). In the preserved condition each of these vesicles contained, at their blind end, a mass of white granular substance, rendering the vesicles very conspicuous on opening the worm. The structure of the tubule and the shape of the funnel (fig. 19) closely resemble those of Lumbricus.

The nephridia behind somite XX are smaller, and the tubules even less coiled than in the one just described.

The nephridia in somites III to IX present a somewhat different arrangement of their parts (fig. 17 n^1—n^7). The tubule is less developed than in the posterior nephridia, and instead of opening into the neck of the vesicle near the external pore it enters the enlarged portion of the vesicle near its blind end. These seven nephridia have very long narrow vesicles, since the tubular portion is carried far back, and lies (as shown in fig. 14, *l*) alongside of, and partly above, the œsophagus and gizzard. A similar difference between the most anterior and the following nephridia is seen in the case of Microchæta Rappi.

Pyriform Sacs.—In each of the somites behind the ninth, i. e. behind the last pair of spermathecæ, there is a pair of small pyriform sacs (fig. 17, *p*) placed between the nerve-cord and the ventral setæ. Each of these has its enlarged free end

filled with a white granular substance similar to that found in the nephridial vesicles; this rounded end is directed outwards, whilst the narrow duct passes at first inwards, then bends backwards and pierces the body wall. By means of transverse sections the external aperture of these organs is found to be situated between the nerve-cord and the ventral couple of setæ on each side (fig. 20, *b*). They were not sufficiently well preserved to make out any details of their structure, but, so far as I could make out, their general structure is as follows:— A delicate membrane surrounds the sac (fig. 21, *a*) and forms its wall, within which is a granular substance (*b*) which was stained only slightly by borax-carmine. In the centre of this substance is an irregular lumen (*d*) lined by short columnar cells (*c*) whose nuclei stained deeply; whether these are ciliated or not I was unable to determine.

The diagram (fig. 20) shows the position in relation to the setæ of this pyriform sac and its pore on one side, and the nephridiopore on the other side.

These pyriform sacs seem quite similar in shape and position to those found only in the posterior region of the body in Urochæta; what their function may be seems quite impossible to say at present.

The vascular system was not followed to any great extent. The dorsal trunk (fig. 14, *p*) is ampullate posteriorly and becomes wider in the region of the pouched intestine; passing forwards this character becomes more marked in somites XIII and forwards. In the intestinal region a pair of vessels is given off in each somite to the intestine. In each of the somites XII and XIII is a pair of moniliform lateral hearts, but anteriorly to these somites these hearts are very thin and no longer moniliform; they pass from the dorsal to the ventral trunk. Beside the dorsal and ventral trunks both a typhlosolar and a subneural trunk are present.

The nervous system exhibits no essential difference from that of Lumbricus. The cerebral ganglia are distinct and placed in somite III.

Thus Urobenus resembles Urochæta in two remarkable

points viz. in the possession of similar intestinal glands, and of pyriform sacs. These led me at first to think that I had to deal with an Urochæta, but the various points of difference —e. g. the setæ, which in Perrier's form are notched at the free extremity, the nephridia, the character of the seminal reservoirs, which are elongated and tongue shaped in Urochæta, the presence of a distinct prostomium, which is not the case in Perrier's worm, and the general character of the alimentary tract,—all these lead me to form a new genus for the worm under consideration.

Diachæta Thomasii, nov. gen. et. sp.

In a bottle containing several small worms from St. Thomas (West Indies) some were very noticeable on account of their stoutness and of the arrangement of the setæ ; this latter peculiarity suggested to me the name Diachæta, since the setæ are far removed from each other as in Plutellus and Ac. multiporus, and moreover alternate from somite to somite, as in the posterior region of Urochæta, and in Schmarda's genus Pontoscolex.

External Anatomy.—The worm is cylindrical with very obtuse rounded extremities and is greatly swollen anteriorly, instead of narrowing towards the prostomium (Pl. VIII, fig. 22). Its length is about 3 inches, and its breadth about $\frac{1}{3}$rd inch, but the worm was very much contracted so that these measurements will not represent its size when living. The body consists of 335 somites, of which those behind the clitellum are very short, whilst in the preclitellar region they are of much greater length ; some of these anterior ones are traversed by two grooves with a slight ridge between them, on which the setæ are placed (fig. 22).

The prostomium is absent, and the anterior border of the buccal somite is not much narrower than the clitellum. It is marked by numerous longitudinal grooves extending backwards for about half the length of the somite.

The clitellum extends through somites xx to xxxiii, and completely surrounds the body (fig. 24), as in Perichæta,

Digaster, &c. The intersegmental grooves in this region are very deep and wide, and the setæ, except those of the ventral row, are not very evident.

Behind the clitellum the body of the worm becomes much narrower and retains this diameter to the end of the body. The five somites immediately in front of the clitellum are very short, and the diameter of the worm is here much smaller, but in front of these it increases and retains the size of the clitellum up to somite II. Somites X, XI, XII, XIII are very conspicuous, and are not annulated as are those immediately anterior to them.

The setæ are eight in each somite and are not in couples, but they are arranged in such a way as to form fourteen rows along the body. In describing this arrangement it will be convenient to adopt M. Perrier's plan which he used in the case of Plutellus,[1] of numbering the setæ on each side. The most ventral seta on each side will be called "seta 1," whilst the most dorsal will be called "seta 7." The series of "seta 1" form a continuous line on each side of the ventral mid-line throughout the body, but the remaining three setæ ("2, 4, 6,") on each side of one somite alternate with the three setæ ("3, 5, 7") of the somite in front and of that behind (fig. 23). In this way we get the fourteen rows of setæ.

As will be best seen from the figures (23 and 24) the setæ of the two sides of one somite are themselves not symmetrical. The setæ 1 and 2 of one somite, or 1 and 3 of the next somite, correspond to the ventral couple of Lumbricus, &c., and setæ 4 and 6, or 5 and 7 to the lateral couple of the same Earthworm. The setæ are very small, being only about ·55 mm. in length; they have the usual shape, but the distal and proximal regions differ in length; the distal (free) region is much shorter than the proximal (embedded) region, and its extremity is more strongly curved than in the ordinary setæ of Lumbricus (fig. 25).

External Apertures.—I could find no apertures whatever, except the terminal mouth and anus. But by dissection I find that the nephridia open to the exterior by

[1] 'Arch. de Zool. Exper. et gen.,' t. ii, 1873, p. 245.

pores place slightly ventrad of and anterior to the line of "seta 4" (fig. 23, *a*), i. e. the lower seta of the lateral couple, as in Urochæta, Microchæta, &c. These pores do not follow the setæ in their alternation, but form a continuous line; the two male pores were also by dissection found to be situated very far back, viz. in somite XXII. Each is placed a little dorsad of and anterior to the line of "seta 3," which is present on one side of this somite, but absent on the other side (fig. 24, *b*). The spermathecæ open at the posterior edges of somites VI, VII, and VIII, in the same line with the nephridiopores (fig. 23, *c*).

Internal Anatomy.—There are five strong infundibuliform septa behind somites VI to X, hiding the œsophagus and gizzard, such as are seen in Titanus, Anteus, Urochæta, &c.; the next ten septa behind these are much thinner, and behind somite XX they are exceedingly delicate. The elongated seminal reservoirs are very conspicuous (fig. 26).

The alimentary tract presents no remarkable points. The œsophagus occupies somite V and part of somite VI. The gizzard occupies the rest of somite VI. Then follows the tubular intestine, which is very narrow; it passes through the five strong septa, and in somite XI becomes enlarged; its walls become thin, and are constricted as the tube passes through the septa; this is the sacculated or typhlosolar intestine. The typhlosole is a simple laterally-compressed fold, carrying the usual blood-vessel.

There are no glands nor cæca opening into the œsophagus or intestine.

The only genital organs which I was able to find are a pair of seminal reservoirs with their rosettes and ducts, and three pairs of spermathecæ.

The seminal reservoirs consist of a pair of very long tongue-shaped sacs (as in Titanus and Urochæta), starting from somite XII, and reaching as far backwards as somite XXXVIII (fig. 26, *g*). This is a most exceptional length for the seminal reservoir, for in Urochæta the elongated sacs only occupy three somites, and in Titanus fourteen somites. As

they pass through the septa the sacs are greatly constricted; so much so in one specimen that the reservoir on one side is cut in two, as not unfrequently happens in Lumbricus.

In another specimen the reservoirs are present, but are empty, and are not of so great an extent as in the one figured.

The ciliated rosettes (*f*) lie in somite XI, one on each side of the intestine; they are not enclosed in the reservoirs in front of which they are placed. This arrangement is very similar to the condition found in Urochæta.

The single sperm-duct on each side is very delicate; it passes backwards along the body wall to its aperture in somite XXII, without having any accessory glands in connection with it.

I could find no testes, nor ovaries nor oviducts. The spermathecæ are six in number (*d*), a pair lying in each of the somites VI, VII, VIII, and having their apertures in the posterior region of these somites. Each is a simple elongated pyriform sac (fig. 29), bent twice upon itself.

The nephridia occur in each somite behind the second. Each consists of a coiled tubule (fig. 27), which is of much greater extent than in Urobenus, and contans parallel lumina within it, having the usual structure. The tubule opens into the cœlom by means of a funnel similar to that of Urobenus; the proximal end of the tubule alters its character before passing to the exterior, the lumen becoming wider and the walls muscular, as in Lumbricus; but here this vesicular region is more highly developed than in that form, which, however, it resembles in that the vesicle is a continuation of the tubule, and not a diverticulum from it, as in Urobenus and Microchæta. The nephridiopores have already been mentioned as being in a line with seta 4.

As is so frequently the case, the most anterior nephridium is greatly modified. Resting against the hinder part of the pharynx in somites IV and V is a large, compact, glandular-looking organ (fig. 26, *c*), the slightly-coiled duct of which opens to the exterior on the anterior edge of somite III. The glandular-looking portion of this modified nephridium is made up

of a compact mass of tubules (fig. 28), or rather of a single very greatly folded tubule (*a*), the folds of which are pressed close against one another, and contain parallel lumina, which in some regions appear to be ciliated. From the outer side of this mass arises the vesicular portion or duct (*b*), which is irregular in diameter, bends upon itself three or four times, and then passes forwards as a narrow, straight duct, lying alongside the pharynx, to its external pore. The structure of this nephridium is quite similar to the unmodified posterior ones. In Urochæta Perrier describes "glandes à mucosité," which have the same position, and Beddard describes a modified nephridium in Ac. multiporus, which opens, not to the exterior, but into the buccal cavity. It seems probable that all these glandular bodies in the anterior region of the body may be modified nephridia.

The vascular system consists of the usual longitudinal vessels, viz. dorsal, ventral, and typhosolar trunks, together with a series of lateral hearts. I could see no lateral longitudinal ("intestino-tegumentary") trunks. The dorsal trunk in the posterior region of the body is only slightly moniliform, but as it passes forwards this condition becomes more marked, especially in somites XVI to XII. In somite XI a pair of moniliform lateral hearts are given off, and similar ones are found in somites X, IX, and VIII, each being smaller than that behind it. In the next two anterior somites the "commissural vessels" are no longer moniliform, and are very much more delicate than the posterior vessels.

The nervous system consists of the usual cerebral ganglia, which are here well marked, and lie in somite III and a series of ventral ganglia. These lie quite close together, so that the intervening cord is very short (fig. 30). This is doubtless due to the greatly contracted state of the worm. Three or four pairs of lateral nerves are given off from each ganglion, but none from the short cord. Probably if the contraction were less and the ganglia longer some of the lateral nerves would appear to come off from the cord.

Just as Urobenus has certain resemblances to Urochæta,

so also there are several points in which Diachæta shows a resemblance to Perrier's genus, viz. in the similarly modified first nephridium, in the single elongated pair of seminal reservoirs (though they are in Diachæta very much longer than in the other worm), and the single pair of "free" ciliated rosettes, and in the position of the nephridiopores. But there are noteworthy differences between the two worms. In Diachæta the body wall is thick, and not transparent. The anterior extremity is not pointed, as in Urochæta, but much swollen and obtuse. There are neither calciferous glands ("glandes de Morren") on the intestine, nor the pyriform sacs on the body wall, nor "intestinal hearts." The nephridia differ considerably. The clitellum is longer than the area supposed by Perrier to represent this structure in Urochæta (but certainly this may differ in the same genus).

The setæ are all simple, and have not the bifid form characteristic of Urochæta; they alternate throughout the body,[1] except the ventralmost, which remain in line. In Urochæta these last alternate as the rest do; but this happens only in the posterior part of the body. It seems, then, that this new worm is very closely allied to Urochæta, and in any classification would have to be placed very near to it among the Intraclitelliani.

Trigaster Lankesteri, nov. gen. et sp.

This worm, like Diachæta, comes from St. Thomas and belongs to the group Intraclitelliani. The single specimen was incomplete, as the hinder part was wanting. Professor Ray Lankester had opened it and made sketches of it.

The most striking point externally is the presence of a deep median ventral fossa, situated in the anterior region of the clitellum; this fossa is bounded on each side by a couple of papillæ; the whole arrangement is doubtless used in copulation.

This worm possesses three distinct gizzards, separated from one another by œsophageal regions; it is this character that

[1] In the genera Pontoscolex, Schm., and Geogenia, Kinb., a similar alternation of the setæ is present in certain regions of the body.

suggested its generic name. Here then we have a condition intermediate between Digaster (E. P.) and Moniligaster[1] (E. P.), but whereas the former is post-clitellian, and the latter aclitellian, Trigaster has its male genital pores situated within the clitellum.

External Anatomy.—The size and number of somites is necessarily unknown, as the only specimen that I have had the opportunity of examining is incomplete.

The setæ are arranged in four couples in each somite (fig. 31); the ventral and lateral couples on one side are nearer to one another than are the ventral couples of the two sides; the eight setæ are all on the ventral surface. The setæ are very small and difficult to see owing to the greatly contracted state of the worm. As seen in a transverse section they are ·2 mm. long, and are less curved than in Lumbricus. The preclitellar somites are quadriannulated, and the third annulus forms a very distinct ridge (*a* in fig. 32); the setæ are placed in the second annulus (fig. 32).

The prostomium is broad, and occupies the whole dorsal border of the anterior extremity; it is not embedded in somite I. The setæ commence in somite II.

The clitellum commences behind somite XIII, and occupies twenty-seven somites, extending as far as somite XL. This is an exceptional length for the clitellum, the nearest approach being Titanus with fifteen somites. The clitellum is incomplete, there being a groove in the mid-ventral line, in which the two ventral couples of setæ are placed (fig. 31). In the anterior part of this groove is the fossa (*g*) above mentioned.

There are no dorsal pores, nor could I find any nephridiopores.

The male pores are four in number, and although not visible externally are indicated by the fossa, and are easily seen in transverse sections. This fossa appears to extend through somites XVII, XVIII, XIX, and XX and part of somite XVI, but the somites are so short in this region and the setæ so difficult to see that the actual number of somites may be less,

[1] 'Nouvelles Arch. de Mus. d'hist. nat. de Paris,' viii, 1872.

as I may have counted annuli by mistake for somites. The side of the fossa is formed partly by two rather prominent papillæ (*f*, *f'*), one of which is formed on somite XVII and part of somite XVI ; the other is formed by somites XIX and XX; between the two papillæ the side of the fossa is formed by somite XVIII, which dips down into the fossa at a level with the base of the papillæ.

The papillæ are in a line with the ventral couple of setæ, though I could see no setæ on the papillæ themselves, and there are no special or "penial" setæ connected with this region. Each male pore lies at the base of a papilla, giving four male pores as in Acanthodrilus.

The pores of the spermathecæ, though not visible externally, lie in somites VII and VIII in a line with the ventral setæ in the posterior region of the somites.

Internal Anatomy.—None of the septa are particularly thick, but those in the anterior region are slightly stronger than the more posterior ones, and tend to hide the gizzard.

The alimentary tract (fig. 33) consists of a short buccal mass, a pharynx which passes through five somites, œsophagus, three gizzards and the intestine. A short œsophagus leaves the pharynx and passes through somite VI; in somite VII it widens out and enters the first gizzard, in somite VIII the anterior region of the somite is occupied by a thin-walled œsophageal portion, and the posterior region by a second gizzard. The same arrangement is repeated in somite IX. Behind each of the gizzards is placed a slightly thicker septum ; each gizzard has the usual structure and is quite separated from the neighbouring gizzards. The tubular intestine commences in somite X, and extends through this and the two following somites. In somite XIII the intestine becomes rather larger and as it passes through the septa is slightly constricted. This forms the sacculated intestine. There are no œsophageal nor intestine glands such as are found in Lumbricus, Microchæta, Urobenus, &c., but there are three pairs of "grape-like glands" around the pharynx and œsophagus, a pair in each of the somites IV, V, and VI. These organs are

shown in situ in fig. 33 and enlarged in figs. 35 and 36. Whether these structures are "salivary glands" and open into the alimentary canal I am unable to say. I could not find any opening though they were embedded in the muscular wall of the pharynx, nor on the other hand could I find any external opening as the alimentary tract had been removed from the body before I received it. Each of these structures is made up of a much branched tubule, each branch ending in a tuft of elongated processes, each of which contains a lumen (fig. 35). The lumen bends round on itself at the apex of the process (fig. 36), and probably is continuous up and down each of the processes; this lumen is intracellular and in transverse section (fig. 37) has an appearance quite similar to that of a nephridium. There is a very abundant vascular supply to these structures and the capillary loops have numerous dilatations on their course, as in the capillaries on the nephridium of Lumbricus, but here they are larger and much more numerous; these dilatations are filled with a granular material (figs. 36, 37) which is probably due to the remains of the blood-corpuscles. I think that there is no doubt that these structures, though apparently in connection with the alimentary canal, are really modified nephridia; even if they do open into the alimentary canal we have the same thing occurring in Ac. multiporus. The nephridia in the rest of the body are extremely minute, and this may have some relation to the great development of these anterior ones. Perrier remarks on the great development of the glandular appendages of the alimentary canal of Perichæta Houlleti[1] and the small development of the nephridia, and suggests that these appendages may take on an excretory function, the products being used for digestive purposes.

The Genital System.—Of the male organs I could find neither testes, nor seminal reservoirs, nor ciliated rosettes, nor sperm-ducts; but in each of the somites XVI and XVIII is a pair of whitish convoluted tubes, having the shape and appearance of the "prostates" of Acanthodrilus (fig. 34, *d*, *d'*). Each

[1] 'Nouv. Arch.,' &c., viii.

of these prostates consists of two regions traversed by a lumen. The distal blind extremity has a looser structure and a more granular appearance, whilst the proximal portion, near the external pore, has a nacreous aspect.

The distal portion is coiled, and has a layer of columnar cells (fig. 42, *b*) surrounding the lumen. Beyond this layer is a deeper one of club-shaped granular cells (*a*), very similar to the cells in the epidermis of the clitellum of Lumbricus, &c., and as in that region there are capillary blood-vessels traversing this glandular layer. The outer wall of this region is formed by thin but firm membrane.

The epithelium of the lumen in the proximal region (fig. 41) consists of tall, narrow, columnar cells, with a highly refracting cuticle, and, instead of a deep layer of granular cells, there is a muscular layer, consisting of oblique fibres, as well as circular and longitudinal fibres. In this region, too, the capillary blood-vessels ramify amongst the epithelial cells. Outside the muscular layer is a thin membrane, as in the glandular region.

I could find no spermatozoa or other contents in these organs. Their proximal portion is probably extrusible, and represents the penial region of the duct in Perichæta, whilst the slightly coiled portion will correspond to the greatly coiled, compact, glandular "prostate" of that genus. The external pores of these "prostates" lie at the base of the papillæ, which form the boundaries of the ventral "copulatory fossa;" a pore being at the base of each papilla.

The two ovaries lie in somite XII (fig. 34), one on each side. The ovary is a grape-like mass of lobules, and is attached to the anterior septum of the somite (fig. 39). Each lobule is supplied by a blood-vessel, around the branches of which are set the ova, embedded in the cœlomic epithelial cells (fig. 40). Each ovum has the characteristic structure.

In each of the somites VII and VIII there is a pair of subglobular white sacs (fig. 34, *a*, *a'*), opening to the exterior in the posterior region of the somite, close to the nerve-cord, in a line with the ventral setæ (fig. 31, *e*, *e'*). When I examined these sacs by teasing them I could find no spermatozoa in them, but

merely a mass of granular, oily-looking granules. But by means of sections I was able to identify these sacs as spermathecæ. The spermatozoa were now seen in bundles, chiefly in the neck of the sack, while in the sack itself they were less numerous, and mixed with the granular globules. The wall of the spermatheca is muscular, and the cavity is lined by tall columnar epithelial cells.

The nephridia are represented in each somite by numerous vascular tufts on the body wall; more numerous in the genital somites than elsewhere. These are apparently independent of one another, and consist of a slightly-coiled tubule containing a narrow, intracellular lumen. Surrounding each tuft is a network of capillaries (fig. 38) without dilatations. As for their communication, either internally or externally, I am unable to say anything. Though I cut sections through the body wall I could not trace the nephridia through it, owing to the badly preserved condition of the worm, nor could I make out any detail of their structure.

I have already described the glandular structures in the anterior somites, which I take to be modified nephridia.

As for the fact of there being a large number of small nephridia in each somite, several Earthworms are now known to possess more than two nephridia to each somite. Beddard has described Ac. multiporus with eight, and I have sections through a Perichæta in which there are very numerous, small nephridia in each somite—a condition also found by Beddard in this genus, who has seen their external pores.

Kinberg's Genera.

In my previous paper I gave the characters of eleven Earthworms, as described, though very inadequately, and, as it now appears, wrongly, by Kinberg[1] in 1866. In the April number of the 'Comptes Rendus'[2] for the present year M. E. Perrier, in a short note, corrects some of Kinberg's descriptions, as observed by himself after examination

[1] 'Ofversigt af. Kongl. Vetensk. Acad. Förhandlgr,' Stockholm, xxiii.

[2] 'Comptes rendus,' cii, 1886, p. 875.

of Kinberg's type specimens. He comes to the conclusion that the only good genera are Tritogenia, which has eight setæ in each somite, and not six, as Kinberg stated; Geogenia, with the clitellum occupying somites XIII to XVIII, and carrying modified setæ; and Eurydame, which has four setæ, and not eight, in a somite. These have a quincuncial arrangement in the posterior region of the body, and are bifurcated, as in Urochæta (E. P.). In other respects Kinberg's descriptions are correct. The remainder of Kinberg's worms belong to other genera. Mandane and Hegesipyle are species of Perrier's Acanthodrilus. Alyattes is a species of Lumbricus. Hypogæon remains indeterminate; whilst the remainder, as mentioned in my previous paper, Perrier considers to be species of Perichæta, Schm. (=Megascolex, Templeton).

Recent Additions to Earthworm Literature.

Since my previous paper[1] was published, several additions have been made to the literature of Earthworms, some treating of the histology of known species and others being descriptions of new forms.

Professor F. Vejdovsky's beautiful work on the Oligochæta ('Systeme und Morphologie der Oligochaeten') contains a comparatively slight reference to terricolous forms, so far as new anatomical details are concerned. Amongst these are drawings of the structure of the clitellum, details as to the development of the setæ, structure of body wall, the spermatophors, &c., in Dendrobæna, Allolobophora, Allurus, Lumbricus, as well as some reference to and drawings of Criodrilus lacuum, Hoffmeister.

In regard to the classification of the group, he puts aside Perrier's arrangement, founded on the relation between the sperm pore and the clitellum, and divides the whole of the Oligochæta into seventeen families (p. 63). Many reasons can be adduced for this plan in preference to the more hard-and-

[1] This Journal, 1886, pp. 213—292.

fast arrangement previously adopted, e. g. species of Acanthodrilus have been described by Beddard ('Proc. Zool. Soc.,' 1885, p. 814), and by Horst ('Notes of Leyden Museum,' vi), in which the male pores are not posterior to the clitellum, as in the three species described by Perrier, but are within the area of the clitellum. Again, Beddard has described a species of Megascolex (Pleurochæta, 'Trans. Roy. Soc. Edin.,' xxx, 1883), in which the genital pore has also this position, instead of the usual post-clitellian position of the remaining species of Megascolex and Perichæta.

It seems better, therefore, to use in the future Vejdovsky's plan, and form the following families for the Terricolous Oligochæta :

1. Pontodrilidæ.
2. Criodrilidæ.
3. Lumbricidæ (= Preclitelliani).
4. Eudrilidæ (= Intraclitelliani).
5. Acanthodrilidæ.
6. Perichætidæ (+ Pleurochætidæ of Vejdovsky).
7. Plutellidæ.
8. Moniligasteridæ.

In February of this year Mr. F. E. Beddard published ('Ann. Mag. Nat. Hist.,' p. 89) a description of a new species of Perichæta, and of Moniligaster, together with some notes on Perichæta Houlleti, E. P., and P. posthuma, Vaillant. P. Ceylonica, Beddard, differs from all other species of this genus with the exception of P. armata, Beddard,[1] in possessing a sac, containing one or more penial setæ, on each side of somite XVIII, in which lie the male pores—an arrangement found, too, in Acanthodrilus.

Moniligaster Barwelli, Beddard, agrees with Perrier's M. Deshayesii in having no clitellum, although the genital organs are mature in both species.

In the new species only the posterior pair of "testes" in somite IX are present. The sperm duct, which appears to have

[1] 'Ann. Mag. Nat. Hist.,' xii, 1883.

no funnel, opens to the exterior at the hinder region of the same somite. In somite VIII is a pair of spermathecæ, occupying the position of the anterior pair of testes of Perrier's form.

In the 'Zool. Anzeiger,' 1886, p 342, Beddard discusses the relation of the ovary to the spermatheca in Eudrilus, as described by Perrier ('Nouv. Arch. Mus. d'Hist. Nat.,' 1872, viii), from whose figures and description it appears that the ovary is grafted on to the spermatheca, from the opposite side of which springs a coiled diverticulum.

Beddard comes to the conclusion that the ovary is in continuity with the oviduct—Perrier's coiled "diverticulum"—and, moreover, that the ovary has a very different structure from that of Oligochæta in general, in that it is surrounded by a fibrous tunic, continuous with the wall of the oviduct, and the cavity is divided by trabeculæ into chambers, in which lie ova in different stages of development.

At a recent meeting of the Zoological Society of London, Beddard pointed out certain variations in the position of the genital pores of Perionyx excavatus, E. P., as seen in a large number of specimens.

The development of the seminal reservoirs in Lumbricus is described by Dr. R. S. Bergh ('Zool. Anzeiger,' 1886, p. 231) in a preliminary note.

It consists essentially of a bulging of the septa of the somites X and XI, so as to give rise to the anterior and posterior sacs on each side of the first, and a posterior sac on each side of the second of these somites [as mentioned in a footnote on p. 259, of my previous paper]. There is a similar arrangement in connection with the ovaries—"receptacula ovorum"—which consists of small backward, saclike protrusions of the posterior septum of somite XIII. Each spermatheca is developed as an invagination of the epidermis, forming a sac which is surrounded by the muscular layers of the body wall.

A memoir by Hermann Ude ('Zeit. für wiss. Zool.,' xlvi, pp. 85—142) on the dorsal pore of Earthworms, deals chiefly with the structure of the body wall; unfortunately figures are

not very freely given. The various layers of the body wall are considered, and a discussion of the opinions of previous authors with respect to each of the layers, forms a great part of the paper. In the epidermis he describes the columnar cells and the goblet-cells; but in the latter he figures no branched base, which, however, I find to exist: the structure of the clitellum is not described.

Below the epidermis he finds a "basal membrane." In the muscular layers he notes an appearance presented in sections across the fibres, which shows the fibre to be made up of fibrillæ. The fibre consists of a denser peripheral portion, in which lies the nucleus, and a less dense central portion. The bundles of muscle-fibres are surrounded by finely granular connective tissue, the perimysium, in which are found small nuclei, whilst the larger nuclei belong to the muscle-fibres.

He draws attention to the fact that the arrangement of the longitudinal muscles in Lumbricus agricola, Hoffm., is not universal in the genus.

The dorsal pore lies on the anterior edge of the somites in which it occurs, and appears in the intersegmental groove. It is absent in the most anterior somites, but the position of the first pore is constant for a given species, e. g. in L. agricola, between somites VIII and IX, in Allolobophora turgida, Eisen, between somites X and XI. In Typhæus orientalis, Beddard, the pore commences only behind the clitellum. It has not been noticed in Anteus, Titanus, Urochæta, or Microchæta.

Vejdovsky states that it is generally absent in the Limicolæ.

In a fully-developed clitellum the pores become closed by a development of cuticular substance around the edge, which gradually increases and fills the pore. In Allol. mucosa, Eisen, however, it remains visible in the clitellum.

Claparède described the epidermis as being invaginated at the dorsal pore, as it is at the seta-follicle; but Ude finds that such is not the case. The dorsal pore is a perforation through the epidermis and muscular layers, and the cœlomic

epithelium passes across these layers and meets the cuticle round the edge of the pore. There is a special set of muscle-bundles, forming a sphincter muscle for the pore.

As to the physiology of the pore, Ude considers that there is not the slightest connection between the dorsal pore and the nephridia, although the former is, to a certain extent, excretory, since the cœlomic fluid can be extruded through it, either in drops, as in Lumbricus, or may be even squirted through the pore to a distance of about a foot, as has been noticed in species of Megascolex and Perichæta by Vordermann ('Natwork. Tijdsche-Nederl. Indie,' vol. ii, p. 111).

The following experiments were undertaken to ascertain whether or not any liquid was taken in through the pore. A worm was dried by lying on blotting paper for some hours; the anterior and posterior extremities were then tied with string and the worm was immersed for fifteen minutes in water. On removal it was found to be greatly swollen, and Ude was led to think, from this, that water was taken in through the pore. But the following experiment caused him to doubt the truth of this opinion. Instead of employing pure water he dissolved some iron oxide in the water, and after the worm had remained in this for some time (after previously being dried) it was killed and the cœlomic fluid was tested for iron by means of cyanide of potassium. No red colour was produced, and hence Ude concludes that no water was taken in through the dorsal pore. The swelling of the worm must therefore have been due to the intaking of water by the genital ducts, nephridia, and mouth. The worm was carefully weighed before and after the various stages of the experiment, and it was found that it weighed about 0·2 grams more (on the average) after immersion than before.

Accompanying this memoir is a bibliography of the subject, and a useful table for determining the species of the genera Lumbricus and Allolobophora is given. The position of the first dorsal pore is given in each species, and appears to furnish a useful specific character.

Two new species are described, Allolobophora longa and A. hispanica.

Rosa ('Bull. Mus. Zool., &c.,' Torino, vol. i) has added a new species, A. celtica, to his previously described forms.

EXPLANATION OF PLATES VIII & IX,

Illustrating Mr. Benham's "Studies on Earthworms."

Microchæta Beddardi.

Fig. 1.—The anterior extremity of the worm, showing three somites from above. (× 1½.)

Fig. 2.—The three anterior somites from the side. (× 1½.) *a, a.* The second and third nephridiopores on the intersegmental grooves. *b.* The first nephridiopore, situated on a slight prominence in somite I. *c.* The lateral setæ. *d.* The ventral setæ.

Fig. 3.—Four somites of the clitellum from below. (× 2.) *a.* The nephridiopores. *b.* The spermathecal pores. *c.* The lateral setæ. *d.* The ventral setæ. *e.* The pores of the oviducts.

Fig. 4.—One of the lateral setæ from a preclitellar somite. Nat. size, 0·39 mm.

Fig. 5.—One of the ventral setæ from somite XIX. Nat. size, 0·715 mm.

Fig. 6.—A spermatheca.

Fig. 7.—Two somites of another specimen, showing the asymmetrical disposition of the spermathecæ.

Fig. 8.—A portion of the intestine, with the intestinal glands.

Fig. 9.—A portion of the intestine, with the intestinal glands, of M. Rappi.

Fig. 10.—A portion of one of a series of transverse sections of M. Beddardi, showing the relative positions of the ovary and its duct: one on each side of the septum XII—XIII. *a.* Portion of the funnel of the oviduct. *b.* Blood-vessel in septum. *f.* Cœlomic epithelial cells, forming the ovary. *o.* Ova amongst these cells. *s.* Septum.

Urobenus brasiliensis.

Fig. 11.—The anterior extremity of the worm (nat. size). *a.* Prostomium

Fig. 12.—Ventral view of the clitellum and neighbouring somites. (× 2.)

a. Ventral setæ. *b*. Lateral setæ. *c*. The nephridiopores. *d*. The pores of the sperm-ducts.

Fig. 13.—Setæ. Nat. size, 0·65 mm. *a*. From the preclitellar region. *b*. One of the ventral setæ from the clitellum.

Fig. 14.—The worm opened along the dorsal surface to show its general anatomy. (× 1½.) *a*. Prostomium. *b*. Pharynx. *c*. Œsophagus. *d*. Proventriculus. *e*. Gizzard. *f*. Intestinal glands. *g*. Tubular intestine. *h*. Pouched intestine. *j*. Sacculated intestine. *k*. Ventral cæca, at the junction of the pouched with the sacculated intestines. *l, l*. Nephridia. *m, m*. Spermathecæ. *n, n'*. Seminal reservoirs. *p*. Dorsal blood-trunk.

Fig. 15.—The junction of (*a*) the pouched with (*c*) the sacculated intestine, seen from the ventral surface in order to show the origin of the ventral cæca (*b*).

Fig. 16.—A portion of the pouched intestine, the dorsal wall of which has been removed, seen from above. *a*. The ventral wall of the axial lumen of the intestine. *b*. A pouch. *c*. The glandular (?) ridge at the junction of a pouch with the axial portion.

Fig. 17.—The alimentary tract has been removed to show the genital organs, nephridia, &c. (× 2.) The seminal reservoirs have been removed from the left side; the anterior nephridia have been drawn aside to show more clearly the somites to which they belong. The posterior nephridia are also somewhat displaced. *a*. Prostomium. *b*. Cerebral or supra-pharyngeal ganglia. *c*. First ventral ganglion. *d*. Ventral nerve-cord. *f, f*. The spermathecæ (there should be lines separating the spermathecæ from the nephridia where they dip into the body wall, on the right side). *g, g*. The seminal reservoirs of the right side. *h, j*. The anterior and posterior ciliated rosettes. *k*. The sperm-duct. *l*. Position of the sperm-pore. *n*, n^1, n^7. The nephridia, of which n^1—n^7 are somewhat different from the following ones. *p*. The pyriform vesicles. *q, r*. Interruptions in the muscular coat of the body wall for the ventral and lateral setæ respectively.

Fig. 18.—One of the posterior nephridia. *a*. The vesicular diverticulum. *b*. The tubule. *c*. The entrance of the tubule into the neck of the vesicle. *d*. The duct leading to the nephridiopore. *e*. Nephridial funnel.

Fig. 19.—A nephridial funnel.

Fig. 20.—A diagrammatic transverse section through the body to show the relative positions on one side of the pyriform vesicle, and on the other of the nephridiopore with the setæ. 1, 2. The ventral couple of setæ. 3, 4. The lateral couple of setæ. *a*. Pyriform vesicle. *b*. Its external pore. *n*. Nephridial vesicle. *o*. Nephridiopore.

Fig. 21.—A transverse section through a pyriform vesicle. *a*. Membrane forming the outer wall. *b*. Granular substance. *c*. Columnar epithelium lining the lumen. *d*. lumen.

Diachæta Thomasii.

Fig. 22.—Dorsal view of the first thirty-nine somites, showing the scattered and alternating arrangement of the setæ. (× 3.) *cl.* Clitellum, occupying somites xx to xxxiii. *r.* Ridge in the somites anteriorly to somite x.

Fig. 23.—View, from below, of a portion of the body wall which has been cut along the dorsal mid-line and pinned out, so as to show the alternating and asymmetrical arrangement of the setæ, which are numbered 1 to 7. (× 10.) *a.* The nephridiopores. *c.* The spermathecal pores. *d.* Setæ, 1, 2, 3, corresponding to the ventral couple of Lumbricus. *e.* The setæ, 4, 5, 6, 7, corresponding to the lateral couple.

Fig. 24.—Ventral view of three somites of the clitellum, which has been cut along the dorsal mid-line and spread out. *a.* The nephridiopores. *b.* The pores of the sperm-duct. *d.* The ventral setæ. *e.* The lateral setæ.

Fig. 25.—A seta; nat. size 0·25 mm.

Fig. 26.—General view of the worm, when opened from the dorsal surface; the spermathecæ have been straightened out in order to show their position; the gizzard, &c., are hidden by the strong septa. (× 3.) *a.* The supra-pharyngeal ganglia. *b.* The pharynx. *c.* The modified first nephridium. *d, d.* The spermathecæ. *e, e.* The strong septa behind the somites vi to x. *f.* The ciliated rosette. *g.* The seminal reservoir. *h.* The dorsal blood-trunk. *j, j.* Nephridia. *k.* The sacculated intestine. *l.* A lateral heart.

Fig. 27.—A nephridium. *a.* The coiled tubule. *b.* Vesicular portion or duct, being a continuation of the tubule. *c.* The portion of the duct leading to the nephridiopore. *f.* The funnel.

Fig. 28.—One of the first pair of nephridia ("glandes à mucosité" of Perrier). *a.* The glandular-looking mass of tubules. *b.* The vesicular portion or duct. *c.* The external aperture which is placed on the anterior edge of somite ii.

Fig. 29.—A spermatheca.

Fig. 30.—A portion of the nerve-cord, showing four closely placed ganglia, with the lateral nerves.

Trigaster Lankesteri.

Fig. 31.—Ventral view of the first fifty somites; the body wall has been cut along the dorsal mid-line and pinned out. (× 2.) *a.* The prostomium. *b.* The mouth. *c.* The lateral setæ. *d.* The ventral setæ. *e, e'.* The spermathecal pores. *f, f'.* The papillæ forming the sides of the median fossa. *g.* The median genital fossa. *h.* The clitellum occupying somites xiv to xl.

Fig. 32.—Two somites further enlarged, to show the three grooves which surround the somites. *a.* A more or less prominent ridge in the anterior somites. *c.* The lateral, and *d.* the ventral setæ.

Fig. 33.—The alimentary tract, together with the septa, removed from the body. (× 4.) *a.* The buccal region. *b.* The pharynx. *c, d, e.* The three

pairs of glandular-looking, modified nephridia. *f*, *f*, *f*. Œsophageal regions, separated by the three gizzards, *g*, *g'*, *g''*. *h*, *h*. The septa. *j'*. The tubular intestine. *k*. The sacculated intestine.

Fig. 34.—The contents of the somites VI to XX after the removal of the alimentary tract. (× 3.) *a*, *a'*. The two pairs of spermathecæ. *b*. The ovary. *c*, *c'*. Vascular tufts, probably nephridia, attached to the body wall. *d*, *d'*. The two pairs of prostates. *e*. The ventral nerve-cord.

Fig. 35.—A group of tubules from one of the modified nephridia of somite VI. *a*. The base from which the tubules arise. *b*, *b*. The capillary blood-vessels with their dilatations on the tubules. *c*, *c*. The branched processes springing from *a*.

Fig. 36.—The free extremity of one of the tubules of a modified nephridium from somite VI. *a*. Connective-tissue wall of the tubule. *b*. Capillary blood-vessel in the wall. *d*. A dilatation of the blood-vessel. *l*. Lumen of the tubule.

Fig. 37.—A transverse section of a tubule of one of the modified nephridia. *a*. A dilatation of one of the capillaries. *b*, *b*. Capillary blood-vessels. *c*. Connective tissue forming the wall of the tubule. *d*. Perforated cell of the nephridium. *l*. Intracellular lumen. *n*. Nucleus of perforated cell.

Fig. 38.—A portion of one of the vascular tufts, indicated at *c*. in Fig. 34. *a*. Connective tissue. *b*. blood-vessel. *l*. Intracellular lumen.

Fig. 39.—The ovary attached to the septum XI—XII. (× 10.)

Fig. 40.—A lobule of the ovary, with the blood-vessel, *b*, branching amongst the cœlomic epithelial cells, *c*, amongst which are the ova, *a*. (Slightly diagrammatic.)

Fig. 41.—A portion of a section through the prostate, near its external aperture. *a*. Cuticular lining. *b*. Columnar epithelial cells. *c*. Muscular coat. *d*. Membrane forming outer wall of prostate. *e*. Blood-vessels lying in this coat, and branching amongst the epithelial cells.

Fig. 42.—A portion of a section through the prostate near its free extremity. *a*. Deep-lying, club-shaped granular cells. *b*. Columnar epithelial cells. *e*. Blood-vessels ramifying amongst the club-shaped cells. *d*. A membrane forming the external coat of the prostate.

Fig. 43.—A portion of a transverse section through one of the intestinal glands of Urobenus. *a*. Various-sized, oily-looking globules (? calcareous) lying in the lumen of the tubules. *b*. Large blood-sinuses surrounding the tubules. *e*. Epithelial cells lining the lumen. *l*. The lumen. *m*. Basement membrane to the epithelium, forming the wall of the blood-sinus.

On Dinophilus Gigas.

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With Plate X.

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, M'Intosh, 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. apatris of Korschelt, differing from it chiefly in the presence of a nervous system and in the histological structure of the ectoderm. The paper of Korschelt[2] 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 platyelminth 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 Korschelt[1] 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, T_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 Hallez[1] 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. metameroides 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 Trochozoon[6] 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 develope skeletal structures; and when once this is ascertained the resemblance to the Molluscan odontophore becomes obvious. Further, in Terebella, and other Polychœts, 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 sems, 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 Rhabdocœl Turbellarians. The body cavity and excretory system especially are in exactly the same condition as those of a Rhabdocœl with well developed cœlomic spaces, such, for example, as Mesostoma.

It is commonly stated that myo-epithelial cells are absent from the ectoderm of Rhabdocœls, 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 Rhabdocœls 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 subsequently[2] 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 proboscidiform 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 Rhabdocœl pharynx (fig. 16).

We seem, therefore, to have in Dinophilus a form which, related on the one hand to the Archiannelids, 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 unmistakeably to a Turbellarian origin.

EXPLANATION OF PLATE X,

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. *sh*^{1}. 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 animul 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 Salensky).
Fig. 15. Navis (schematised from Vejdovsky).
Fig. 16. Vortex (schematised from von Graff).

The Development of the Mole (Talpa Europea).

STAGES E to J.

By

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With Plates XI, XII, and XIII.

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

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

To facilitate reference I have described the development of the embryo in stages, which, in continuance with the stages of growth described in a former paper (No. 8), will be called Stages E, F, G, H, and J. A summary of the various sections of this paper will be found on p. 150.

External Features.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The anterior fold of the amnion (*vide* p. 146) is the so-called pro-amnion of Beneden and Julin (No. 2). It must be noted that up to the close of Stage J no signs of a folding off of the tail end of the embryo can be observed, and, indeed it is not until considerably later that this process takes place.

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

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

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

The Epiblast.

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

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

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

During Stages E to G the median portion becomes thickened

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

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

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

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

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

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

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

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

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

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

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

(1) The median medullary groove.

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

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

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

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

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

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

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

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

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

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

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

The Brain. — When the medullary groove first closes in

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

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

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

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

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

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

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

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

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

The Hypoblast.

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

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

The formation of the deep medullary groove in Stage D and

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

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

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

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

The Alimentary Canal.—The first trace of the alimentary canal appears during Stage D (*vide* No. 8, fig. 46) at the anterior end of the embryo as a short tubular diverticulum. In the paper referred to I described this tube as the notochord, an error which I have corrected here and in more detail in p. 136 of the present paper in the section devoted to that organ.

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

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

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

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

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

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

At the close of Stage J there is a still further change in these relations. The lateral outgrowths of the fore-gut are now directed towards invaginations of the epiblast which correspond to the grooves mentioned in the description of a surface view of an embryo of this stage (Stage J).

The outgrowths are directed outwards and downwards from the lateral portions of the lumen of the canal (fig. 46).

The hypoblast and epiblast have met and are partially fused in the case of the anterior diverticula, although there is as yet no perforation constituting a definite cleft, but in the more posterior diverticula the hypoblast does not meet the epiblastic involution. Now also the fore-gut is a little longer, and the fusion of epiblast and hypoblast on the ventral surface near the front end is closer, although the perforation to form the mouth has not yet taken place (fig. 49).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The Mesoblast.

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

At the periphery these mesoblastic plates are split into two layers, an upper somatic and a lower splanchnic layer, along the whole of their extent posterior to the cephalic plate. The split is entirely peripheral, however, and does not extend into the embryonic area (*vide* former paper No. 8).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Anterior to the protovertebræ scattered mesoblast cells

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

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

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

The muscle-plates are therefore first formed anteriorly.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

These two pits constrict the blastoderm and partially divide

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

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

The Amnion.—The amnion is first formed, as I have before described (p. 127), at the hind end of the embryo; extending thence forwards, and being met by the lateral folds of the amnion which also grow, in the first place, from behind forwards (figs. 26, 28, 35, and 50). This portion of the amnion is formed as in the Chick of a double fold of somatopleure. Immediately upon the junction of the two lateral folds and the formation of true and false amnion, the epiblast of the false amnion, which is shown in fig. 28, unites eventually with the neighbouring uterine tissue, and the thin sheet of somatic mesoblast alone remains between the uterine wall externally and the splanchnic mesoblast within.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Summary.

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

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

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

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

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

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

The Cranial and Spinal Nerves are not described.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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DESCRIPTION OF PLATES XI, XII, & XIII,

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

List of Reference Letters.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Fig. 27. A section through the anterior protovertebra.

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

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

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

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

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

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

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

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

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

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

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

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

Fig. 39. In the middle region: the notochord is not yet separated from the hypoblast in the middle line, although it is so separated at either edge. The lateral ingrowth of the hypoblast is shown.

Fig. 40, from the posterior region, shows relations similar to those seen

in Fig. 39, only the notochordal mass is itself considerably larger the lateral ingrowth of the hypoblast is here also indicated.

Fig. 41. From still further posteriorwards: the notochord is not yet isolated from the hypoblast, but formed into an arc.

Fig. 42. From the hind end of the embryo, immediately in front of the primitive streak: the notochord is a large thickened axial mass, with no indication of the growth of the hypoblast below it.

Fig. 43, Stage H.—A transverse section through the medullary cord of an embryo with eleven protovertebræ, from the region in front of the first protovertebra and behind the hind-brain. Between the lateral mesoblast plate and the cord is a small space, in which several nuclei are seen. The space is continuous with blood-vessels in process of formation, and the nuclei show a tendency to pass into the medullary cord. One such nucleus is shown in the drawing.

Figs. 44 and 45, Stage J.—Transverse sections through the hind-brain of an embryo with fourteen protovertebræ. The alimentary canal is narrow in front (Fig. 44), and wider posteriorly (Fig. 45). The two grooves in the epiblast on the under surface in the anterior section converge in a single deeper groove in the posterior section, where the fusion of the epiblast and hypoblast takes place, and where the mouth will eventually be formed. The dorso-ventral elongation of the fore-gut and the notochord is due to the plane in which the section was cut, caused by the cranial flexure. The presence of mesoblast cells between the notochord and the floor of the brain is to be noticed.

Fig. 46, Stage J, is a section, not completely transverse, through an embryo with fourteen protovertebræ, passing through the hind-brain and the auditory involution. The first aortic arch is shown on one side, and a lateral prolongation of the fore-gut to form the first visceral cleft on the other side.

Fig. 47, Stage J.—Transverse section through an embryo with thirteen protovertebræ in the region of the second protovertebra. Fore-gut crescent shaped. Anterior cardinal veins and dorsal aortæ present. Embryo is completely folded off from the yolk-sac. The heart is enclosed in the pericardium. The thick outer wall and flattened epithelial layer of the heart are here seen to be connected by fine processes of the cells forming one or other of these layers.

Fig. 48, Stage J.—A transverse section through the primitive streak of an embryo with fourteen protovertebræ. The thickened lateral mesoblast will be seen, by comparing this section with that drawn in Fig. 50, to be concerned in the formation of the allantois. Allantoic vessels (*all. v.*) are to be seen in this section.

Fig. 49, Stage J.—A median longitudinal section through the head of an embryo with fourteen protovertebræ. The division between the fore- and mid-brains and the folded floor and thin roof of the hind-brain is shown. The

cranial flexure, ventral prolongation of the anterior end of the fore-gut, and the hooked anterior end of the notochord is also indicated. The notochord is seen to be continuous at its anterior end with the hypoblast and epiblast at the point where the mouth will eventually be formed. The existence of mesoblast cells between the notochord and the floor of the brain is to be noticed.

Fig. 50, Stage J.—A median longitudinal section through the hind end of an embryo with fourteen protovertebræ. The allantoic cavity has increased in size, and numerous blood-vessels are seen in its walls. The mesoblast surrounding the allantoic cavity is derived from the primitive streak mesoblast. The relation of the epiblast, hypoblast, notochord, and mesoblast at the front end of the primitive streak is seen to be precisely the same as I indicated in a diagram (Fig. 50) of my former paper (No. 8). The neurenteric canal is obliterated.

Fig. 51, Stage J.—A transverse section of a portion of the muscle-plate of an embryo with fourteen protovertebræ. The cells of the muscle-plate (*msc. pl.*) are extended into long processes which are continuous with the epiblast cells (*ep.*) lying above them. These processes are commencing muscular fibres.

Fig. 52, Stage J.—Longitudinal section through an embryo with fourteen protovertebræ. The section is taken through a line about half way between a perpendicular and horizontal longitudinal line, and bisects the muscle-plates and aorta of one side of the body. The hypoblast lining the alimentary canal is cut through slightly to one side of the middle line, and the notochord therefore is not shown. The arched muscle-plate and muscular processes of its cells, the aorta and its dorso-lateral prolongations between the protovertebræ are shown.

On the Presence and Structure of the Pineal Eye in Lacertilia.

By

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With Plates XIV, XV, XVI, XVII, XVIII, XIX and XX.

The following work has been carried on in the morphological laboratory of the University of Oxford. It has been made possible solely through the kindness of Professor Moseley, whose invaluable assistance in various ways, especially in procuring from different sources the necessary specimens, I here desire to acknowledge with sincere thanks.

Historical Account.—Though it was impossible for the external indication of the important organ which forms the subject of the following pages to escape the notice of naturalists and more especially of those dealing with the classification of the group, consisting as it does in the modification of a median scale upon the dorsal surface of the head, yet it is strange that only within a very recent period has there been any thorough investigation of the structures lying beneath. This is perhaps chiefly to be accounted for by the fact that the structure in question lies usually within the parietal foramen, enclosed tightly by bone and connective tissue, and is thus left intact within the skull on removal either of the skin from the external or the brain from the internal surface.

Brandt,[1] writing in 1829, uses the following words when describing the skull of Lacerta agilis. "Hinterhaupts-

[1] 'Medizinisch Zoologie,' 1829, Bd. i, p. 160.

schilder 4; selten nur 3; die beiden mittelsten hintereinander stehenden, die kleinsten, das obere, grössere regelmässig 5-eckig, meist mitten, mit einer runden, vertieften stelle," and he adds in a foot-note, " Eine eigne Drüsenstelle bezeichnend." The external marking on the surface of the head is not represented in the drawing of L. agilis (fig. A, Tf. xix), but his description shows that he recognised the presence of an internal modification corresponding to the specialised scale.

Milne Edwards[1] and Dugès[2] both figure the external modification in certain lizards, but neither, strangely, make the slightest mention of it in their descriptions of the animals.

Leydig,[3] writing more than forty years later, is apparently the first to point out with any clearness the presence of the organ, and to give some account of its structure and of the development of the epiphysis, though he entirely failed to discover the relationship existing between the two. Under a high power, he says, the body in question which lies " above the thalamencephalon or the region of the third ventricle," is seen to consist of long cells similar to those of a cylindrical epithelium, so arranged that they form altogether a shallow pit with a circular outline; the rim of the pit is turned upward, and has a thick black girdle of pigment; " welcher schon für das freie Auge das Organ sehr bemerklich macht." After stating that it has a special blood supply, he goes on to say: " Das Organ ist keineswegs, woran Man zunächst denken könnte, die embryonale Zirbel, dem diese folgt erst darunter und ist von ganz anderer Beschaffenheit."

" Fragliches Gebilde entspricht ferner der Stelle, wo sich am skeletirten Schädel des fertigen Thieres im späteren scheitelbeine, das oben schon erwähnte kreis runde Loch befindet."

He examined the organ in Lacerta agilis, L. muralis, L. vivipara, and Anguis fragilis. On pl. xii (fig. 159) he

[1] " Recherches Zoologiques pour servir à l'histoire des Lèzards," 'An. Sci. Nat.,' 1829, tom. xvi, p. 50.

[2] " Mémoire sur les espèces indigènes du genre Lacerta," 'An. Sci. Nat.,' 1829, tom. xvi, p. 337.

[3] 'Die Arten der Saurier,' 1872, p. 72, Taf. 12.

draws a section at right angles to the long axis of the head in L. agilis, passing through the organ in question, which he calls the "Stirn Organ," and the pineal gland. Speaking of this section he says: "Man gewinnt dadurch die Ueberzeugung das es sich um eine innerhalb der Epidermis besonders abgegrenzte Partie handelt; und zwar einer solchen, welche von kugeligen Umriss und zelliger Zusammensetzung über der Oeffnung im Scheitelbein ruht. Unmittelbar unter dem Knochen in der gleichen senkrechten Linie steht die Zirbeldrüse. Sollen etwa die Lagen des Schnittes genauer aufgezählt werden, so folgt von aussen nach innen zuerst die Hornschicht der Epidermis; dan die Schleimschicht und das kugelige zellige, Organ in ihr; darauf die nicht ossificirte, stark schwarz pigmentirte Theil der Lederhaut; alsdann der Knochen mit seinen Markraümen, welche gegen die Oberfläche geöffnet sind. Unterhalb des Knochens kommt die wieder stark gefärbte harte Hirnhaut, und unter dieser, ihr angeheftet die Zirbel; sie verbindet sich durch zwei nervöse Schenkel mit dem Gehirn."

He describes also the presence of the organ in Anguis fragilis; it is present as a small dark spot on the thalamencephalon of very young embryos (cf. Tf. xii, fig. 160), whilst in somewhat older embryos (fig. 162), in addition to the spot, a dark streak is present lying above the unpigmented part, which he recognises as the true pineal gland as well as "ein kleiner unpigmentirter Körper, wie ein winziger Hügel bemerkbar" (fig. 163, c).

These three parts are distinct from the epiphysis itself, and can be seen on removal of the skin from the head. Further, it is evident that the black spot and the black streak are of a similar structure, the walls of each are composed of long cylindrical cells so arranged in the streak as to bound a clear space (see fig. 163), whilst at the black spot they enclose a pit "die vielleicht als Ausgang jener Lichtung zu deuten ist." The cells of both structures have pigment at their inner ends bounding the cavities, the pigment in those of the "spot" being much deeper than in those of the "streak." With regard to the epiphysis, he says: "Die Zirbel deren Stiel aus

zwei Schenkeln besteht, liegt unterhalb des 'Punctes' und 'Streifens,' und zeigt sich als etwas von beiden wohl verschiedenes. Ihre Oberfläche hat das schon gedachte, fältige Aussehen, das ich auf eine Zusammensetzung aus gewundenen Schlaüchen bezog. Doch erhielt ich auch den Eindruck, als ob es sich um eine blasige Bildung mit Faltung der Oberfläche handle. Die Zirbel ist vollig unpigmentirt." As to the nature and function of the structure, he says: "Wie das Organ zu deuten sie, wird im Augenblick wohl Niemand zu sagen sich im Stande fühlen. Doch kann ich nicht umhin, einstweilen an die 'Stirndrüse' der Batrachier zu denken und etwas dieser Bildung verwandter zu vermuthen."

In 1882 Rabl Rückhard,[1] dealing with the development of the epiphysis in the Trout, stated that the pineal gland appears early in the median line on the dorsal surface of the brain, between the first and second brain vesicles, as an outgrowth which admits of close comparison with that of the primary optic vesicles. This resemblance led him to the idea of the possibility, supposing certain secondary developments of epiblast (to form a lens) and of mesoblast took place, that the pineal gland might become transformed into an eye just as are the optic vesicles. This result—the formation of an eye more or less closely similar to the paired eyes—is of course precisely that which does not obtain in Lacertilia, where no such secondary development from epiblast and mesoblast takes place.

He says: "Allein während diese unter Mitmirkung des sich zur Linse einstülpenden Ectoderms und des Mesoderms complicirte Veränderungen eingehen, die schliesslich zur Entwickelung des höchst entwickelten Sinnesorganes, des Auges, führen, sehen wir an der Zirbeldrüse trotz der günstigen Lage ihres distalen Endes dicht unter dem Ectoderm nichts dergleichen. Mann denke sich eine ähnliche Wucherung und ihre Folgen, wie an dem die Augenblasen bedeckenden Ectoderm, das Auftreten von Pigment im sich betheiligenden Mesoderm und nichts steht der Vorstellung im Wege, dass

[1] "Zur Deutung und Entwickelung des Gehirns der Knockenfische," 'Arch. f. Nat. und Phys.,' 1882, p. 111.

sich aus der Zirbel ein den Augen ähnliches unpaares Sinnesorgan entwickelt. Interessant ist, dass diese Gegend in einen bestimmten embryonstadium bei Reptilien (Lacerta, Anguis) eine ähnliche Entwickelung wenigstens andeutungsweise zeigt und dass hier am Scheitelbeine des fertigen Thieres sich ein kreisrundes Loch befindet."

In a subsequent paper, which I have not had the opportunity of seeing, but a quotation from which is given by the author in a recent note to the 'Zoologischer Anzeiger,'[1] he apparently makes a further suggestion with regard to the pineal gland, and says "Das Schädeldach der riesigen fossilen enaliosaurier des Lias des Ichthyosaurus und Plesiosaurus besitzt ein unpaares Loch, welches seiner Lage nach mit dem Loch in Scheitelbein der Saurier übereinzustimmen scheint. Vielleicht lag auch hier das viel entwickeltere Zirbelorgan mit seinem distalen Endtheil zu Tage, und man könnte sich vorstellen, das seine Leistung nicht sowohl die eines Sehorgans als die eines Organs des Wärmesinnes war, dazu bestimmt, seine Träger vor der zu ntensiven Einwirkung der tropischen Sonnenstrahlen zu warnen, wenn sie in träger Ruh, nach Art ihrer noch lebenden Vettern der Crocodile, sich am Strande und auf den Sandbänken der Liassee sonnten."

Ahlborn[2] has described carefully the structure of the epiphysis in Petromyzon, giving a series of drawings to illustrate the histology of the part and its attachment to the brain.

He follows Scott in saying that it arises as a glove-finger-shaped outgrowth on the hinder part of the roof of the thalamencephalon in front of the posterior commissure and behind the ganglion habenulæ.

In the adult, according to Ahlborn, the basal proximal part is reduced to a mere rudiment, whilst the most distal portion of the pineal gland has acquired a secondary fusion with the

[1] 'Zool. Anzeig.,' 21st June, 1886, "Zur Deutung der Zirbeldrüse." The paper referred to here was, of course, published subsequently to Ahlborn's paper, "Ueber die Bedeutung der Zirbeldrüse," published in 1884.

[2] "Untersuchungen über das Gehirn der Petromyzon," 'Zeit. f. Wiss.,' 1883, p. 230, Tf. 13 and 16.

terminal division of the left ganglion habenulæ, whereby is simulated the existence of a primitive genetic connection of the epiphysis with the anterior roof of the thalamencephalon.

In the epiphysis Ahlborn states that three parts can be distinguished clearly separated off from each other.

(1) A hinder thread-like stalk.

(2) Two anterior vesicles lying upon one another (Taf. xiii, fig. 2, and Taf. xvi, figs. 43, 44, 46, and 47). The latter form, the "Weisse kuchenartige Masse," which Wiedersheim recognised as the primitive pineal gland, and lie above the point of the beak-shaped roof of the thalamencephalon. The thread-like stalk is attached to the upper vesicle, and corresponds to the proximal and median part of the epiphysis of Selachians and Amphibians (and we may now add to the stalk connecting the "eye" with the dorsal surface of the thalamencephalon of Lacertilia).

The distal portion of the epiphysis consists of two vesicles, of which the upper is the larger; their cavities, save in rare cases, do not communicate with each other. Ahlborn describes the upper vesicle as being a delicate hollow structure, flattened out dorso-ventrally, and placed close to the skeletogenous roof of the cranial cavity. The cells of the lower wall are always much thicker and deeper than those of the upper, and in his figures (Taf. xvi, figs. 44, 46, and 47), though he does not describe them minutely, are seen to have their long rod-like ends free from nuclei, and turned towards the cavity, whilst the nuclei are all placed close to their external extremities. These rod-like structures, however, are quite devoid of pigment, and, moreover, have a thin but well-marked layer of nervous matter present between them and the cavity of the vesicle, which is itself apparently occupied by strands of nervous tissue passing from the posterior to the thin anterior wall. There is nothing comparable to a lens.

The under vesicle is attached on its ventral surface to the left ganglion habenulæ (the whole organ is placed asymmetrically, and lies on the left side), whilst its upper wall is fused with the larger upper vesicle. This secondary fusion with

the brain roof necessitating the closure of the epiphysis within the cranial cavity.

Ahlborn[1] has also, in a separate article, discussed the nature of the pineal gland. He does not agree with Van Wijhe, who, following Goette's work on Amphibia, had regarded the pineal gland as "Ein Umbildungsprodukt einer letzten Verbindungs des Hirns mit der Oberhaut" (a mistake corrected later by Van Wijhe (see *infra*). He agrees, on the other hand, with Balfour,[2] who stated that the epiphysis arose as an outgrowth from the dorsal surface of the thalamencephalon, and says, himself: "Das Neuralrohr is relativ lange vor dem Auftreten der ersten epiphysenanlage vollständig geschlossen, der Porus ist nicht mehr vorhanden." He states, further, that the cavity of the primitive pineal gland is a new structure formed as an outgrowth of the neural canal, and "ist also nicht ein Rest der vorderen Verschlussöffnung des Gehirns;" hence it cannot be compared with the anterior neuropore of Ascidians and Amphioxus; but he says: "Durch den Vergleich der Epiphysis cerebri mit einer primitiven Augenblase glaube ich nun eine Reihe sehr gewichtiger Gründe für eine neue und wie es scheint richtige Deutung der Zirbeldrüse gefunden zu haben." He then draws attention to the fact that both the pineal gland and the optic vesicles agree in origin as hollow outgrowths, the only difference between the two being that the optic vesicles are large and laterally placed, whilst the pineal vesicle is small, dorsal, and median. After giving in detail other reasons, he says, Alles zusammengenommen komme ich nun aus folgenden gründen:

(1) Nach mit dem Augenblasen übereinstimmenden Entstehung der Epiphysis durch eine hohle Austülpung der Hirnwand;

(2) nach dem Ursprung und der Verknüpfung der Epiphysis mit der optischen Hirnregion, speciell mit dem Thalamus opticus;

[1] "Ueber die Bedeutung der Zirbeldrüse," 'Zeit. f. Wiss.,' 1884, Bd. xl, p. 331.

[2] 'Elasm. Fishes,' p. 177.

(3) nach der morphologischen Aehnlichkeit des Organs mit einer primitiven Augenblase (Bläschen und Stiel) ;

(4) nach der angenähert peripherischen Lage des Bläschens bei den Selachiern, Ganoiden und Petromyzonten und nach einer volkommen peripherischen Lage bei den Amphibien (ausserhalb des Schädels auf gleicher höhe mit den Augen ;

(5) nach dem ursprunglichen zusammenhang der Epiphysis mit der Nervenleiste (van Wijhe) ; zu der Vermuthung, das die glandula pinealis als das Rudiment einer unpaaren Augenanlage anzusehen ist. Wenn dieser Schluss richtig ist, so besitzt die Epiphysis als Rudimentäres Stirnauge, wie mir scheint, noch jetzt ein funktionirendes Analogon in dem unpaaren Auge der Tunicaten und vielleicht auch des Amphioxus."

Van Wijhe, dealing with the development of Selachians, stated first that the anterior neuropore (the spot at which the brain remained last in connection with the epidermis during closure of the neural canal) corresponded to the pineal gland as was stated by Goette to hold true for Amphibia. In his more recent paper,[1] wherein he describes the results arrived at by working with duck embryos, he corrects his first mistake, and states that in birds, though the neuropore exists till the stage with twenty-eight somites, it then completely disappears, whilst when twenty-nine somites are present, the earliest rudiment of the epiphysis appears.

Hoffmann[2] states that in representatives of nearly all classes of Vertebrates it has been proved that the epiphysis arises as an evagination of the roof of the thalamencephalon, and figures its earliest stages in various reptilian embryos (Tropidonatus natrix and Lacerta) ; showing also that it is perfectly distinct from, though present at the same time as, the anterior neuropore. The latter, he says, indicates the position where the "Vorderhirn" joins the "Zwichenhirn" whilst the epiphysial

[1] "Ueber den vorderen Neuroporus und die phylogenetische Function des Canalis Neurentericus der Wirbelthiere," 'Zool. Anzeig.,' 1884, p. 683.

[2] "Weitere Untersuchungen zur Entwickelungsgeschichte der Reptilien,' 'Morph. Jahr.,' Bd. xi, 1885, p. 192.

rudiment is situated where the "Zwichenhirn" and the "Mittelhirn" unite. He states further: "Die vordere Ausbruchtung der Epiphysis schnürt sich volständinge von den Hirndach ab; sie bildet eine kleine, runde, selbstandige Blase von plattgedruckter Form und stellt die Anlage des sogenannten Leydigschen Organes von Strahl hat dieses zuerst erkannt und ich kann seinen Befund bestatigen."

The most recent, as well as most interesting work upon the pineal gland is that of de Graaf,[1] to whom is certainly due the merit of having first clearly shown that in one particular animal (Anguis fragilis) the pineal gland actually is modified into a structure comparable to an Invertebrate eye. He says: "Dem zufolge gleicht bei Anguis fragilis das ganze abgeschnürte Stück etwa dem Auge eines höher entwickelten wirbellosen Thieres, wie uns z. B. Cephalopoden, Pteropoden und Heteropoden bekannt ist."

According to de Graaf the Epiphysis, in Amphibia and Reptiles (Lacertilia), arises as a hollow outgrowth of the thalamencephalon,[2] never passing much beyond this stage in Urodeles (Pl. 2, figs. 13—18), but in Anura and Lacertilia becoming divided into two parts. In the former, growth results in the formation of a distal bladder-shaped portion and a solid stalk connecting this with the brain-roof (Pl. 2, figs. 22—29); the distal part is gradually constricted off from the stalk and comes to lie excerebrally and finally without the cranium and close beneath the skin; the stalk, on the other hand, lies permanently within the brain membranes and thus enclosed in the skull cavity.

In the adult, he says, the cut-off portion of the epiphysis ("Stieda's gland") lies embedded in the cutis close beneath the epidermis, is surrounded by a specially close-woven case,

[1] (*a*) "Zur Anatomie und Entwickelung der Epiphyse bei Amphibien und Reptilien," 'Zool. Anzeig.,' 29th March, 1886. (*b*) 'Bijdrage tot de kennis van den bouw en de ontwickkeling der epiphyse bij Amphibiën en Reptiliën,' van Henri W. de Graaf, Leiden, 1886.

[2] He thus differs from Goette in regarding the epiphysis as a secondary outgrowth, having nothing to do with the neuropore.

and shows retrogressive metamorphosis, undergoing fatty degeneration. What Goette regarded as the epiphysial stalk is, according to Graaf, nothing more than a branch of the Ramus supra-maxillaris of the fifth nerve, and always terminates in the connective-tissue case, never in the organ itself. The extra-cranial part, though present in the adult Rana esculenta, R. temporaria, Alytes obstetricans, Bombinator ingens, and Bufo cinerea, is completely wanting in the full-grown Hyla arborea. In Reptilia the development of the epiphysis takes place as in Amphibia, the distal portion being, according to de Graaf, completely cut off from the proximal stalk; it lies between the brain membranes and has the form of a small, roundish, more or less flattened out vesicle, and shows cellular structure. The wall lying in contact with the parietal foramen is thickened and lens shaped, whilst the hinder wall is pigmented on its inner side.

De Graaf describes in some detail and figures (Pl. 4, figs. 32—34) the organ in Anguis fragilis. Reference to this description will be made later on.

Results of the present investigation.

I desire in the first place to acknowledge the kindness of Dr. Günther, to whom I am indebted for the gift of examples of different genera (indicated by an asterisk in the list below) from the duplicate specimens of the British Museum; my thanks are also due to Professor Stewart for the opportunity of examining specimens of Iguana and Varanus from the collection of the Royal College of Surgeons.

To E. B. Poulton, Esq., of Keble College, and to F. Beddard, Esq., of the Zoological Society, I am indebted for specimens of Hatteria.

My thanks also are due to Professor Westwood for the gift of a fine Chameleo vulgaris, and for the opportunity of examining C. bifurcatus; and to G. C. Bourne, Esq., of New College, for a specimen of Gecko mauritanicus.

I have also to acknowledge gratefully the gift of various species of Lacertilia, prepared especially and sent to me from the Bahamas by J. Gardiner, Esq.; they arrived too late for the results of their examination to be included in the present article, but I hope to be able to publish an account of the structure of the organ in these forms in a short time.

The forms investigated have been the following:

Hatteria punctata.
*Varanus giganteus.
„ bengalensis.
Monitor (sp.?).
Ameiva corvina.
Chameleo vulgaris.
„ bifurcatus.
Gecko verus.
„ mauritanicus.
Anolis (various species).
Leiolœmus tenuis.
*Uraniscodon (Plica) umbra.
*Iguana tuberculata.
Draco volans.
*Ceratophora aspera.
Lyriocephalus scutatus.
*Calotes versicola.
* „ ophiomaca.
*Agama hispida.
*Stellio cordylina.
*Grammatophora barbata.
*Moloch horridus.
Leiodera nitida.
Anguis fragilis.
Cyclodus gigas.
Lacerta ocellata.
„ viridis.
„ (Zootoca) vivipara.
Seps chalcidica.

The material has, in the great majority of cases, consisted of spirit specimens in a better or worse state of preservation so far as histological structure was concerned, so that in many instances it has been impossible to do much more than ascertain the presence or absence of the organ, its connection or separation from the proximal part of the epiphysis, and perhaps a few details with regard to its histological structure. Even in fresh specimens the organ lies so deeply embedded in connective tissue and so closely shut in by bone, which must be removed along with it to prevent injury to the structure, that there is great difficulty in rapidly reaching it with reagents. Of two of the most important forms—Hatteria punctata and Varanus giganteus—I have had the great advantage, through Professor Moseley's kindness, of examining fresh specimens, and have thus been able to investigate more carefully the structure of the retina.

In the account which follows the structure of the organ is described separately in the different forms examined; this structure, as might have been expected to be the case in an organ of this kind (which must be regarded as in a more or less rudimentary condition), shows considerable variation, even amongst species of the same genus. I hope on a future occasion to describe the organ in other forms of Lacertilia.

Hatteria punctata, Pl. XIV, figs. 2, 3, 4, and 5; Pl. XV, figs. 7 and 8; Pl. XX, fig. 7.

(1) External Appearance.—There is in Hatteria but very little external trace of the eye, no special scale being modified into a "cornea;" an absence of pigment, however, in the skin of the median line, slightly posterior to the level of the paired eyes, indicates the position of the parietal foramen; this external indication being more evident in some than in others.

(2) Position of the Eye.—The foramen itself is filled up by a plug of connective tissue, which, notwithstanding the absence of pigment, must effectually prevent the organ lying beneath from functioning as an eye in the ordinary sense of the word; light would more easily penetrate the skin at this than at any other portion of the surface of the head, but yet it is perfectly impossible for an image to be formed upon the retina. The fibres of the connective tissue in the foramen may be divided into two sets—(1) an outer set (Pl. XV, fig. 7, Ct^1) arranged on the whole at right angles to the surface of the head, and which on the inner side of the foramen are connected with (2) an inner set lying immediately in front of the eye, and arranged so as practically to form a hemisphere, part of the internal surface of which forms the anterior boundary of a capsule enclosing the eye (figs. 2, 7, and 8, Ct^2). The hinder half of the capsule which thus lies in the lower part of the foramen is formed of somewhat loosely aggregated fibres with well-marked nuclei scattered irregularly amongst them, and is drawn out in the direction of the optic stalk, which, together with a blood-vessel, pierces the capsule wall at its

most posterior point (fig. 2); the extreme length of the capsule is 1·4 mm. Special fibres cross from the capsule wall to the edge of the lens, and, being connected with the tissue immediately surrounding the retina, may serve the purpose of keeping the eye in position, and thus represent the rudiment of a structure of importance when the eye was fully functional. The capsule in its hinder part contains much irregularly scattered connective tissue with nuclei, its anterior part, however, being free from them. Within the capsule breaks up an artery (figs. 2 and 7, *B.v.*) whose branches ramify amongst the fibres behind the eye; this special blood supply is a prominent feature in connection with the organ in all the forms examined.

The eye lies with its long axis directed upwards and forwards in the most anterior part of the capsule; figs. 7 and 8 show the relative position of the eye in its capsule with regard to the brain and the parietal foramen.

Structure of the Eye.—Through the kindness of Prof. Moseley I have been able to examine the structure in a fresh specimen, and, notwithstanding the fact that the organ cannot now be fully functional, the retina is fairly well developed.

The eye has, roughly speaking, the shape in section (Pl. XIV, fig. 2) of a cone, the base of which lies turned towards the surface, whilst the pineal stalk is connected with the apex. The walls of the optic vesicle are divided into two parts, (1) an anterior; (2) a posterior; of which the former forms the lens, and the latter the sensitive structures.

(1) Lens.—The lens of the pineal thus differs markedly from that of the paired eyes, where it originates as a secondary structure by invagination of the epiblast, whilst in the former it is apparently directly the product of the brain wall itself, and equivalent in position to that part of the paired optic vesicles which after invagination forms the retinal elements.

De Graaf has likened the eye to that of such Invertebrates as Cephalopods and Pteropods; but, apart from other differences which exist between the two in regard to both development and structure, the lens is not in the least degree com-

parable in the two cases, being in the Invertebrates mentioned formed as a cuticular secretion.

In Hatteria as in all forms examined it is distinctly cellular, the nuclei being prominent and numerous (fig. 2). The median cells are elongate so as to give the lens a curious cone shape, the base corresponding to the front of the eye and the apex lying in the optic axis; the cells are further arranged in a definite manner as shown in fig. 2, and are, as the latter indicates, directly continuous with those of the retina.

(2) Retina. — The retinal elements are arranged in the manner typical of Invertebrates, i. e. the rods lie on the inner side bounding the cavity of the optic vesicle, the nerve entering posteriorly and not spreading out in front of the rods.

Within the same vertebrate animal we thus find eyes developed on both vertebrate and invertebrate types, both being also formed from the modification of the walls of hollow outgrowths of the brain.

The retina consists of the following elements (Pl. XIV, figs. 2, 3, 4, and 5):

(1) A layer of rod-like bodies (R) enveloped in deep pigment, which when the rods are separated (fig. 5) is seen to be so deposited upon them as to produce a striated appearance. The pigment is specially densely deposited around the margin of the retina in contact with the lens, extending here through the whole thickness of the wall. A curious specialisation takes place in connection with the rods lying in the optic axis, which also obtains in the pineal eye of many other forms. The rods in this portion are elongated (R^1) to at least twice the length of the ordinary ones, and are in connection at their outer ends with a special group of nucleated cells (n^3) which lie enclosed by a somewhat definite membrane in the pineal stalk, with the fibres of which they are directly connected (fig. 4).

(2) A double and, in parts, triple row of spherical nucleated elements (n^1), which appear to be connected by processes, on the one hand with the rods, and on the other with the layers external to them. They surround posteriorly the elongate rods,

and their processes in this region run in many cases (n^4) directly into connection with the fibres of the optic stalk. The layer gradually thins out anteriorly until that part is reached where, in the neighbourhood of the lens, the pigment is present through the whole breadth of the wall. In its thickest part the whole layer (consisting of the double or triple row of elements) is about the same breadth as the layer of rods.

(3) External to the spherical elements lies a thin layer consisting of a fine punctated material, which takes the stain (hæmatoxylin) with difficulty but contains numerous scattered fine pigment granules. Into this, which may be called the Molecular layer,[1] pass processes from the retinal elements on either side (fig. 2, *mo.*). The layer in question is a very thin one in Hatteria punctata, but forms, when seen in section (fig. 2), a definite boundary line separating the retinal elements into an internal and an external division. Posteriorly the layer spreads out and surrounds the specially elongated rods in the optic axis, anteriorly it reaches as far forwards as the ring of pigment surrounding the lens. It is possible that this layer and many of the processes passing into it may be of the nature of supporting structures.

(4) A layer of nucleated spherical elements (fig. 3, n^2) lying close to the molecular layer, and distinguished from those on the inner side by their greater size; they are arranged so as to alternate (the alternate arrangement is, however, by no means perfectly constant) with

(5) A layer of cone-shaped bodies (*Co.*) in which no nuclei can be detected. They lie with their broad ends externally, and gradually taper internally till their pointed ends are closely in contact with the molecular layer into which processses from them run (fig. 3).

(6) Between the bases of the above are a series of spindle-shaped elements with nuclei, from which processes pass off internally, which may either run directly into the molecular layer or into the spherical bodies on its external side. At the

[1] Cf. de Graaf, Pl. 4, fig. 34, *gl.*

posterior part (i. e. near the pineal stalk) the cone-shaped elements seem to be absent, and their place to be taken by large nucleated spindles (*Co*[1]), which, as it were, bend round internally (fig. 5) and give off processes running directly into the fibres of the stalk.

Connection with the Brain. — It has hitherto been stated by all writers that the distal part of the epiphysis becomes separated from the proximal which forms the pineal gland of the adult, and that the former comes to lie (as shown by de Graaf in Anguis fragilis) external to the cranial cavity in the parietal foramen. De Graaf[1] figures in Anguis the eye as fitted closely into the parietal foramen encased by connective tissue, but separated by a considerable interval from the proximal hollow epiphysial stalk from which in development it has been cut off.

In Hatteria, as also in several other forms to be described below, longitudinal vertical sections show clearly that the highly developed eye is connected with the epiphysis by a solid and well-marked stalk, which may be called the pineal stalk.

This runs in the median line backwards and slightly downwards; it enters the eye at the posterior end, the walls of the optic vesicle being here (fig. 2) drawn out somewhat backwards. The relationship of the elongated rods to the stalk has been already described; passing backwards from the eye the stalk makes a decided bend upwards, then pierces the wall of the eye capsule at its most posterior point and runs straight back to the epiphysis; its fibres enter the latter, being apparently connected with the cells of the apex and the under surface. The pineal stalk contains elements which have much the appearance of those found at an early stage in the developing nerve of the paired eyes, that is, they much resemble cells which are undergoing a process of elongation so as to form long fibres (figs. 2 and 4); some having undergone considerable elongation, others being yet spindle shaped.

There can be little doubt that this median, azygos, nerve

[1] Pl. 4, figs. 31, 32, 33, and 34.

represents the originally hollow process uniting the proximal with the distal portion of the epiphysis, and which, losing its connection with the optic vesicle in some forms (e. g. Anguis), is in others (e. g. Hatteria) transformed into a solid stalk serving as the nerve of the pineal eye. It has been sufficiently demonstrated that the latter is the distal portion of the epiphysis, and we are thus presented with a new sensory structure —the pineal eye—agreeing precisely with the paired eyes in (1) its development as an outgrowth from the walls of the neural canal, and (2) the formation of its nerve by the gradual solidification of the primitively hollow tube connecting the distal vesicle with the proximal portion of the outgrowth. In the case of the paired eyes the whole of the outgrowth save the vesicle is transformed into a nerve; in the pineal eye only the median part of the outgrowth is thus metamorphosed, the proximal part retaining its originally hollow nature.

Varanus giganteus, Pl. XIV, fig. 1, fig. 6; Pl. XV, fig. 10; Pl. XIX, fig. 34.

External Appearance.—In a large specimen of this animal, measuring six feet one inch from the snout to the tip of the tail, which I was enabled to examine in the fresh state through Professor Moseley's kindness, the external indication of the eye is so clear that it is remarkable that no one has hitherto examined the organ lying beneath. The head is covered with small, deeply-pigmented tubercle-like scales, save in the median line, where, somewhat posterior to the paired eyes, a single large scale is present, standing out prominently by reason of its creamy whiteness (fig. 10).

The scale is roughly hexagonal in shape, measuring 5 mm. across, and has upon it a slightly-raised circular rim, the area within which has the appearance of a transparent membrane drawn tensely over a cavity beneath. A dark circular spot in the middle, visible in the living animal, indicates the position of the eye, and is, as will subsequently be shown, due to the presence of a mass of pigment in the lens. In the matter of

external indication of the structure Varanus thus differs much from Hatteria in the possession of this scale, modified to form a cornea.

Position of the Eye.—The cornea thus formed lies immediately above the parietal foramen, the space in which is tightly filled by connective tissue, in the midst of which again lies the pineal eye. There is thus no real cavity beneath the cornea, but the pigment, which elsewhere is abundantly present in the skin, is here entirely absent, so that by this means the passage of light to the organ is much facilitated. Beneath the epidermis and the rete mucosum the connective-tissue fibres of the cutis vera are arranged in two definite sets, as in Hatteria: (1) a series running parallel to the anterior surface of the eye from side to side of the foramen (Pl. XIV, fig. 1, Ct^2), interlacing with each other, and thus forming a dome-shaped structure above the eye; and (2) a series of bundles (Ct^1) at right angles to the former, upon which they spread out at their internal ends, whilst externally they run outwards to the rete mucosum. Obliquely directed strands pass from one bundle to another, and the irregular spaces thus left are filled up by a meshwork of indefinitely arranged fibres.

Immediately below the level of the first series of fibres is placed the eye itself, but, instead of lying, as in Hatteria, in a capsule, the connective tissue closely invests it. The tissue within the parietal foramen may be divided into three parts: (1) a series (Ct^3) bounding the sides of the parietal foramen, and continuous with the upper series (Ct^1); these follow in their course the outline of the bone; (2) irregularly arranged fibres (Ct^4), filling up the greater part of the foramen; (3) a series forming a special encasement for the eye, to the sides of which their long axes are parallel (Ct^5, the arrangement of these is scarcely made sufficiently prominent in the figure).

In Hatteria is found a special capsule in the space within which the eye is situate. Even in this form a certain amount of connective tissue lies within the capsule, whilst a still greater development of the tissue would lead to the condition which obtains in Varanus giganteus.

In addition to the connective tissue within the foramen a large blood-vessel is present, which, accompanying the optic stalk till the foramen is reached, breaks up in this into numerous branches ramifying in the connective tissue (*B.v.*), a branch finally passing from either side in front of the eye (fig. 1), whilst one pierces the connective-tissue dome.

Structure of the Eye.—The eye is, though the size of the two specimens of Hatteria and Varanus are so different (Hatteria under 2 ft., Varanus 6 ft.), as nearly as possible precisely the same size in both, measuring, in the line of the optic axis, ·4 mm., but in Varanus the eye is compressed somewhat in this direction, so that it is broader from side to side slightly than in Hatteria (cf. figs. 1 and 2).

Lens.—The lens is distinctly cellular in structure, the cells being elongated in the direction of the optic axis, and having the appearance of stretching the whole breadth of the lens, their nuclei, which are very prominent, being situated so that in section (fig. 1) they form a well-marked line across the lens from side to side somewhat nearer to the inner than the outer surface. The whole lens has the appearance represented in fig. 1, being thickest in the median line and thinning away rapidly at each side where it joins the retina.

Right in its very middle is present a large, more or less globular mass of small spherical cells, deeply pigmented (fig. 1, *pig.*), and lying directly in the optic axis. The presence of these must of necessity interfere with the action of the organ as an eye, in fact, the whole structure is characterised by the presence of a great amount of pigment deposited in every part. It is this pigment in the lens which causes the eye seen through the transparent cornea to appear like a black spot, and its presence, which must be regarded as due to degeneracy in the tissues, indicates that the organ is now in a rudimentary condition.

Structure of Retina.—The rods line the cavity of the vesicle and form a very definite layer, being deeply embedded in pigment, which renders it difficult to distinguish their outlines. Processes pass from them, often accompanied by pigment granules, into the external-lying layers. As in Hatteria

certain of the rods become elongated; this lengthening is confined in the former to those lying in the optic axis, but in Varanus takes place at two points, one of which, the most prominent, lies in the optic axis, whilst the other lies to the anterior side, each being connected with the entrance of a separate nervous strand into the eye.

Amongst the rods are scattered numerous spherical masses of pigment. There is not the slightest indication of any structures lying internal to the rods embedded in pigment, such as are described by de Graaf in Anguis; on the other hand, the internal limit of the layer of rods is so well marked as to present the appearance of a definite membrane lining the cavity. The latter was most probably filled during life by a fluid, the coagulated remains of which are seen attached as an irregular structureless coagulum to the inner ends of the rods. External to the rods is a layer of finely punctated material (*Mo*) apparently corresponding to the much narrower layer in Hatteria. This layer, together with the rods, occupies as nearly as possible one half of the breadth of the retina. In this layer are situated spherical elements (n^1), which in some cases can be traced into connection with the rods; no arrangement in two or more rows, as in Hatteria, can be detected, but they appear to be placed somewhat irregularly. External to the molecular layer, the outer limit of which is somewhat sharply defined, lie a series of spherical-shaped elements (n^2). The appearance of these as seen in section is given in fig. 6. Some of the elements resemble those lying within the molecular layer (n^1), others have processes passing straight through to the rods on the internal and the nerve-fibres on the external side, whilst others again are connected with one another and with the layers on either side by irregularly branching processes.

Certain of the nerve-fibres pass round behind the vesicle and then enter the retinal elements, but apparently the greater number are directly connected with the two above-described bundles of elongated rods.

Within the external layers of the retina are many large

spherical masses of deep brown pigment (*pig.*[1]), connected in some cases with the pigment enclosing the rods; beyond this, again, a certain amount of pigment in minute granules is scattered irregularly amongst the external spherical elements, and completely external to the optic vesicle posteriorly is massed around the entrance of the nerve a great amount of pigment deposited in branchial cells (*pig.*[2]).

Nerve.—The pineal stalk is well marked in Varanus giganteus and differs moreover from anything met with amongst other forms (even other genera of Varanus).[1] Instead of being single there are three distinct nervous strands entering the vesicle posteriorly; two of these are more prominent than the third, which appears to be in connection with the anterior of the former; the single posteriorly placed nerve entering very nearly but not quite in the line of the optic axis. The larger and smaller anterior strands join together, and then, after a marked curve, shared in by the posterior one, they join the latter and run back as the solid pineal stalk to the proximal part of the epiphysis.

At first it seemed possible that the appearance described might be due to the cutting in longitudinal section of the walls of a hollow stalk distorted somewhat by reagents, but an examination of a continuous series soon showed that this was not the case, and that the pineal stalk, single proximally, broke up distally into two, and finally into three separate nerves entering the optic vesicle.

The most noticeable features in the eye of Varanus are:

(1) The great development of pigment in all parts, and more especially in the lens.

(2) The curious nature of the retina, which has really the form of a cellular network; the cells being in connection with one another by branched processes, the nuclei being scattered somewhat irregularly and giving rise, together with the protoplasm around them, to the spherical elements of the retina.

[1] The only other lizard as yet examined, in which anything comparable to this is found, is Lacerta ocellata, to be described later on.

Reference to this structure of the retina will be made again when dealing with the epiphysis in Cyclodus.

(3) The triple nature of the pineal stalk.

Varanus bengalensis, Pl. XV, fig. 12; Pl. XVI, fig. 17; Pl. XIX, figs. 37 and 41.

External Indication.—In the several specimens of Varanus examined (in addition to V. giganteus) the external indication of the eye was very clear indeed, consisting of a large, modified, median scale (Pl. XIX, fig. 37), lying somewhat posterior to the level of the paired eyes, and having at its centre a circular dark space, surrounded at a short distance by a dark circular line. The central part, which is to a certain extent transparent, acts as a cornea for the eye placed beneath.

Position of the Eye.—In small specimens of Varanus, when the skin is removed from the head, the pineal eye is removed with it and may be examined whole. Fig. 12 represents a portion of the skull roof of a very young specimen of V. bengalensis viewed from the under surface, the bone being very thin indeed. The portion surrounding the parietal foramen is represented in the figure, together with the pineal eye, lying in the latter and viewed as a solid object. The foramen has a somewhat oval shape and backwards from it leads a groove in the median line. The specimen from which this is taken was not in good histological preservation, and no connection with the brain can be traced. The eye is circular in outline and depressed from above downwards, and shows, when viewed by transmitted light, the rods embedded in pigment and forming a very definite layer. Since they line a space within the vesicle, circular in outline, those at the sides, when the object is viewed from above or below, form a circle (*R*), external to which lie the other elements of the retina. In the optic axis posteriorly lies a prominent mass of rods more deeply pigmented than elsewhere, and which indicate most probably a series of elongated rods connected with the union of the pineal stalk; the latter may have been pulled away along with the

brain membranes when the surface of the skull was removed from above the brain.

The connective tissue lying external to the eye is quite transparent, and being placed as it is immediately beneath the skin, the entrance of light is thus made possible; in fact, it is impossible to prevent the light from entering, not only in this but in the case of the pineal eyes of all other Lacertilia, when they are placed so near to the skin.

In section, the eye of a somewhat larger V. bengalensis shows the following structure differing much from that of V. giganteus, a difference the more noticeable since it exists between members of the same genus.

Fig. 41 represents a longitudinal section along the median line of the head passing through the parietal foramen; the results are represented somewhat diagrammatically. The eye lies within the foramen tightly enclosed again within connective tissue, no special capsule being present. A very noticeable feature is the entire absence of pigment above the eye, though this is present in abundance in the skin elsewhere (*Ct, pig.*) in the connective tissue of the cutis vera. The eye itself is depressed dorso-ventrally, so that but comparatively little space remains within the vesicle; the latter lies directly above the anterior extremity of the proximal part of the epiphysis, which runs right up into the foramen from the dorsal wall of the thalamencephalon lying some distance posteriorly.

Fig. 17 gives a more detailed representation of the foramen with its contents. Beneath the cuticle (*cu.*) the epidermis is seen (*ep.*), then the rete mucosum, the nucleated cells of which are in this part somewhat longer than those elsewhere; beneath this lies the connective tissue of the cutis vera (*Ct*). On either side of the foramen are numerous pigment cells (*Ct, pig.*), and the fibres as before may be divided into two series—(1) a set running at right angles to the long axis of the head, and (2) others forming a roof for the foramen, and connected with those lying within the latter, which form a close investment for the eye (Ct^5).

Within the foramen also is a much branched blood-vessel which enters along with the epiphysial stalk; a small branch passes for-

ward on either side in front of the eye just as in V. giganteus. The figure shows the specialisation in the connective tissue above the eye, and the entire absence of pigment-bearing cells in the same position, though they are present on both sides in the section.

Lens.—The lens has very much the same structure as in V. giganteus, being distinctly cellular with well-marked nuclei, forming in section a double or triple row from side to side, the cells appearing to run the whole breadth, whilst in the middle of the lens a great mass of pigment is deposited in the line of the optic axis. The pigment masses are spherical on the external, and more rod-like on the internal surface.

Structure of Retina.—The specimen being preserved in spirits without special reference to histological work, it was somewhat difficult to make out many points with regard to the structure of the retina. The rods are well developed and prominent, lining the cavity of the vesicle, and having their long axes arranged as indicated in the figure, those in the optic axis being at right angles to the external surface, the eye itself being immovably fixed, so as to look directly upwards. They are embedded in pigment, and none amongst them appear to be specially elongated (associated, doubtless, with the absence of connection with any nerve, such as is present in V. giganteus or Hatteria). No trace of any definite structure internal to the rods can be seen.

External to the rods lie a series of spherical-shaped elements (*n'*), corresponding, presumably, to the same in Hatteria and V. giganteus, and at intervals amongst these can be detected spindle-shaped bodies, which, together with the former, stain easily (with hæmatoxylin and borax-carmine). Both these lie within a layer, consisting, as in V. giganteus, of finely-punctated material, whose external limit is well defined. It is difficult to ascertain precisely the structure of this particular layer, which in these two (as well as in other forms) has the appearance of a ground substance, in which lie the external ends of the rods and the spherical elements, but its constant presence and character renders it unlikely that it is the result simply of reagents; it is here called the molecular layer, but may, perhaps, differ in nature from the layer to which the same name is applied in Hatteria.

External to this lies a series of cone-shaped bodies (*Co.*), the pointed internal ends of which abut against the molecular layer, their broader external extremities being placed against the limiting membrane of the eye, where a certain amount of pigment (*pig.*[1]) is deposited in the form of fine granules.

In some cases a connection (not well drawn in the figure) can be traced between the cones and the rods, or, in other cases, the spherical elements. This connection is best developed in the optic axis.

Epiphysis.—In a preliminary communication to the Royal Society[1] the eye of one specimen of V. bengalensis was described as connected with the brain by a hollow epiphysial stalk. Further investigations have shown that this statement must be modified. It is by no means easy to determine the point, and possibly with a fresh specimen a connection between the eye and the proximal portion of the epiphysis may be shown to exist. The two come very close together (closer than is represented in fig. 17), and there is a decided appearance of a connection between them. Further study of my sections has failed to establish the point, and fig. 17 represents, as far as can at present be ascertained, the actual state.

The epiphysis (fig. 41) may be divided into three parts: (1) the distal, separated off as the pineal eye; (2) a short, hollow, proximal portion, arising from the roof of the thalamencephalon, and running at right angles to this; and (3) a median portion running forward from the end of the latter along the roof of the cranial cavity enclosed in the brain membranes. This part also is hollow, and its walls consist of a single layer of distinctly nucleated columnar cells. Its distal extremity lies immediately beneath the pineal eye, and is swollen out and filled with blood-corpuscles, the cells in the wall of this part being somewhat cubical in shape. Passing backward the walls approach one another until they come into contact, and for a short distance a solid stalk is formed; further back, again, the walls part from each other, and in this region the cells lengthen out very much until they pass into the proximal part (fig. 41).

[1] 'Proc. R. S.,' "Preliminary Communication on the Structure and Presence in Sphenodon and other Lizards of the Median Eye, described by de Graaf in Anguis fragilis," June 10th, 1886.

Monitor (sp. ?).—In the Monitor examined there was no external trace of the organ to be discerned, though when the skin was removed from the dorsal surface of the head and viewed by transmitted light, an absence of pigment and general transparency in the spot overlying the parietal foramen indicated the position of the eye. The latter could be easily distinguished as a small black spot lying within the foramen, which was itself, in the form examined, extremely small. Unfortunately the specimen was in a bad state of preservation histologically, and the tissues very dry, so that it was again impossible to make out the details of the structure. The eye, which is deeply pigmented save anteriorly, where is the lens, appears to be placed at the distal extremity of a pineal stalk which, as in Varanus giganteus, runs up vertically through the foramen, accompanied as usual by a large artery.

Chameleo vulgaris, Pl. XVI, fig. 21; Pl. XIX, fig. 40; Pl. XX, fig. 6.

In this form a curious modification takes place, an optic vesicle being formed but not reaching any high degree of development. In the short account written in 'Nature,'[1] it was stated in a note that the organ was present in Chameleo vulgaris—a statement of which de Graaf has subsequently denied the truth. He says that though the parietal foramen is open in the young form it becomes closed as the adult state is reached, and that there can be thus no organ remaining in connection with the proximal part of the epiphysis. Before reading his note, and subsequently to the publication in 'Nature,' three more adult specimens were cut in section (the first note was based upon a dissection), with the result that each one has fully confirmed the statement that the organ is present in Chameleo, and moreover remains in connection with the proximal part of the epiphysis, though it certainly is in a comparatively low state of development.

External Indication.—The presence of the organ is indicated in both Chameleo vulgaris and Chameleo

[1] 'Nature,' May 13th, 1886.

bifurcatus by a tubercle slightly depressed below the level of the surrounding ones, and having a very transparent appearance;[1] it lies in the median line just in front of the anterior end of the strongly marked ridge, which occupies the dorsal surface of the head posteriorly.

Fig. 40 gives a diagrammatic view of the relationship of the different parts; the parietal foramen is not large but is still clearly present, and very easily distinguishable in sections. Within it and lying immediately beneath the modified tubercle is the optic vesicle; elsewhere as usual the skin is deeply pigmented, but the pigment cells are entirely wanting above the vesicle, a fact which is especially noticeable in sections of this animal, the cells having long processes and being closely packed together (fig. 21). It is this absence of pigment which produces the transparent effect in the tubercle. The surface of the latter is very convex, and beneath it the layers of the skin are arranged as usual, a series of special connective-tissue fibres forming an encasement for the vesicle. Within the foramen there is the customary well-marked and branching blood-vessel (*b.v.*), which accompanies the pineal stalk.

Structure of Vesicle.—In Chameleo the structure of the vesicle is very simple. It has the form of a hollow sphere whose walls have been compressed dorso-ventrally, so that its greatest length lies in the line of the long axis of the head. Its walls are formed of elongated distinctly nucleated cells, those facing into the cavity bearing long cilia; no pigment is present and there is no differentiation into lens and retina, the cells of the anterior and posterior walls of the vesicle being alike. Posteriorly the inner wall of the vesicle is, as it were, drawn downwards (fig. 21), a small horn-like space being thus formed, turned somewhat towards the pineal stalk; its general appearance conveys the idea of the vesicle having at first had the relationship to the then open pineal stalk which is at present shown by the swollen distal extremity to the epiphysial tube in Cyclodus. By the meeting of the walls of the epiphysial tube the vesicle would become closed, and the solid

[1] The external indication is much clearer in some than in other specimens.

pineal stalk formed; this would be attached primitively to the posterior end, and the bending of the cells of the vesicle wall (fig. 21) make it appear as if a subsequent drawing down of the stalk to the ventral surface had taken place. In the specimens examined the stalk is seen to end anteriorly somewhat sharply against the under surface of the vesicle, at any rate, in this part none of its fibres could be traced into the cells above, though, as the specimens examined were not specially preserved for histological purposes, it is quite possible that with fresh ones a connection might be demonstrated. Posteriorly, however, where the drawing down of the wall occurs the fibres and cells are in connection with each other.

The pineal stalk itself is a very definite structure, running from the under surface of the vesicle downwards and slightly backwards, till just without the parietal foramen, where it joins the hollow epiyhysial stalk running backward to the roof of the thalamencephalon. In structure it resembles closely that of Hatteria.

Gecko verus.

Neither in the adult nor in the embryo is there the slightest external trace of the organ, the skin being tuberculated and capable of being lifted up from the head without remaining attached in the position of the parietal foramen. There is no discernible trace of the latter: in lizards in which it is present the skin cannot be removed wholly from the surface of the head.

Sections show that the epiphysis is a well-marked structure in Platydactylus arising from the roof of the thalamencephalon and running straight upwards till it comes into contact with the roof of the cranial cavity. This portion corresponds to the proximal part of the structure in other forms, and apparently the pineal stalk, which usually runs forward from this along the dura mater, as well as the distal portion modified into the pineal eye, are both absent in Gecko. The epiphysis is hollow and its cavity gradually increases in size as it passes further from the roof of the brain and approaches the skull, against which it ends blindly; there is no differentiation in its walls, so far as could be discerned, to form an optic vesicle.

The same structure is present in Gecko verus and Gecko mauritanicus.

Ameiva corvina.

Ameiva externally agrees with Platydactylus in the absence of a modified scale to function as a cornea; the skin of the head is also easily removable, not being attached in the position of the foramen, which is also wanting in this species. I have not yet examined it by means of sections, but as far as can be told it agrees with Gecko.

Anolis, Pl. XV, fig. 11; Pl. XVII, fig. 24.

It is not my intention in this paper to describe the structure of the eye of Anolis in any great detail, as before long I hope, by the kindness of Mr. J. Gardiner, to be enabled to describe, by means of specimens prepared carefully by him, the eyes of several species of Anolis from the Bahamas. The eye of one specimen has, however, been figured viewed as a solid object from beneath (fig. 11). The brain membranes are represented, the dura mater having branched pigment-cells scattered over it, and having a specially dark ring around the margin of the parietal foramen in which lies the eye. The latter is somewhat elliptical in shape, its long axis lying in the same line with that of the head: the eye is compressed dorso-ventrally, and when compared with the organ in Varanus bengalensis (fig. 12), placed by its side, the rods are seen to be much larger than in the latter; the cavity within the optic vesicle, whose size is indicated by the circular space bounded by the inner ends of the rods, being hence considerably less in Anolis than in Varanus.

Fig. 24 (Pl. XVII) is a drawing of the eye of another species of Anolis from the West Indies. The organ lies in the foramen with its upper surface close beneath the surface of the head. Its shape is unlike that of any form described hitherto, being elongated in a dorso-ventral direction. The lens is cellular and its hinder border is deeply convex towards the cavity of the vesicle, calling to mind somewhat the shape of the structure in Hatteria; in the optic axis certain of the

cells are apparently undergoing degeneration, pigment being deposited in them.

Retina.—The hinder wall of the vesicle forming the retina is thinnest where it joins the lens and thickest posteriorly. The whole is noticeable by reason of a great development of pigment, which appears to surround all the elements. The rods (*R.*) are very well marked and in some cases, especially in the line of the optic axis, present the appearance of being striated; in the latter position also they are especially elongated. At their external ends they seem to be connected with spherical elements (*n*), also embedded in pigment; these are united by means of processes, rendered evident again by pigment deposited upon them, with a layer of elements apparently corresponding to the cone-shaped bodies of other retinas (*Co.*). In its most posterior region the elements seem to be in connection with the fibres of the optic stalk (*Op. s.*), which runs downwards and backwards within the vacuolate tissue filling up the parietal foramen.

Leiolœmus tenuis.

The external indication of the eye is very clear in the specimen; the scale is in the usual position and surrounded by a series arranged in a circular manner around it as a centre, the two posterior ones being larger than the other four. In the middle of the eye-scale itself lies the circular cornea, white and dome-shaped. Sections show that the eye is present beneath, the walls of the vesicle being differentiated into a transparent cellular lens anteriorly and a retina posteriorly; the rods are enveloped in pigment, and the latter is deposited also through the whole thickness of the retina. The whole organ had shrunk so much that it was impossible again to do more than recognise the presence of the structure, and the fact that it was differentiated into an eye; the proximal part of the epiphysis stretches, in the dura mater, very nearly to the eye, but whether there is or is not any connection between the two could not be determined. In this form also pigment is present in great abundance in the skin, and its absence above the eye is a marked feature in sections.

Plica (Uraniscodon) umbra.

In this the external indication is particularly clear. The scales on the dorsal surface of the head are small, save one whose great size renders it prominent; in the centre of this a small, white, slightly dome-shaped structure indicates the position of the eye beneath.

Position of the eye.—The organ lies very far forward on the dorsal surface, being placed (Pl. XIX, fig. 35) over the anterior region of the cerebral hemispheres; it is situated within the parietal foramen, the size of which is far greater than that of the eye itself, which lies embedded in connective tissue. The usual absence of pigment immediately above it is to be noted.

Structure.—The organ was not in a good enough state of preservation histologically to render any detailed examination of its structure possible. So far as could be discerned the connection of the eye with the epiphysis is retained, the solid pineal stalk (*Op. s.*) running backward immediately within the skull cavity. Attention may be drawn to one curious point—close to the eye is a small secondary and deeply pigmented vesicle (*op*^{1}.). It may be possible that in the specimen examined this is merely due to a shrinkage of the walls of the whole optic vesicle, whereby the anterior and posterior have come into close contact, and thus simulated the appearance of two vesicles, but, as far as could be ascertained, this was not the case. The deep pigmentation of the anterior as well as the posterior wall is strong evidence against this view.

Iguana tuberculata, Pl. XV, figs. 15 and 16; Pl. XXVII, fig. 23.

The full description of the organ, which is present highly developed in Iguana is not given in this paper. I hope before long to have the opportunity of examining its structure in a living specimen.

External Indication.—The usual modified scale is present and in large specimens is very conspicuous. In smaller ones (Pl. XV, fig. 16) a slightly raised central portion is present,

which is devoid of pigment, and transparent enough to allow of the eye beneath being seen as a dark spot. In larger specimens (fig. 15) the central part is still more raised, and forms a dome-shaped structure. In the figure, which is twice the size of the original, the scales from the dorsal surface of the head are represented, and the prominence of the scale with its modification to form a cornea can be seen. The only wonder again is that long before this a careful examination of the structure has not been made.

Structure.—The eye lies within the parietal foramen, which is well developed in Iguana, surrounded closely by connective tissue, there being no capsule present. The eye is so placed that its optic axis is as nearly as possible in the vertical line. In shape it simply resembles an inverted cup with the lens, which has a flattened external surface, occupying the anterior end. The organ is usually more cup-shaped and symmetrical than the one figured (Pl. XVII, fig. 23); but this, which is drawn without any of its surroundings, will serve to demonstrate the structure as far as it will be described in the present communication.

Lens.—The lens is convex posteriorly, and almost—due to its anterior surface being flattened—plano-convex in shape; it is distinctly cellular, with well-marked nuclei scattered irregularly in section. On either side it thins out to join the walls of the posterior part, in which, at the line of union, a specially deep circular ring of pigment is deposited.

Retina.—The rods (*R*) are well marked and embedded in deep pigment. In the line of the optic axis is a bundle of specially elongated ones (R^1); externally they are in contact with spherical elements (n^1), which are as usual of, roughly speaking, the same size as the nuclei of the lens cells. These elements, together with the external ends of the rods, appear to be surrounded by a molecular layer of punctated material, clearly distinguishable, but yet not so well marked as in Varanus giganteus. Most externally is a layer of cone-shaped bodies (*Co.*), the internal ends of which taper off into processes connecting them either with the spherical elements

or with the rods. Their flattened bases rest upon the connective-tissue investment of the eye.

At its posterior extremity enters the pineal stalk. The appearance of this in one form examined is given in fig. 23, where it had the form of a simple nervous strand, much as in Hatteria, the specialised rods running down into it, though there was no group of nucleated bodies to be seen at their external ends.

Draco volans.

The eye is present in Draco volans, though the specimens examined did not make it possible to investigate the structure in detail, the vesicle walls having apparently shrunk and come to lie close together, so as to obliterate the internal cavity. The whole is in a condition, as far as could be ascertained, which resembles that seen in Chameleo or Lyriocephalus.

The vesicle is ovoid in shape, and placed with its long axis in the median line of the head within the parietal foramen; its walls are composed of cells with very distinct nuclei, but no further differentiation to form retina or lens could be distinguished, and the vesicle itself was remarkable for the absence of pigment in its walls, a feature already noticed in Chameleo and Lyriocephalus. The only pigment present lay in the dura mater, and surrounded the very posterior extremity of the vesicle in the position in which the pineal stalk would enter, though it was not possible to determine the existence of this.

Externally specimens of Draco differed somewhat in their indication of the organ, its position being in most cases easily determined by the presence of a specially modified scale in the usual position, and bearing a cornea-like space.

Ceratophora aspera.

The organ is indicated externally in the usual manner by a scale modified to form a cornea. The structure of the epiphysis is interesting, being unlike that met with before. In the specimen examined, though the external indication was present, the parietal foramen was seen, when sections through the head

were cut, to be closed. Its position is indicated by a large blood-vessel which branches on the internal surface of the skull as it enters the bone exactly as the vessel accompanying the pineal stalk branches on entering the parietal foramen, the two branches thus formed pass through to the external surface. The parietal foramen appears simply to have closed up, the blood-vessel remaining and piercing the bone.

In many forms such as Leiolœmus the optic vesicle is placed quite on the internal side of the foramen; in such a form as this were the bone to grow and close up the foramen the vesicle would be left on the internal surface; this is exactly what appears to have taken place in Ceratophora aspera. The epiphysis has the usual form, being well developed and consisting of a proximal portion at right angles to the roof of the thalamencephalon, whilst, from the further end, the distal portion runs forward along the under surface of the dura mater as the pineal stalk until it ends in a slightly swollen portion immediately beneath the parietal foramen. This corresponds to the optic vesicle of other forms; in structure it appears to be solid and to consist of rounded elements, resembling very closely those present and figured by de Graaf in Rana esculenta.

There is this important difference, however, between Amphibia and Lacertilia, that in the former the distal portion of the epiphysis becomes completely cut off from the proximal and is placed externally to the skull, whilst in Lacertilia, on the other hand, the distal part not only remains in connection with the proximal but is permanently closed within the skull cavity after closure of the parietal foramen.

Lyriocephalus scutatus.

The usual external indication is present though not so prominent as in many other forms, the scale being somewhat smaller than those by which it is enclosed posteriorly, which form a V-shaped ridge behind it, the point of the V being directed backward; on the scale a circular, slightly raised, transparent part is modified to form a cornea.

Internally the structure of the optic vesicle resembles more

that of Chameleo than any other, there being no differentiation of the walls to form a lens and retina. The shape of the vesicle is, however, unlike that of Chameleo, being elongated dorso-ventrally. Its walls consist of nucleated columnar cells, and are thicker anteriorly than posteriorly, where there is present a small amount of pigment on the external surface of the cells.

The whole structure lies in the parietal foramen, and has the form, viewed as a solid object, of a small ovoid body whose anterior end is closely apposed to the connective-tissue, forming a roof to the parietal foramen, between which and the cuticle no pigment is present. The pineal stalk is a prominent structure, entering the posterior end of the vesicle where it unites with the cells; unfortunately, in the specimen examined the part with the optic vesicle and portion of the pineal stalk attached to it was torn away from the underlying structures, but their can be little doubt from the similarity between this form and such as Chameleo, that the stalk simply passes back to join the proximal portion of the epiphysis, the upper part of which is seen running forward in the dura mater directly towards the optic vesicle.

Calotes, Pl. XV, figs. 13 and 14; Pl. XVIII, figs. 31 and 33; Pl. XX, fig. 8.

In smaller species of Calotes the external indication of the eye is most clear. A large median scale is so modified (fig. 13) as to present precisely the appearance of an eye. In its centre is a circular black space, within which lies a white ring enclosing a dark space resembling exactly the pupil. This effect is produced by reason of the central part of the scale being transparent and slightly raised into a dome-shaped cornea, while beneath it lies the pineal eye which, on removal of the scale, is seen to have a globular form. The external surface is covered with a glistening white substance, save anteriorly, where the transparent lens is placed; the internal cavity is lined by the rods embedded in deep pigment, and hence appears intensely dark when seen through the lens, the whole eye having

thus the appearance, viewed from above, of a white rim surrounding a dark circular space, and lying immediately beneath the scale, is easily visible on the dorsal surface of the head.

Calotes ophiomaca and C. versicola.—In both these species the external indication is very clear, the modified scale with its corneal, central part forming a prominent object on the surface of the head: internally the structure is practically the same in both forms, and the description which follows is that of the first mentioned of the two species.

Position of the Eye.—The organ is considerably smaller than the foramen in which it lies, and is enclosed in connective tissue; the inner fibres of the cutis vera are so arranged as to form a dome-shaped structure above the eye (*Ct.*^{2}) whilst there is the usual marked absence of pigment between the latter and the external surface, which is also dome-shaped. The cells of the rete mucosum are noticeably elongated and columnar immediately above the eye (*R.M.*).

Structure.—The whole organ is considerably compressed, in the dorso-ventral line, its longest axis (Pl. XVIII, fig. 33) lying in the same line with that of the head.

Lens.—The lens is distinctly cellular though the nuclei of the component cells are not clearly visible (fig. 33, *Le.*) as in other form such as Seps (fig. 32). The structure is concavo-convex in shape, its anterior surface being convex outwards, whilst certain of the cells on the inner side have become pigmented (*pig.*) and thereby assumed a striking similarity to the rods.

Retina.—The rods (*R.*) are very well developed, facing into the cavity of the optic vesicle; from their external ends prominently marked processes pass to an outer layer of cone-shaped bodies (*Co.*), the broad bases of which lie upon the external limiting structure of the eye. There is an absence of any spherical elements such as are seen in other forms. As before said, no nuclei can be recognised in the lens, and the failure to detect both may very probably be due to the fact that the specimen was not in a very good state of histological preservation rather than to their being absent.

In connection with the eye a large blood-vessel (*B. v.*) is developed which runs up by the side of the epiphysial process to the foramen.

Epiphysis.—The eye is, as far as could be told, completely separated off from the brain; the proximal part of the epiphysis runs, as usual, at right angles to the dorsal surface of the brain, whilst the median part corresponding to the pineal stalk of other forms runs forward from the former along the upper surface of the cranial cavity, ending blindly before the foramen is reached (fig. 31, *Ep*^{1}., *Op. s.*).

Agama hispida, Pl. XIX, fig. 39.

The external indication of the eye is very clear in this form, consisting, in a specially large scale placed medianly on the head posteriorly to the paired eyes, in a slight depression and surrounded by small tubercle-like scales. A raised white rim encloses a circular space marked by a curious hour-glass shaped, dark looking patch.

Sections show that the organ lies within the parietal foramen and is almost spherical in shape; above it the connective tissue of the cutis vera is modified as in other forms (e. g. Varanus bengalensis), and is entirely free from pigment, the cells of the rete mucosum being also somewhat elongated above the eye; the latter is surrounded immediately by vacuolate tissue as in Cyclodus or Anolis (figs. 18 and 24). It is difficult to determine the structure of the eye owing to the fact that not only the rods, which are long and well marked, but also the external part of the retina is deeply pigmented; it appears as if nearly all the elements lying external to the rods had degenerated into pigment-bearing cells, amongst which at intervals spherical elements corresponding to those of other forms can with difficulty be distinguished. In many cases processes, also pigmented, pass from the rods to the pigment masses lying external to them.

The lens is distinctly cellular and forms the transparent anterior boundary to the optic vesicle, though as the walls of the latter are comparatively thick the cavity is small; even in

some of the cells of the lens pigment is deposited. It is difficult to determine whether the organ is or is not yet connected with the proximal part of the epiphysis, owing to the great development of pigment in the dura mater surrounding the upper part of the epiphysis, and leading from this to the eye; it was not possible to say definitely whether in the specimen examined this did or did not contain a process from the proximal part of the epiphysis.

Grammatophora barbata.

The scale modified to act as a cornea is present and prominent on the surface of the head.

The eye is present beneath and has apparently (having been only examined as a solid object) the form of a bulb, very similar indeed to that already described in Calotes; in fact, the figures of this as a solid object (fig. 14) would serve also for that of Grammatophora. Externally the bulb is covered with a glistening white substance, whilst internally it is lined by deep pigment in which the rods are embedded. Above the eye, which does not appear to be connected with the epiphysial stalk, pigment is, as usual, entirely wanting in the skin.

Moloch horridus, Pl. XIX, fig. 36.

External Appearance.—In the specimen examined the external indication was very well marked, consisting of a circular dark space, surrounded again at a short distance by a dark circular line, and lying upon a small smooth space in the median line dorsally amongst the stiff horn-like processes covering the head.

Position of Eye.—Longitudinal sections at once showed (Pl. XIX, fig. 36) that this space corresponded roughly in extent to that of the parietal foramen, and that within this and close beneath the surface lay the eye. Unfortunately like many others this specimen was in too bad a state of preservation to do more than enable me to ascertain with certainty the presence and general outline of the organ. It is remarkable for its spherical shape, deep pigment, and com-

parative size. These points are indicated in fig. 36 where the eye is drawn as a solid object. It will be seen that it lies close beneath the surface, the skin being here completely devoid of pigment and quite smooth, forming in fact a cornea (*Cor.*). As far as could be ascertained, though the point could not be determined with certainty owing to the state of preservation of the specimen, the eye is connected, as represented diagrammatically with the proximal part of the epiphysis by the solid pineal stalk (*Op. s.* ?).

Leiodera nitida, Pl. XVII, fig. 22; Pl. XIX, fig. 38.

External Appearance.—The specialised scale (Pl. XIX, fig. 38) forms a prominent feature in the median dorsal line of the head, bearing in its centre a small dome-shaped structure perfectly white, and hence standing out in clear contrast to the deeply-pigmented scale, of which it is a specialised portion.

Position of the Eye.—The organ lies closely embedded in connective tissue, and not really filling up the parietal foramen, than which it is considerably smaller. The layers of the skin above it are so modified as to form the external dome-shaped structure already noticed, whilst pigment is markedly absent from this part, though present on either side (fig. 22, *Ct. pig.*). A very striking feature in section is the peculiar elongation of the cells of the rete mucosum (*R. M.*), whose internal ends appear in many cases to be prolonged downwards, each cell being so placed that its long axis is at right angles to the surface at that particular spot. The connective tissue, further, very closely invests the eye, whilst no such well-developed blood-vessel is to be recognised as is met with in most cases.

Structure.—The organ has a very definite shape shown in fig. 22, being depressed dorso-ventrally, as a result of which the cavity of the vesicle is very small.

Lens.—The lens is well developed, and, as usual, cellular, the nuclei of its cells being prominent in section, and so arranged that they form in the main a line from side to side.

It is thickest in the line of the optic axis, and thins off to each side, where it joins the retina. The lens is, in fact, doubly convex, its anterior surface being in close contact with the investing connective tissue, and parallel to the surface of the dome-shaped cornea above.

Retina.—The retina, owing to the compression of the eye, may be likened in shape to the walls of an oblong box, the lid of which is formed by the lens. The rods line the internal surface, and are very clearly marked; none appear to be especially elongated; their external ends are in connection with a layer of spherical-shaped elements (n^1), as in other forms these elements being also of the same size as the nuclei of the lens. Most externally lie a layer of cone-shaped bodies (*Co.*), whose inner ends taper off into processes passing to the spherical or rod elements, whilst their broad bases lie upon the external limiting membrane of the eye.

Epiphysis.—The eye appears to be completely separated off from the proximal part of the epiphysis, which consists of (1) a proximal part with walls of distinctly nucleated cells, which extends vertically from the thalamencephalon to the roof of the brain cavity; and (2) of a solid thin part running forward along the brain roof from the proximal part towards, but not reaching as far as, the parietal foramen; it is enveloped in pigment, and, being very thin, is somewhat difficult to trace.

Anguis fragilis, Pl. XVII, fig. 25.

This form has been described and figured in detail by de Graaf,[1] but in certain important points I am unable to agree with him.

Fig. 25 represents, somewhat diagrammatically, a longitudinal vertical section through the foramen, the eye, and the epiphysis. The eye in the specimen figured was considerably smaller than the foramen, and the epiphysis was remarkable for running forward until very close to the eye, whilst its distal rounded extremity was invested by pigment cells (*Ep. pig.*).

[1] Op. cit., pl. 4, fig. 34.

As described by de Graaf, the eye is separated off from the epiphysis. In his figure the lens is shown completely separated off from the retina, which overlaps it anteriorly. This does not appear to be the case; but, on the contrary, the eye, as far as could be told, agreed with all other forms examined in having the lens directly continuous with the posterior walls of the vesicle.[1]

The most important point of difference, however, is concerned with the retina. De Graaf figures this (Pl. 4, fig. 34) as having a layer of unpigmented rods (*sl.*)—his "Staafjeslaag"—together with a layer of unpigmented cells (*cep.*)—his "Cyllindercellenlaag"—lying internal to the pigmented rods. Of neither of these two layers can I succeed in finding any trace, either in Anguis fragilis, or in any of the forms yet examined. In every instance all that can be discerned within the rods is merely the remains of what may be supposed to have been during life the fluid contents of the vesicle. In coagulating this does in some instances appear to attach itself to the parts of the rods facing into the cavity, but never forms, in any specimen examined hitherto, any structures so definite as to be interpreted into the "Staafjeslaag" or "Cyllindercellenlaag" of de Graaf.

Cyclodus gigas, Pl. XV, fig. 9; Pl. XVI, figs. 18, 19, and 20; Pl. XVIII, fig. 29; Pl. XX, fig. 5.

In Cyclodus the epiphysis is not developed into an eye, but the structure is nevertheless in an interesting state, showing most probably a stage passed through during the development of the eye in other forms.

External Appearance.—In fig. 9 is represented a portion of the scale specially modified in connection with the organ. It lies, as in all other forms, in the median line posterior to

[1] In my first communication to 'Nature,' the lens of Hatteria was described as separated from the retina, but examination of a fresh specimen showed at once this was a result due to slight post-mortem degeneration of the tissues, and that in reality the two were perfectly continuous, a result which subsequent investigations of many forms has fully confirmed.

the paired eyes, and is easily discernible in the living animal, one of which I was enabled to examine. It consists of a dark patch, having again the appearance of a membrane stretched tensely over a cavity, surrounded by an irregular, slightly-raised, white border, represented in the figure, in which is drawn only the central part of the scale.

Thus the modification to form a "cornea" is, as reference to the figure will show, in a rudimentary state, and foreshadows the similarly rudimentary condition of the organ beneath. In fig. 29 is represented a solid side view of the brain, showing the position of the pineal gland; it lies enclosed in the brain membranes, and fitting closely into the parietal foramen, out of which it is easily removed along with the dura mater. The epiphysis is very long, and stretches far forward beyond the roof of the thalamencephalon, almost to the anterior extremity of the cerebral hemispheres, its distal extremity being deeply embedded in pigment in the dura mater, and having the appearance, as in fig. 29, of a dark, swollen mass.

In section it is seen that the epiphysis is hollow throughout its whole course, the cavity being in direct communication with the third ventricle; the cells composing its walls are all columnar in nature and distinctly nucleated, cilia also being easily distinguished in most parts.

The whole may be divided into two parts: (1) a proximal portion, stretching from the roof of the thalamencephalon in the form of a tube to the parietal foramen; and (2) a swollen distal extremity lying in the latter, and closely invested by vacuolate tissue. In other words, the epiphysis in Cyclodus has the form of a vesicle attached to the brain by a hollow stalk. The vesicle may be regarded as homologous with the eye of other lizards in a rudimentary state,[1] and the hollow connecting process with the solid pineal stalk and proximal part of the epiphysis of such a form as Hatteria.

In figs. 19 and 20 is represented, on a larger scale, the structure of the anterior and posterior walls of the vesicle (by the anterior wall is meant that nearest the external surface).

[1] It may also be closely compared with the condition in adult Elasmobranchs.

In both, the cells are seen to be much elongated with very distinct nuclei; in the case of the anterior ones, save for the presence of well-marked cilia, they differ but little from those of a lens. An elongation of those lying in the middle would, in fact, transform this into the lens of such a form as Lacerta ocellata (Pl. XVIII, fig. 30).

Passing to the posterior surface, however, a curious but interesting modification takes place (cf. figs. 19 and 20), the nuclei all pass to the external surface, whilst the ends of the cells, which are left facing into the cavity of the vesicle, bear a close resemblance to the rod-like structures of the retina of other forms.

It is possible that we have here a stage in the development of the retina. The internal portion of the cell forms the "rod," the nucleus passes to the external end, and with the protoplasm lying around it forms the spherical-shaped element of the retina, still retaining its connection with the rod. Other cells, lying on the opposite side (supposing the wall of the vesicle, as in Cyclodus, to be more than one cell thick), become transformed into the external-lying elements of the retina, their protoplasm becoming in part drawn out into processes, which enter into connection with those of other cells, in part remaining around the nuclei, forming thus the external spherical elements and the processes connecting these with each other. This development would give exactly such a structure as has been already described in Varanus giganteus. In this form it is noticeable that the spherical elements of the retina consist of nuclei with a small amount of protoplasm around them, the nuclei being identical in size with those of the lens, the greater part of the protoplasm of the cells seeming to be developed into processes connecting the various elements. By this means is developed a network of branched cells, connected on the one hand with rods, and on the other with nerve-fibres.

In Cyclodus the stage is reached and retained in which the rods have begun to be formed by a removal of the nuclei to the outer ends of the cells, where they form, together with

those of the external-lying cells, a prominent layer (figs. 18 and 20, *n*.).

Lacerta.

Two species of this genus have been examined.

(1) Lacerta viridis, Pl. XVII, fig. 26.

In this form the external indication, though recognisable, is not at all prominent, consisting merely in a dark circular space upon a median scale.

The organ lies immediately beneath this within the foramen; it is flattened out dorso-ventrally and embedded in deep pigment, as represented in fig. 26, where it is drawn as a solid object. Its smallness and the great deposition of pigment rendered it very difficult to examine the structure in detail, and the backward extension of the pigment towards the epiphysis made it also difficult to distinguish any pineal stalk, though in parts there were indications of its existence (*Ep.*[1] ?); this pigment may, however, be a deposition in the brain membranes which must once have surrounded the stalk connecting the vesicle with the epiphysis, and which persist after the separation of the two has taken place.

(2) Lacerta ocellata, Pl. XVIII, figs. 27, 28, and 30.

In this the external indication of the organ is far more conspicuous than in L. viridis; the scale with its dark central circular space, surrounded by a slightly raised light-coloured rim, forming a well-marked feature on the dorsal surface of the head.

Position of the Eye.—This differs somewhat from that of other forms inasmuch as it lies closer to the external surface; the connective tissue in which it lies completely fills up the foramen, and when the brain, together with its membranes, are pulled away internally from the skull, the eye is brought away with them (Pl. XVIII, fig. 28). The position within the foramen is represented in fig. 27, where the eye is drawn as a solid object surrounded by a great number of branched pigment cells. The foramen is supposed to be cut in half longitudinally and vertically, one side being removed to show the

eye; the connective tissue enclosing it being omitted for the sake of clearness.

Structure.—In shape the eye resembles more than anything else a hemisphere, the equatorial plane being occupied by the lens, which is, in shape, almost concavo-convex, its outer, anterior surface being flattened. The bulb is encased closely by the connective tissue of the dura mater (*D. M.*), a thin layer passing in front of the lens, whilst all the posterior surface is surrounded by branched pigment cells (*pig.*[3]).

Lens.—The lens has the usual cellular structure, being thinnest round the margin where it is continuous with the retina; the nuclei of its component cells form a well-marked layer running across it in section from side to side.

Retina.—Within the retina a considerable deposition of pigment in various parts indicates, to a certain extent, degeneracy, and at the same time renders the examination of its structure difficult.

The rods (*R*) are well marked, and in places present the appearance of being striated. Two bundles of rods are slightly elongated (R^1), being in connection with two distinct nervous strands entering the retina posteriorly. External to the rods lie spherical nucleated elements arranged in two layers, an inner (n^1) and an outer (n^2), whilst amongst them much pigment is scattered in small granules, rendering their detection difficult; in parts still larger masses of pigment are present, which may perhaps be due to the degeneracy of the spherical elements into pigment-bearing cells.

Epiphysis.—As before said, two distinct nervous strands may be seen entering the retina posteriorly and close together (*ne*), one being larger than the other; back from these two, which soon unite, may be traced a single nervous strand which it is extremely difficult to follow, owing to its close investment by connective tissue of the dura mater, but which I believe runs downwards and backwards until it joins the proximal part of the epiphysis (*Ep.*), which is considerably swollen and has a curious development of pigment in its walls.

Along with the pineal stalk runs the usual blood-vessel,

which on nearing the eye bulb breaks up into numerous branches which ramify (figs. 27 and 30, *B. v.*) amongst the pigment cells encasing the eye.

Zootoca vivipara.

The presence and structure of the eye in this form has been described to a certain extent by Leydig, though he failed to recognise its connection with the epiphysis, and did not apply to it the name of eye. The presence of deep pigment in the specimen examined makes it impossible to describe in detail the structure of the retina. Pigment is also thickly deposited in the skin, but it is seen in section to end abruptly on each side of the parietal foramen; so thick is the layer of pigment that no light, save for this provision, could possibly reach the pineal eye.

The eye has the usual form of a hollow vesicle with the lens anteriorly, lying immediately beneath the specialised scale. Pigment runs from the proximal part of the epiphysis to the eye, but, as far as could be told, the latter is separated from the brain.

The eye is present in early stages, before any definite indication of the parietals can be distinguished; in an embryo whose head measured 6 mm. in length, the eye is a prominent object on the dorsal surface of the head, immediately beneath the skin. It is flattened in the dorso-ventral line so that the cavity is small; anteriorly the lens is differentiated and its cells are perfectly continuous with those of the vesicle behind, which are being transformed into the retinal elements, though the fine pigment granules already deposited throughout their substance (and absent from those of the lens) render it difficult to distinguish the different elements; facing into the vesicle, however, the rods can be seen around which the pigment granules are thickest; external to these lie spherical elements massed closely together and not yet separated into definite layers. These may very probably be regarded as the nuclei of the cells whose internal parts are becoming transformed into rods. The eye appears to be connected with the

proximal portion of the epiphysis by a fibrous strand, such as is represented by De Graaf as connecting the distal with the proximal portion of the epiphysis in Bufo cinerea.

Seps chalcidica, Pl. XVIII, fig. 32; Pl. XX, fig. 5.

External Appearance.—The external modification is not so evident in this form as in some others. If one of the median scales posterior to the paired eyes on the dorsal surface of the head be examined it will be found to have upon it a dark-coloured oval patch (hence distinguishable from the yellow-brown surface of the scale); this, which has the characteristic appearance of a membrane stretched over a space beneath, indicates the position of the eye lying beneath it.

Position of the Eye.—The eye lies somewhat on the inner side of the foramen (Pl. XVIII, fig. 32), there being as usual no pigment between it and the external surface. It is remarkable in being the only one in the forms yet examined, which is larger than the foramen; its relation to this is shown in the figure, where it is seen that the parietal bones overlap it on each side to a small extent; if by any reason the foramen became closed then the eye would be situated intracranially, whilst in Amphibia the position is always extracranial, when the distal vesicle of the epiphysis becomes, as in Anura, separated off from the proximal. The eye is surrounded immediately by a great development of pigment bearing tissue which fills up what part of the foramen is not occupied by the organ itself.

Structure.—The whole eye is, in longitudinal vertical section, seen to be elliptical in shape, the long axis corresponding in position with that of the head and hence forming a strong contrast to such an eye as that of Anolis (Pl. XVIII, fig. 24).

Lens.—The lens is distinctly cellular, the nuclei of the constituent cells forming a line prominent in section across from side to side, slightly nearer to the inner than to the outer surface; the whole is doubly convex in shape, thickest in the line of the optic axis, and thinnest where it is continuous with the retina.

Retina.—The specimen not being in very good order histo-

logically, the structure of the retina could not be determined with any great amount of accuracy. The rods as usual formed the most prominent feature; at their external ends in certain parts spherical elements could be distinguished (n^1), whilst, most externally, elements corresponding doubtless to the cone-shaped ones of other eyes were present (Co.). In many parts external to the rods masses of pigment (*pig.*[2]), indicating doubtless degeneracy in the tissues of the retina, were present.

Connection with the brain.—The eye is apparently completely separated off from the brain, no pineal stalk being recognisable.

General Account of the Structure in Lacertilia.

The above account reveals the epiphysis within the group Lacertilia as a structure of very varied development, in some forms presenting merely the appearance of a hollow process from the roof of the thalamencephalon, in others being modified into a well-marked eye, whilst between these two extremes various intermediate forms are found. In taking a short general review of the results detailed above we may deal with them under the four following heads:

(1) General Form of the Epiphysis.[1]—The simplest form seen is in Platydactylus, where it has merely the structure of a hollow outgrowth running at right angles to the surface of the thalamencephalon until it reaches the dura mater lining the cranial cavity. In Hatteria, on the other hand, we have a form in which specialisation is carried to its furthest extent, with the result that the epiphysis becomes modified into three parts—(1) a proximal part, still hollow, and connected with the brain roof, (2) a median, solid pineal stalk, serving to connect the former with (3) the distal portion differentiated into an optic organ. These forms may be taken as two extremes, the gap between which is filled up by various modifications: thus in Cyclodus the epiphysis instead of running straight upwards turns forwards, and at the distal end swells out into a vesicle whose walls show a trace

[1] Compare the diagram showing the development of the epiphysis in various forms on Pl. XX.

of differentiation into lens anteriorly and retina posteriorly; the hollow connection with the brain persisting through life. In such forms again as Calotes, Seps, or Leiodera the same differentiation into an optic organ with retinal elements takes place as in Hatteria, but the connection with the brain is lost. In a few forms further, such as Chameleo vulgaris and Lyriocephalus scutatus, the development of the epiphysis is carried to a great extent, resulting in a division into three parts, as in Hatteria, but the distal vesicle is not differentiated into an eye, its walls retaining their primitive structure.

In Varanus giganteus a peculiar modification takes place, seen in no other form examined; the pineal stalk, which is well developed, breaking up into three divisions before the eye is reached, whilst in V. bengalensis the eye is apparently separated from the proximal portion of the epiphysis, and the part equivalent to the pineal stalk of the other species is hollow and ends beneath the optic vesicle in a slight swelling.

(2) State of Retinal Elements.—Dealing with the state of development of the retinal elements, the eyes are found to differ to no little extent in this respect; thus in Hatteria it is better developed than in any other form examined: in Varanus, on the other hand, while the elements can be distinguished the whole eye is marked by a great deposition of pigment; even in the centre of the lens a large globular mass is present which must effectually prevent the entrance of light to the vesicle in the line of the optic axis, whilst, in addition to this, many of the retinal elements degenerate into pigment-bearing cells. In others, such as Anolis, almost all the elements are enveloped in pigment, whilst in others, as Agama hispida, so great is the deposition that it is not possible to distinguish any elements save the rods. In contrast to this we find in some genera, such as Chameleo and Lyriocephalus that no pigment is present at all, and, accompanying this absence of pigment, it is found that the vesicle is not developed into an eye, its walls retaining their

primitive structure of columnar cells, ciliated internally. In Cyclodus again we find another modification present, the epiphysis having apparently reached a stage passed through in the development of the eye of other Lacertilia; a vesicle is formed distally, but the pineal stalk remains widely open, very little pigment is present amongst the cells, and no true eye is found. In Ceratophora, lastly, the distal extremity of the epiphysis is placed within the cranial cavity beneath the spot, corresponding in position to the parietal foramen of other forms; the portion equivalent to the optic vesicle is present, forming a slightly swollen mass at the distal extremity of the pineal stalk (?), consisting of rounded elements very similar to those present in the extracranial part of the epiphysis of Bufo cinerea.

(3) External Modification.—When we come to deal with the external modification it is very remarkable to notice that a high development in this is by no means necessarily accompanied by, or the index of, a highly-developed sense-organ beneath. In Varanus giganteus the external indication is extremely well marked, whilst the eye beneath is also well developed, and connected with the pineal stalk; in Hatteria, on the other hand, the eye is still better developed, the retinal elements being more clearly differentiated, whilst there is quite absent that great development of pigment which must indicate to a certain extent degeneracy in the eye of Varanus. Despite this there is in Hatteria no external modification, or, at all events, only a very slight one present indicating the position of the eye beneath; the latter also lies deeply embedded in connective tissue—deeper still than in the case of Varanus—though, as in every other form, there is a marked absence of pigment between the eye and the surface of the head. Thus, in the one of these two forms in which the organ is most highly developed, we find that the external modification is much the least evident. If, again, such genera as Calotes, Seps, Leiodera, and Anolis be taken, in these the modified scale is so prominent as to form the most noticeable feature on the dorsal surface of the head, and to resemble a cornea; below it

the eye is in a more or less highly developed state, its elements often obscured by deposition of pigment, but revealing in all cases, even when best developed, its rudimentary nature by the absence of any nervous connection with the brain.

(4) Position of the Eye.—With regard to the position of the eye considerable variation is seen. In such forms as Calotes, Leiodera, Anolis, or Agama, for example, the eye is close beneath the external surface, lying in the upper part of the well-marked parietal foramen. In Varanus the eye lies somewhat deeper, whilst in Hatteria it is placed deeper still on the inner side of the foramen, and in both forms a great development of connective tissue takes place, the latter being in every instance arranged in a definite manner. In Lacerta ocellata and Cyclodus the eye is placed within the parietal foramen, fitting it closely, the foramen having the form (see Pl. XVIII, fig. 27) of a truncated cone, whose apex lies externally. In Ceratophora aspera finally, the parietal foramen is closed, and the modified distal portion of the epiphysis lies quite within the skull cavity. The connective-tissue encasements of the eye also show some variations. In Hatteria is seen the highest development in this respect, the eye lying in a definite capsule, and having special supporting fibres stretching across to it from the walls in the anterior part. In Varanus the arrangement of the connective-tissue fibres appear to indicate the fact that a capsule formerly enclosing the eye, as in Hatteria, has become filled up with fibres, so that the eye is now immovably fixed. In other forms, again, such as Cyclodus, Anolis, or Anguis, it lies surrounded by vacuolate tissue, whilst in others, as Chameleo, Lacerta ocellata, Leiodera, Monitor, Uraniscodon, Calotes, and various other genera, the connective tissue, without any trace of capsule, closely invests the eye, no space being left within the parietal foramen.

If now we take typical examples from amongst the Lacertilia, and consider the state of development in each with regard to the above four points, it is seen that no one form shows a high

state in all, some being well developed in one and some in another respect, but each being degenerate in at least one of the four features.

Referring to the latter under the numbers (1), (2), (3), and (4), and taking first Hatteria, we find that it shows in (1) and (2) a high, in (3) a low, and in (4) a somewhat low state.

Varanus giganteus shows in (1) a high, in (2) a considerably degenerate, in (3) a high, and in (4) a somewhat low state.

Calotes shows in (1) a degenerate (i.e. connection with brain lost), in (2) a somewhat degenerate, in (3) a very high, and in (4) a high state of development.

Chameleo vulgaris shows in (1) a high, in (2) a low, in (3) a fairly high, and in (4) a somewhat low state.

The same result exactly is obtained when each form is tested in the same way, showing that the organ is never present in a perfectly functional state, but always presents some one feature, at least, in which it is more or less imperfect.

We are thus brought to the conclusion that the pineal eye in Lacertilia is a rudimentary structure—that at the present time it is not so highly developed as it must have been at some previous period when fully functional.

It is, indeed, difficult to ascertain whether the structure is now functional at all. In lizards, whose paired eyes are closed, no result is obtained by rapidly focussing a strong beam of light on to the modified eye scale, and thus on to the pineal eye; in fact, strong light suddenly focussed into one of the paired eyes merely causes the lid to be drawn down without any further apparent result, whilst in the pineal eye there is no protecting lid, and no movement whatever takes place to remove the eye from the direction in which the light is coming.

Wiedersheim has, since the greater part of this paper was written and the preliminary communication to the Royal Society published, attempted to show that the organ is functional and not rudimentary; he bases his conclusions upon the study of several forms such as Varanus, in which, as pre-

viously described by myself, a most noticeable feature is the absence of pigment between the eye and the exterior. This is certainly very clearly marked and, further, is perfectly constant; but there can be no doubt, in face of the descriptions given above, that, if we use the term "rudimentary organs" to include such as are now from change in their structure less capable of fulfilling their function than they have been at some previous time, then within this category must certainly be included the pineal eye of Lacertilia. Such features as the great development of pigment in, for example, Varanus, or the loss of connection with the brain in many, such as Calotes, are surely only capable of explanation on the supposition that the organ is rudimentary.

One of the most prominent features in connection with the organ is its Invertebrate structure. This was pointed out by de Graaf in Angius fragilis, but in none of the forms examined have any structures equivalent to the rod-like bodies placed internally to those embedded in deep pigment, described and figured by him as present in the above-mentioned species, been found. There is often, however, a structureless substance resembling a coagulum present within the vesicle, which doubtless represents the remains of a humour which was fluid during life; in certain cases it appears to have attached itself to the inner ends of the rods and thus simulates to a certain extent elements lying internal to and connected with them. Further, there seems to be but little ground for likening the eye to that of Cephalopods and Pteropods, as is done by de Graaf; the structure of the retina is different, and that of the lens essentially so, being formed as a cuticular secretion in Mollusca, whilst in Lacertilia it is distinctly cellular and formed directly from the cells of the neural canal.

As before[1] pointed out the development and structure of this organ is extremely interesting, as showing that out of the walls of a vesicle originating as a hollow outgrowth from the neural canal, may be formed an optic

[1] 'Nature,' May, 1886.

organ of the Invertebrate type, whilst from the walls of a precisely similar vesicle, and within the same animal, may be formed an eye of the Vertebrate type.

In both cases the nerve-fibres enter into connection with the retinal elements lying on the side remote from the rods; in one case, however, important secondary developments take place which are wanting in the other, and to which are further entirely due the differences existing between the two types of eyes.

In the case of the pineal eye, first, we have a vesicle, the anterior portion of the walls of which are transformed into the lens; of the cells forming the walls of the posterior half, those facing into the cavity give rise to the rods, whilst external to these are formed the other retinal elements, into connection with which enter the fibres of the pineal stalk; the primary optic vesicle persists, and there is thus formed an eye on what is usually spoken of as "the Invertebrate type," i. e. the rods facing directly into the cavity of the vesicle, and the nerve entering into connection with the external lying elements.

In the case of the paired eye, however, we find that, whilst up to a certain point it agrees in development precisely with the pineal eye, after that point is reached secondary structures appear which have an important influence on its final form. The retinal elements are formed out of the cells of the vesicle wall; the lens, however, is not, but arises as an invagination which pushes before it the external wall; whilst there is this difference between the lenses of the two forms, we see at once, when dealing with the retinal elements, that they are formed in a similar position to that in which they are present in the pineal eye—that is, the cells facing into the optic vesicle give rise to the rod-elements, whilst the external lying cells give rise to what are really the outer layers of the retina (nuclear and molecular layers, &c.). It is simply the formation of the lens as an invagination which causes the rods to assume what appears to be an external position, but is external only when regarded in connection with a secondarily

formed cavity, the primary optic cavity which persists in the pineal eye entirely disappearing in the paired eyes.

There is, however, this difference, that in the pineal eye the posterior portion of the vesicle wall forms the retina, in the paired eyes the anterior; the lens of the pineal eye being a structure totally distinct from that of the paired eyes.

In the pineal eye both light transmitting and light receiving structures are formed out of the walls of the neural canal; the absence of this in the paired eye does not perhaps constitute so great a difference as appears at first sight, for though the lens is not formed out of the neural wall it is formed out of epiblast cells exactly as this is, and in such forms as the Amphibia, where the epiblast is divided into two layers, nervous and epidermic, then the lens is formed solely by the cells of the nervous layer.

In both cases, finally, the nerve-fibres are in connection with the external lying elements and retain this connection throughout life, to do which, after invagination has taken place in the paired eyes, they must pierce the walls of the secondary vesicle; there is thus produced the phenomenon of the nerve-fibres spreading out "in front of" (as it is called), and internal to, the retinal elements, though, morphologically speaking, they are behind and external to them, exactly as in the pineal eye.

Significance of the Organ.

In all forms of Vertebrates the epiphysis arises at an early stage as a hollow outgrowth from the roof of the thalamencephalon. Goette stated that the epiphysis was identical in position with the anterior neuropore—the part at which the walls of the neural canal remained longest in connection with the epiblast —but there seems to be no doubt whatever that this is not the case and that the rudiment of the epiphysis is formed at an early period in a position some little way posterior to that of the anterior neuropore. There can thus be no connection between the persistent anterior neuropore of Amphioxus and the epiphysis of other animals, supposing the former to be equivalent to the neuropore of remaining Chordata, which cannot be

regarded as perfectly certain when its relationship to the anterior end of the notochord is considered.

In Petromyzon, according to Ahlborn,[1] the epiphysis arises as a hollow outgrowth from the roof of the thalamencephalon, which in subsequent development becomes divided into three parts, (*a*) a proximal solid stalk, (*b*) two distal vesicles of which the larger is the uppermost, whilst the smaller acquires a secondary connection with the left ganglion habenulæ. The whole lies within the cartilages enclosing the brain, and though a certain rod-like appearance is subsequently produced in the cells, particularly those of the upper vesicle, still no pigment is developed and no differentiation into retina or lens takes place.

In Elasmobranchs[2] the epiphysis stretches forward as a hollow outgrowth with a dilated end, which may remain within the skull cavity or be enclosed in the cartilage of the roof.

In Amphibia[3] the same development takes place in early stages, the organ remaining in Urodeles as a mushroom-shaped structure, whilst in Anura it is differentiated into a vesicle distally and a solid fibrous stalk proximally, the former being afterwards cut off and occupying an extracranial position immediately beneath the skin.

In Reptilia it arises in all forms as a hollow, forwardly directed outgrowth, which becomes most highly differentiated in Lacertilia, where, in many forms, its distal vesicular portion forms an optic organ.

In Aves the structure also stretches forward, originating as a hollow outgrowth, and being subsequently divided into a distal part which becomes vascular and a proximal solid stalk.

In Mammalia, finally, the structure is much less developed, the process being shorter than in the lower forms and directed backwards.

[1] "Untersuchungen über das Gehirn der Petromyzon," 'Zeit. f. Wiss.,' Bd. xxxix, p. 230, Tf. 13 and 16.

[2] Balfour, 'Elasm. Fishes,' p. 17.

[3] Henri de Graaf, op. cit., p. 23 (Urodeles) and p. 27 (Anura).

Taking thus the animal kingdom as a whole, we see that the epiphysis presents in all forms below mammals the following two points in common with regard to its structure.

(1) It originates as a hollow vesicular outgrowth stretching forward from the roof of the thalamencephalon.

(2) It becomes divided during development into two main divisions.

(*a*) A distal vesicle.

(*b*) A stalk (hollow or solid) connecting (*a*) with the brain roof.

In Mammalia the first of these two points obtains (except that the structure stretches backwards instead of forwards), but degeneration of the tissues sets in at an early period and secretion of solid material takes place in the part corresponding to the hollow vesicle of other forms.

In Aves both points obtain, but in course of development the distal vesicle becomes solid and highly vascular. Below Aves it apparently remains vesicular throughout life save in the Anura where the distal division separates off, becomes solid, and lies extracranially.

So far as is known to us at present the distal portion becomes most highly modified in Lacertilia; further investigations into its structure in other groups is needed, but, as far as our present knowledge goes, we are justified in saying that in Lacertilia alone, amongst living forms, the distal part of the epiphysis is modified into an eye and the tissues between it and the external surface are modified so as to allow of the easy transmission of rays of light to the organ.

In Petromyzon certainly the structure of the organ as figured by Ahlborn resembles somewhat an eye, but closer examination reveals considerable differences between it and the eye of any Lacertilian.

(1) Its division into two vesicles, one above the other, is a point of some importance, indicating that in this case development takes place along another line from that pursued in Lacertilia, where the vesicle always remains single.

(2) The absence of true retinal elements or lens is remark-

able. At first sight Ahlborn's figures of the organ, especially of the walls of the upper vesicle, call to mind the rod elements of other forms, but a closer examination again reveals important points of difference; they do not, as in Lacertilia, face into the cavity, being bounded internally as well as externally by nervous matter, and, more important still, there is an entire absence of pigment, which is the prominent feature possessed in common by the rods of all Lacertilian eyes. Further, again, the cavity of the optic vesicle is traversed by strands of nervous matter passing from the anterior to the posterior wall, a feature entirely wanting in any pineal eye, however degenerate, amongst Lacertilia.

On the other hand, these rod-like structures occupy the hinder wall of the vesicle, the proper position, supposing them to be true but degenerate retinal elements; and it may be remembered that amongst Lacertilia, which must be regarded as descended from ancestors possessed of pineal eyes, we do know of one form (Cyclodus) in which the eye now stops at a stage of development in which the cells of the posterior wall much resemble those of Petromyzon, and are devoid of pigment. The absence of lens also is paralleled in the case of Cyclodus.

(3) The organ is completely enclosed within the cartilaginous cranium, and acquires a secondary connection with the brain (its lower vesicle fusing with the left ganglion habenulæ) which is quite unknown amongst any Lacertilian.

The conclusion to be drawn from these facts[1] is that at the present time the epiphysis of Petromyzon certainly does not become modified into a pineal eye at all comparable to that of

[1] For our knowledge of the structure of the epiphysis of Petromyzon we must rely on Ahlborn's description here quoted; it is, of course, possible that, viewed in the light of recent work, the structures described by him might be found to bear another interpretation. I have not at present been able to study the structure, but would suggest the possibility of what Ahlborn figures as nervous material lying internal to the rod-like structures, and as strands of tissue crossing from the anterior to the posterior wall of the vesicle, being in reality only the coagulated remains of the fluid contents of the vesicle.

Lacertilia, in which its double nature and secondary fusion with the brain are quite unparalleled. At the same time it is of course possible, though we have no direct evidence of the fact, that the epiphysis is in a rudimentary state, and may be the degenerate representative of a once well-developed pineal eye. The general structure of the organ—a distal vesicular part with a solid proximal stalk—being in favour of this view, as is also the resemblance—upon which, however, too much stress must not be laid, between the walls of the upper vesicle and those of the swollen extremity of the epiphysis in Cyclodus.

Further investigations into the structure of the epiphysis are much needed amongst Pisces. At present it is known that amongst Elasmobranchs the structure developes as a hollow outgrowth from the roof of the thalamencephalon. This, as figured by Balfour in Scyllium,[1] stretches forward right over the cerebral hemisphere, and comes finally to consist of (1) a swollen distal extremity, and (2) a hollow stalk connecting (1) with the brain roof. The swollen extremity may further remain, as in Raja, external to the cranium, or become embedded within the cartilage, as in Acanthias. There is thus a striking similarity between this and the epiphysis at a certain stage of development in Lacertilia and the final stage persistent in Cyclodus.

When, however, we come to the Amphibia we find that amongst these the epiphysis passes through precisely the same forms in development, but (1) remains very rudimentary indeed in Urodela, and (2) after reaching a considerably higher stage of development in Anura undergoes great degeneration. The structure in the latter becomes differentiated into a distal vesicle, connected by a solid pineal stalk with the brain; the stalk soon, however, disappears, and the distal portion lies completely separated extracranially, its constituent cells undergoing degeneration. Never at any period does it become developed in any living Amphibian into an eye.

The word living is used and emphasised, because it is by no means certain that the same remark can be applied to all

[1] 'Comp. Embryology,' vol. ii, p. 355.

the extensive group of extinct forms classed together as Labyrinthodonta, and usually regarded as the extinct representatives of the class Amphibia. On the contrary, one of the most interesting features in the cranial skeleton of these is the possession of an extremely well-marked and prominent parietal foramen,[1] which is proportionately quite as large, and in many cases larger, in comparison to the size of the skull than in living Lacertilia.

There is no doubt that the presence of a parietal foramen is intimately associated with a high state of development of the epiphysis, and we are thus brought without hesitation to the conclusion that, whilst amongst living Amphibians the epiphysis is present only in a rudimentary and degenerate condition, in extinct Amphibia (Labyrinthodonta) the epiphysis was in a high state of specialisation. Further, the only group of living animals in which, as before said, a parietal foramen is present, is Lacertilia. Within this group, though various degenerate forms are seen, yet, inasmuch as

> The organ is found in genera of every family, ancient and recent alike (in Hatteria, in Calotes, in Agama, in Moloch, in Anolis, in Iguana, in Anguis, in Varanus, in Seps, in Lacerta), in which a foramen is developed; whilst again, in such as Gecko, Ameiva, and Ceratophora, where no foramen persists, the organ is absent,

It may be further said that the presence of a parietal foramen, as a structure typical of the skulls of a group of animals indicates the presence of a pineal eye within that group.

It is quite true that in three forms described—Cyclodus, Chameleo, and Lyriocephalus—the foramen is present, and though the epiphysis is, in certain respects, highly developed in each case, the distal portion retaining its connection with the brain roof, yet no true eye is formed. This, however, need present no difficulty in the way of acceptance of the above statement. Regarding the present families of Lacer-

[1] See especially the drawings of Fritsch in 'Fauna der Gaskohle und der Kalkstein,' Prag., 1885.

tilia as descendants of some common ancestor, we can come to no other conclusion, inasmuch as the more primitive and specialised forms agree at the present time in the possession of a parietal foramen occupied by a pineal eye, and that this is, further, a characteristic of the nearest allies of the forms mentioned, than that the ancestral form possessed both these structures, and that the condition seen in Chameleo, Cyclodus, and Lyriocephalus is not typical but secondary; they possess a parietal foramen simply because their ancestors possessed a pineal eye, which in them is in a rudimentary condition, as indeed the external modification in Cyclodus (Pl. XV, fig. 9) seems to show in the case of this form in particular.

When, therefore, we find the parietal foramen exceedingly well developed throughout all the group Labyrinthodonta, we are justified in concluding that in them a pineal eye was in all probability present, even though we may grant the possibility (an unlikely one under the circumstances) of its occasional presence, as in Lacertilia, in a low state of development.

In living Reptilia the presence of the foramen is confined to one group, but amongst the extinct forms, which may be regarded as the ancestors of the Reptilia now living, whilst some, at all events, may further be regarded as intimately connected with the ancestors of living birds, we find that the foramen is a well-developed structure. Judging from its present condition in the relatively small Lacertilia of the present day, we may imagine that in the huge extinct forms of Mesozoic periods—in such as Ichthyosaurus and Plesiosaurus, the walls of whose foramina even present rugosities as if for the insertion of muscles—the pineal eye attained a development and importance quite disproportionate to that with which we are now acquainted in any living form.[1]

[1] I am indebted to Professor Moseley for calling my attention to the paper upon "The Brain of a Theromorphous Reptile of the Permian Epoch," by Cope, in which is figured a cast of the brain of one of the Diadectidæ. Perhaps the most remarkable feature is the huge comparative size of the cavity within

The walls of the foramina are lifted above the level of the parietal bones, and it is perfectly possible, if not certain, that the organ itself, enclosed in the eye capsule, projected considerably beyond the surface.

With the gradual extinction of these forms and of the Deinosauria (i. e. Iguanodon, &c.), after the Cretaceous period was passed, the organ, we may suppose, began with the rapidly dwindling size of the specialised tertiary and later Reptilia and Aves to lose its importance, until, degenerating in various degrees in different groups, it retained traces of its original eye-like structure in the only groups in which, amongst living reptiles, the parietal foramen persists; its preservation being intimately connected with and dependent upon the presence of this structure. The foramen is preserved amongst no group whatever of existing Aves, and hence in these the epiphysis undergoes considerable degeneration, though in its development it still reaches a stage when, as in Reptilia, it consists of a distal vesicle connected with the brain roof by a solid stalk.

In Mammalia the degeneration is far more complete, and all trace of the ancestral importance is completely lost.

There now remains for consideration the two classes, Urochorda and Cephalochorda; with regard to the latter it is very difficult, if not impossible, to homologise any part of its nervous system with the epiphysis of higher forms; the persistent anterior neuropore described by Hatscheck may perhaps be homologous with that of other forms of Chordata which closes during development, though even this must be regarded as extremely doubtful owing to its position considerably posterior to the anterior end of the notochord; neither can it for the same region be considered the homologue of the epiphysis, which again lies posterior to the neuropore. The azygos pigment spot described as an "eye" has no apparent

the parietal foramen, presumably filled during life by the epiphysis. In addition to this, Professor Cope points out a large posterior process leading back towards the optic lobes and roof of the thalamencephalon, and which recent work on living forms can scarcely leave room to doubt represents the flattened pineal stalk.

resemblance in position or structure to the pineal eye of Lacertilia.

As figured by Langerhans[1] and Nüsslin,[2] it is a pigment spot within the walls of the neural canal, and lies anterior to the part shown subsequently by Hatschek to be the anterior neuropore; whereas if it were the homologue of the azygos eye of Tunicata it must lie posteriorly to this.

Turning to the Urochorda a structure is at once met with which naturally suggests comparison with the pineal eye. Yet, however tempting it may be to homologise the azygos Tunicate eye with the latter, it cannot be too clearly pointed out that the two organs differ fundamentally in structure and position, and we have not the slightest reason for supposing that the pineal eye is the direct representative of the Tunicate eye. In the first place, the internal position of the latter clearly distinguishes it from the pineal eye; even supposing the tunicate organ to, in some way, undergo evagination there still remains the difficulty that the retina corresponds in position to the part which after evagination would give rise to the lens, whilst the latter structure is perfectly distinct in nature and formation from that of the pineal eye.

The curious formation of the lens in Tunicates from the union of two or more separate parts, differing in shape and quite distinct from that of Lacertilia in their relationship to the retina, is an important point of difference, and renders it quite impossible, whatever may be the case with the retina, to homologise the lens in the two forms. At the same time there is considerable analogy between the two lenses, inasmuch as each is formed directly out of the walls of the neural canal, a point in which they at once agree with one another, and differ from every other Vertebrate. Notwithstanding this it must, I think, be admitted that the vesicular nature of the eye in Lacertilia and the formation of the lens out of a portion of the vesicle, constitutes a difference of fundamental importance between the two eyes in their fully-developed condition.

[1] 'Arch. f. Mikr. Anat.,' Bd. xii, Tf. 12, fig. 17.

[2] 'Zur Kritik des Amphioxusauges,' Otto Nüsslin, Tübingen, 1877.

Whilst it must be admitted that we are without evidence sufficient to warrant us in regarding the pineal as the direct representative of the azygos Tunicate eye, it is, perhaps, worth suggesting that there may be some connection between the larval eye of Tunicata and the epiphysis of higher forms. It may be pointed out, first of all, that the position of the eye and that of the rudiment of the epiphysis is the same with regard to the anterior end of the notochord, both, further, being situated on the dorsal surface of the "brain," applying this term to the anterior vesicle of the neural canal in Tunicata. It must, however, be also noticed that the eye of the latter is placed not exactly medianly, but slightly to the right side.[1] There still remains the great difficulty of the transformation of the internally placed eye into an external hollow process of the brain roof.

According to Kowalewsky,[2] the Tunicate eye first appears as a thickening of the dorsal wall of the brain cavity, in one particular portion the cells becoming cylindrical and much elongated, and pigment appearing at their internal ends. The refractive structures forming the lens are produced subsequently, so that at first the eye is merely a specially thickened part of the roof of the brain cavity, and only at a later period appears to assume its distinctly internal position, bulging out into the cavity (cf. figs. 32, 34).

Turning now to the epiphysis, we find that it arises as a hollow outgrowth from the brain roof, presenting, as a rule, nothing comparable to the structure of the Tunicate eye. In one form, however, amongst Amphibia, it is just possible that we meet with an indication of a connection existing between the two. A further examination in other forms, particularly those of Pisces, might possibly reveal a similar

[1] Ahlborn draws attention to the slightly asymmetrical position of the epiphysis in Petromyzon, where it becomes, by secondary growth, united to the left ganglion habenulæ; but since the eye of Tunicates is on the right side, it is difficult to imagine any connection between the two.

[2] 'Arch. f. Mikr. Anat.,' Bd. vii, 1871, pl. xii, figs. 32 and 34.

method of development; at any rate, without laying undue stress upon the example to be quoted, it is worth while drawing attention to it, inasmuch as it reveals to us the possible path by which the epiphysis of higher forms has been developed out of a structure similar to the larval Tunicate eye at an early stage. De Graaf, in his recent memoir,[1] figures and describes the development of the epiphysis in Bufo cinerea. His figures are, unfortunately, not drawn with such regard to histological detail as could be desired in the present instance, but, so far as they go, they indicate the possible existence of a connection between the epiphysis of Bufo and the azygos eye of the embryo Tunicate. He shows the epiphysis as arising at first as a thickening of the roof of the thalamencephalon, which soon assumes the form of a slight hollow outgrowth. On the inner surface of the cells, sharing in the thickening and subsequent outgrowth, is a small but well-defined mass of pigment. This pigment very soon entirely disappears, and a hollow process—the epiphysis—is formed, which gradually increases in size, and becomes differentiated into a vesicular distal portion and a solid stalk, the former gradually becoming constricted off. Is it not possible that in these phenomena we have an indication of the change from the internally situated Tunicate eye into an externally placed hollow process? As before said, the Tunicate eye arises as a distinct thickening of the brain-roof, the cells forming the thickened portion bearing pigment on their internal ends. Just the same phenomena are witnessed in the case of the epiphysis of Bufo cinerea, but, instead of developing into an eye internally placed, the cells, whose external ends already form a bulging on the outer surface, form into a well-defined evagination, the internally placed pigment disappears, and the epiphysis, as present in all the higher groups of the Chordata, is developed.

Whether we are here presented with an epitome of the steps passed through during transformation of the internally-placed eye of a transparent organism into the externally-lying evagina-

[1] Op. cit., pl. iii, figs. 22 and 23, p. 27.

tion of a creature whose skin has become opaque, and to whom an eye within the brain has become useless, it would be extremely difficult to say with certainty;[1] it is, however, worth while calling attention to the fact that the epiphysis in very early stages in its development in Bufo cinerea resembles the Tunicate eye before the appearance of refractive elements, whilst subsequent loss of pigment and evagination transforms it into the epiphysis of the adult.

If there be any truth in the above hypothesis it follows that we must start with a form which may be regarded as the common ancestor of present Tunicata and the higher Chordata; in this, which closely resembles an embryonic Tunicate, certain cells of the dorsal wall of the neural cavity are specially elongated and bear pigment at their internal ends, just as in the embryo Tunicate eye and Anuran epiphysis. From this point development leads in two directions—(1) to the highly developed internal eye of present Tunicata with its secondarily developed refractive structures, and (2) by evagination and loss of pigment to the epiphysis of higher Chordata. Subsequent differentiation in the latter results in the formation of a distal vesicle united to the brain roof by a stalk, at first hollow and afterwards solid, whilst finally the distal vesicle becomes modified into the pineal eye.

The evolution of the epiphysis is represented diagrammatically

[1] It will be seen that this differs from the suggestion of Professor Lankester that the internal eye of Tunicates by evagination forms the Vertebrate eye. In the first place I suppose the evagination to give rise to the epiphysis, subsequent differentiation of the distal vesicle of which gives origin to the pineal eye. Secondly, I assume the development of the Tunicate eye and the epiphysis out of an ancestral form common to Tunicata and the higher Chordata, development taking place along two different lines and being possibly connected with the transparency of the one and the opacity of the other form. At the same time it may be pointed out that it is possible that the paired eyes may be formed by evagination of paired internal eyes similar to the one which becomes transformed into the epiphysis. The vesicles giving rise to the paired and pineal eyes are precisely similar to each other, and may have originated in the same way, the two types of eyes being entirely the result of the development of secondary structures.

in the figures on Pl. XX, which show the various stages passed through before the highest form of development is reached, and also the various forms as the result of degeneration. Each stage save the earliest ones (1, 2, and 3) which are found in the development of Tunicata and Bufo cinerea, represent the permanent condition of the epiphysis in some living form.

The question now arises, is it possible to determine at what period or rather within what group of animals the distal vesicle first became differentiated into a pineal eye. There must clearly have been a period during which the hollow epiphysial evagination was not functioning as an eye, precisely in the same way in which the primary optic vesicles must have existed as hollow outgrowths of the brain before they, in like manner, were differentiated into optic organs ; in fact, the three distinct stages of (1), a hollow bladder-like evagination (fig. 4) ; of (2), a distal vesicle connected by a hollow stalk (fig. 5) to the brain ; of (3), a vesicle connected with a solid stalk (fig. 6), must necessarily all have intervened before the final stage (fig. 7) was reached. When in any particular form we find one of these three stages are we to assume that in that given form, and hence in the closely allied members of the same group, the epiphysis has never in its philogenetic history reached a higher stage of development than the one in which it is now present ? Suppose, for example, that we find an animal in which the epiphysis has the form represented in fig. 5, must we take it for granted that in that animal and its ancestors no higher stage of differentiation has ever been reached. Taking the animals in which this particular stage is permanent, we find that they include certain Elasmobranchs together with Cyclodus gigas amongst Lacertilia. Now we have clear evidence that, in the forms from which we must suppose Cyclodus in common with all other lizards to be descended, as well as in its nearest living allies, the epiphysis is developed into a pineal eye. To what conclusion must we come in the case of Elasmobranchs; certainly the non-development of a pineal eye in living examples is no proof whatever that such a structure was not present in its ancestors. It must at once

be granted that an Elasmobranch, such as Raja or Acanthias, differs from Cyclodus inasmuch as none of its living allies have the organ more highly developed, whilst in forms allied to Cyclodus it is in a high state of development; yet even this is by no means of so great importance, as to make us conclude that living forms present us with the highest stage yet reached in Elasmobranchs. If we turn to the Amphibia we find a group of animals amongst whom in no living form is there a pineal eye present, and yet we may feel perfectly sure that in the great group of extinct Amphibia (Labyrinthodonta) one was not only present but most probably developed to its highest point. It must be admitted that we have at present no direct evidence of the existence of pineal eye within the group Pisces: until our knowledge is far greater with regard to the development of the structure in, more especially Dipnoi and Ganoidei, it will be impossible to determine the question of the presence or absence of the structure within the group. Meanwhile, the varied state of development seen in such forms as Petromyzon on the one hand, and Acanthias, Raja, and Scyllium on the other, may perhaps be taken as evidence tending in favour of the view that in its present form the organ is rudimentary. All that may now be rightly insisted upon is that the absence of the eye in living forms, either of this or of any other class, is no proof that one has not been present at some period in the phylogenetic history of the group.

The conclusions, finally, to which we are brought are the following:

(1) Our present knowledge is not great enough to allow us, in Amphioxus, to homologise any structure either with the Tunicate azygos eye or with the epiphysis.

(2) The epiphysis of higher Chordata is the homologue of the larval Tunicate eye.

(3) The pineal eye is produced as a secondary differentiation of the distal part of the epiphysis.

(4) There is not sufficient evidence to prove or disprove the existence of the organ within the group Pisces; it was present in extinct Amphibia, and is found amongst living forms only in Lacertilia.

(5) In all forms at present existing it is in a rudimentary state, and though its structure is better developed in some than in others, it is perfectly functional in none.

(6) It was present most highly developed in

(1) Extinct Amphibia (Labyrinthodonta), and

(2) The large group of extinct forms (as Ichthyosaurus, Plesiosaurus, Iguanodon, &c.) which may be regarded as ancestors alike of living Reptilia and Aves.

(7) The pineal eye may probably be most rightly considered, as peculiarly a sense organ of pre-Tertiary periods.

EXPLANATION OF PLATES XIV, XV, XVI, XVII, XVIII, XIX, & XX,

Illustrating Mr. Baldwin Spencer's Paper on "The Presence and Structure of the Pineal Eye in Lacertilia."

List of Reference Letters.

Ant. (*Le*). Cells of anterior wall of distal vesicle of epiphysis. *C.* Cilia of cells lining epiphysis. *Ca.* Capsule of connective tissue enclosing the pineal eye. *Car.* Cartilage within skull in Hatteria. *Cb.* Cerebellum. *C. H.* Cerebral hemispheres. *Co.* Cone-shaped bodies of pineal eye. *Co.*^{1} Modified cone-shaped bodies lying near the pineal stalk. *Cor.* Cornea. *Ct.*, *Ct.*^{1}, *Ct.*^{2}, *Ct.*^{3}, *Ct.*^{4}, *Ct.*^{5}, *Ct.*^{6} Connective tissue in various positions in connection with the parietal foramen and pineal eye. *Ct. pig.* Pigment in the cutis vera. *Cut.* Cuticle. *De.* Dermis. *D. M.* Dura mater. *Ep.* Epidermis. *Ep.*, *Ep.*^{1} Epiphysis. *Ep.* 1. Swollen distal end of epiphysis. *Ep.*^{1} (*ops.*) Portion of epiphysis equivalent to the pineal stalk. *Hum.* Humour of eye. *Inf.* Infundibulum. *Le.* Lens. *Md.* Medulla oblongata. *Mes.* Mesencephalon. *Mo.* Molecular layer of eye. *N.* Nuclei of cells of epiphysis walls. *N.*^{1}, *n.*^{1} Internal row of nuclei in retina. *n.*^{2}, *N.*^{2} External row of nuclei in retina. *n.*^{3} Specialised nucleated elements in pineal stalk of Hatteria. *ne.* Nerve-fibres. *N. ct.* Nuclei of connective tissue. *Olf.* Olfactory nerve. *Op.*, *Op. v.* Optic vesicle. *Op. S.* Pineal stalk. *Op. L.* Optic lobe of brain. *Pa. for.* Parietal foramen. *Pa.*, *Par.* Parietal bones. *Post.* (*R.*) Cells of posterior wall of swollen end of epiphysis in Cyclodus. *pig.*, *pig.*^{1}, *pig.*^{2}, *pig.*^{3}, *pig.*^{4} Pigment developed in various positions in connection with the eye. *Proc.* Processes uniting various retinal elements in Varanus. *Re.* Retina of pineal eye. *R.*, *r.* Rods of retina. *R.*^{1} Specialised rods in connection with entrance of nerve-fibres. *R. Mp.* Rete mucosum. *S.* Spindle-shaped elements of retina. *Thl.*, *Th.* 3*rd vent.* Thalamencephalon and 3rd ventricle. *Vent.*^{3} 3rd ventricle.

PLATE XIV.

Fig. 1.—Longitudinal vertical section through the parietal foramen of Varanus giganteus. The right side lies posteriorly, the left anteriorly, and the parietal bone enclosing the foramen is shaded yellow. The connective tissue is seen forming a dome to the foramen and filling up the latter. The pineal eye is cut through in the median line, showing the lens with its special development of pigment in the optic axis and the retina with the elongated rods where the nerves enter the vesicle. The nerves are three in number, two

joining into one and the two main strands then uniting to form the solid pineal stalk. The large blood-vessel accompanying the stalk enters the foramen, together with the latter.

Fig. 2.—Longitudinal vertical section through the connective tissue capsule containing the pineal eye of Hatteria punctata. The right side is the anterior, the left the posterior, the external surface of the head being parallel to the breadth of the plate. The capsule is formed anteriorly by the connective tissue filling up the parietal foramen. Into the capsule passes a blood-vessel, which ramifies amongst loosely scattered connective-tissue fibres. The anterior part of the capsule is comparatively empty, but special fibres pass from the walls to the sides of the lens. The optic vesicle is cut through in the median line, showing the cone-shaped lens and the elements of the retina together with the pineal stalk entering posteriorly.

Fig. 3.—Section through the retina of Hatteria punctata. The left is the internal, the right the external surface. Internally the shade indicates the fluid within the vesicle, bounding the cavity of which are the rods lying in pigment. External to the rods lie the inner spherical-shaped elements, then the molecular layer, and external to the latter larger spherical bodies together with conical and spindle-shaped bodies, the latter two being in connection with nerve-fibres. (In the figure the nerve-layer has been drawn so as to appear more prominent than it is in reality.)

Fig. 4.—Section through the portion where the pineal stalk enters the walls of the vesicle. The specialised bundle of rods lying in the optic axis, with the nuclei in connection with them, are seen together with the retinal elements around the entrance of the nerve-fibres of the stalk. The fibres run round in front of the capsular-like structure which contains the specialised nucleated elements, sending some to these on either side, the remainder passing on and either (1) entering into connection with the elements nearest to the pineal stalk, or (2) passing farther on to form a layer of nerve-fibres on the external surface of the vesicle.

Fig. 5.—Separated rods from the retina of the pineal eye of Hatteria punctata. The pigment is so deposited as to produce the effect of striations.

Fig. 6.—Section through the retina of Varanus giganteus. The rods lie embedded in pigment on the internal surface, passing into the cavity of the vesicle; the shade on the left indicates the humour within the latter. The reticular nature of the retina external to the rods is seen, the nuclei of the spherical elements being coloured red. The internal spherical elements are situated within the molecular layer; amongst the external ones are large masses of pigment; more external still is a thin layer of nerve-fibres, and outside this the connective-tissue fibres enclosing the optic vesicle.

PLATE XV.

Fig. 7.—Longitudinal vertical section through the median line of the head in Hatteria punctata in the region of the parietal foramen. The relative positions of the epiphysis, the pineal stalk, and pineal eye, are seen together with the plug of connective tissue filling up the foramen. In front of the epiphysis is the vascular roof of the thalamencephalon.

Fig. 8.—Diagrammatic side view of the brain of Hatteria punctata. The brain is lying in its cartilaginous case. From the thalamencephalon between the cerebral hemispheres and the optic lobes arises the epiphysis, which at first running almost directly upward, turns forwards on reaching the cartilaginous roof as far as the parietal foramen, where the pineal stalk pierces the cartilage and enters the optic vesicle, which is seen lying in its capsule.

Fig. 9.—External view of the modified eye-scale of Cyclodus, showing the modification to form a cornea.

Fig. 10.—External view of the scales in the median line of the head of Varanus giganteus, showing the scale modified as a cornea.

Fig. 11.—The pineal eye of Anolis (sp.?) removed, together with the brain membranes, and viewed as a solid object by transmitted light.

Fig. 12.—The pineal eye of a small specimen of Varanus bengalensis, ying within the parietal foramen and viewed from the under surface.

Fig. 13.—The modified eye-scale of a small Calotes (sp.?), with the transparent cornea in the middle through which the eye is seen.

Fig. 14.—The pineal eye of the same Calotes, whose scale is figured in Fig. 13, removed with the dura mater and viewed as a solid object.

Fig. 15.—Scales from median line on head of a large specimen of Iguana tuberculata, showing the modified eye-scale with cornea.

Fig. 16.—Modified eye-scale of a young Iguana, showing the transparent central portion with the eye beneath as a dark spot.

PLATE XVI.

Fig. 17.—Longitudinal vertical section through the parietal foramen of Varanus bengalensis, showing the pineal eye and the hollow epiphysial stalk immediately beneath. The yellow shade indicates bone. *Ep.*[1] Hollow epiphysial stalk.

Fig. 18.—Longitudinal vertical section through the distal part of the epiphysis of Cyclodus, showing the swollen extremity and the hollow epiphysial stalk connecting this with the brain. *Ep.* 1. Swollen extremity. *Ep.*[1] Epiphysial stalk.

Fig. 19.—Section through a part of the upper wall of the swollen extremity of the epiphysis in Cyclodus. *C.* Cilia of cells bounding the cavity of the epiphysis. *Ant.* (*Le*) The elongate cells, equivalent to those forming the lens of the parietal eye in other forms. *n.* Oval nuclei of the cells.

Fig. 20.—Section through portion of the under wall of the same. *Post.* (*R.*) Ends of the cells facing into the cavity in the position of the rods of other forms. *n.* Circular nuclei of the cells.

Fig. 21.—Longitudinal vertical section through the parietal foramen of Chameleo vulgaris, showing the optic vesicle, pineal stalk, and epiphysis. The yellow shade indicates the parietal bone.

PLATE XVII.

Fig. 22.—Longitudinal vertical section through the parietal foramen and pineal eye of Leiodera nitida. The great elongation of the cells of the rete mucosum is drawn, and the entire absence of pigment from the cutis vera above the eye indicated.

Fig. 23.—Pineal eye of Iguana tuberculata, cut in section and removed from the parietal foramen. When in position the optic axis looks directly upwards.

Fig. 24.—Longitudinal vertical section through the parietal foramen of Anolis (sp.?), showing the pineal eye lying within the vacuolate tissue, together with the pineal stalk.

Fig. 25.—Longitudinal vertical section through the parietal foramen of Anguis fragilis, showing the pineal eye separated from the proximal portion of the epiphysis and the forward extension of the latter.

Fig. 26.—The eye of Lacerta viridis, viewed as a solid object lying within the parietal foramen.

PLATE XVIII.

Fig. 27.—The pineal eye of Lacerta ocellata, viewed as a solid object lying within the parietal foramen, one half of which has been cut away. The eye lies within a mass of branched pigment cells, amongst which ramify the branches of the blood-vessel which accompanies the pineal stalk.

Fig. 28.—The brain of Lacerta ocellata, with the pineal eye lying in the dura mater, viewed from the side.

Fig. 29.—The brain of Cyclodus gigas, viewed from the side, with the epiphysis stretching forwards and upwards and ending in a swollen part within the parietal foramen surrounded by pigment. (The foramen should not be closed above.)

Fig. 30.—Longitudinal vertical section through the pineal eye of Lacerta ocellata, showing the double nature of the nerve.

Fig. 31.—Diagrammatic longitudinal vertical section through the brain of Calotes ophiomaca, to show the pineal eye lying within the parietal foramen and its relationship to the epiphysis, and of this to the brain.

Fig. 32.—Longitudinal vertical section through the pineal eye of Seps chalcidica, showing its relationship to the foramen and its surrounding of deep pigment.

Fig. 33.—Longitudinal vertical section through the pineal eye of Calotes ophiomaca, showing its relationship to the parietal foramen and the blood-vessel within the latter.

PLATE XIX.

Fig. 34.—Diagrammatic longitudinal vertical section through the parietal foramen of Varanus giganteus, showing the eye within the parietal foramen and the pineal stalk.

Fig. 35.—Diagrammatic longitudinal vertical section through the median line of the brain of Plica umbra, to show the eye and its relationship to the pineal stalk and epiphysis.

Fig. 36.—Diagrammatic side view of the brain and pineal eye of Moloch horridus, viewed as a solid object, the eye lying within the parietal foramen.

Fig. 37.—Modified median eye-scale of a small Varanus bengalensis.

Fig. 38.—Modified median eye-scale of Leiodera nitida.

Fig. 39.—Modified median eye-scale of Agama hispida.

Fig. 40.—Diagrammatic longitudinal vertical section through the brain of Chameleo vulgaris, to show the distal vesicle with the pineal stalk.

Fig. 41.—Diagrammatic longitudinal vertical section through the brain of Varanus bengalensis, showing the eye lying in the parietal foramen, and the pineal stalk with its swollen extremity beneath the eye.

PLATE XX.

Diagram illustrating the development of the epiphysis from an internally placed eye in the "brain" of an ancestor common to Tunicata and higher Chordata. Figs. 1—7 illustrate the evolution of the organ till its highest stage of development is reached. Figs. 2 and 3 are diagrammatised from those of two stages in the development of the epiphysis in Bufo cinerea, as given by de Graaf. Fig. 1 represents an early stage of development, according to Kowalevsky, in Tunicates, before the formation of a lens. In higher Chordata loss of pigment and evagination produce the epiphysis, which may in various forms reach different stages shown in the figures. The cross-line shading indicates the parietal bone. Figs. 9—12 representing various stages of degeneration in forms in which the parietal foramen becomes closed. All the figures are, of course, perfectly diagrammatical.

On the Life-History of Pedicellina.

By

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With Plates XXI and XXII.

During the summer of 1885, spent in Rocquaine Bay, Guernsey, I succeeded in obtaining material for the study of the metamorphosis of Pedicellina echinata, a form which occurs in great abundance (in Rocquaine Bay) on Coralline growing under the shade of other seaweeds in tide-pools.

The larvæ of Pedicellina invariably refused to fix themselves when kept in a small quantity of water, and I therefore ultimately adopted the following method for procuring the various stages necessary for the investigation.

Adult colonies were placed, after the removal of all superfluous parts of the Coralline on which they were growing, in a small vessel, the mouth of which was closed by a piece of linen. The vessel was then left for a day or more in a tide-pool, after which a careful search (with the aid of a low power) over the Coralline was generally rewarded by the discovery of several young Pedicellina, which had resulted from larvæ hatched in the tide-pool, and which, owing to their inability to escape from the vessel in which they were confined, had been obliged to fix on the Coralline. After preservation with corrosive sublimate and decalcification of the Alga, sections were easily prepared. In this manner, I succeeded in obtaining numerous individuals of various ages, fixed under perfectly normal conditions.

My study of the metamorphosis of Pedicellina has led me in the main to a complete confirmation of the account already given by Barrois (No. 3), and summarized on pp. 312 and 313 of my previous paper on Loxosoma (No. 4), where I have ventured on a criticism of Barrois' conclusions which I do not find to be justified by my own investigation of the subject. In opposition to my previous opinion, I must now conclude that the post-larval changes consist in a remarkable metamorphosis, and that the first bud is formed after the primary individual has acquired its adult characters. Barrois has published no figures illustrative of his statements, the actual details of the process being difficult to understand from his very short description, whilst the morphological nature of the changes remains entirely obscure. The subject appears to me, therefore, to deserve further consideration.

The structure of the larva is well known from the researches of Hatschek,[1] and it will be unnecessary to describe it in more than a few of its details.

In the swimming attitude of the larva, the ciliated ring is everted to the exterior, whilst from the oral face project two prominent structures;—the epistome, with its tuft of long cilia, and the anal cone, on which opens the anus. During the retracted condition, however, the ciliated ring is reflected to the interior of the large vestibular cavity, whose outer walls are formed by the fold of skin which bears the ciliated ring itself (cf. Pl. XXI, fig. 1). The floor of the vestibule is constituted by the ventral or oral surface of the larva, being specially depressed between the base of the epistome and the anal cone, and at the sides of the latter.

As Barrois has correctly stated, fixation takes place by the oral surface, the larva being meanwhile in its "retracted" condition. Pl. XXI, fig. 1, a median longitudinal section, will serve to illustrate the method of fixation. It will be noticed that the long axis of the stomach is approximately parallel to the surface of attachment.

[1] *Vide* the summary of Hatschek's results in Balfour, 'Comp. Emb.,' vol. i, pp. 242—246.

Fig. 3 represents a horizontal section of a larva not long after its fixation: the occurrence of brain (= "dorsal organ," *v.* No. 4), œsophagus, and rectum in the figure sufficiently defines the level of the section. The epistome is cut in the region of its greatest thickness, whilst at the summit of the anal cone is seen the depression into which opens the anus. By comparing this with fig. 1, it will be observed that the anus has already altered its position, since it is now directed somewhat forwards, the rectum being more nearly parallel to the stomach than before. The cells of the vestibular epithelium are very high at the sides of the anal cone, and are characterized by the special readiness with which they take up colouring matters.

Fig. 5 represents a horizontal section through the apices of the epistome and anal cone of another individual of the same age. The epistome is here seen to be continuous, at each side, with a fold of vestibular epithelium; epistome and folds together forming (as seen in this section) a horseshoe-shaped ridge partially embracing the sides of the anal cone, in which region the two lateral folds become evanescent. The result of this arrangement is the formation of a somewhat deep ciliated groove (*o. g.*) running round the greater part of the vestibule, and passing in front into the transversely elongated, funnel-shaped mouth. Posteriorly, however, owing to the disappearance of the lateral folds, the oral grooves fade away at the sides of the anus, where vestibule and oral grooves consequently appear continuous in such a section as that represented in fig. 5. The relations of these structures will become more clear on reference to fig. 4, a larva somewhat older than those previously described, the section passing transversely through the region of the anal cone, in the plane A B in fig. 1. At the sides of the anal cone are the two lateral portions of the vestibule (*l. v.*), these structures being separated from the oral grooves by the folds already mentioned. In the more anterior sections of the series, the lateral folds become continuous with the epistome, and the oral grooves with the mouth. Further back, on the contrary, the folds become lower, and finally dis-

appear, so that the oral grooves are not distinguishable in the post-anal region of the vestibule. The above description, together with a reference to fig. 5, will thus show that the deep post-anal groove (*m. v.*) of fig. 1 is continuous equally with the oral grooves and with the general vestibular cavity. For further clearness, dotted lines in the same figure indicate the position and relations of the right lateral fold as it would appear by looking at the wall of the vestibule from the inside of the latter. The relations of half of the ciliated ring and of the right oral groove are also shown in the figure.

Fig. 2 represents a longitudinal section of a recently-fixed larva, passing in the direction of the line C D in figs. 3 and 4. One of the lateral folds, owing to its projection inwards into the vestibule, separates the latter into two portions, containing respectively the mouth (and oral groove) and part of the epistome. The latter portion obviously corresponds to one of the lateral regions of the vestibule (*l. v.*) in fig. 3. Fig. 2 further explains the continuity of the tip of the epistome with the lateral folds (cf. figs. 1 and 5). In more median sections of the same series the latter are not seen, the epistome being perfectly free at its apex, whilst the separation of the vestibular cavity into two parts is not apparent.

A considerable portion of the base of the epistome and of the sides of the anal cone is formed of a remarkable tissue, composed of large cells, with transparent contents, hardly staining with colouring matters (fig. 2, *x*). The nature of this tissue (which atrophies during the metamorphosis) is unknown to me.

The revolution (about to be described) of the alimentary canal was obviously well understood by Barrois, although I did not formerly succeed in making out his exceedingly concise statements on this head.

Figs. 8 and 9 represent two sections of an obliquely longitudinal series through a more advanced stage. Fig. 9 involves the rectum, whilst fig. 8 shows the mouth and œsophagus. In the latter figure is seen one of the deep portions of the vestibule lying at the sides of the rectum, which is itself of course

not visible. The dorsal organ and the sucker have both degenerated, and are represented merely by the "globules" described by Barrois in various parts of the larva after its metamorphosis. These "globules" are rounded nucleated cells, which do not stain readily with reagents, their general form being shown in fig. 8, &c.

It is obvious, from an inspection of the two sections figured, that the stomach has now taken up a position inclined to the surface of attachment, the concavity of the alimentary canal being directed somewhat backwards.

Remarkable changes, already described in part by Barrois, have by this time occurred.[1]

Fig. 9 shows that the aperture of the vestibule has closed, so that this cavity has no longer any communication with the exterior. The vestibule is partially divided into three portions, which do not, however, quite correspond with those described by Barrois. The most ventral portion (*v. v.* in fig. 9) corresponds to the region near the previous vestibular aperture, and is destined to atrophy completely. The next portion (*v. or.*) is in connection with the mouth (fig. 8), whilst the most dorsal portion (*v. an.*) contains the anal cone, and is at this stage and later the largest and most important part of the vestibule. The second or oral division still communicates with the ventral portion, whilst it is almost separated from the dorsal or anal division by the growth of the epistome and of the lateral folds.

In another section of the series it is seen that the oral and anal divisions of the vestibule still communicate by a small aperture, as in the diagram, fig. 16 (*a. v. v.*).

The anal portion of the vestibule is very large, and is growing, at the previously posterior end of the larva, away from the surface of attachment. The cells lining this part of the vestibule are obviously engaged in active growth and multi-

[1] The following statements will be more readily understood with the assistance of Pl. XXII, fig. 16, representing in a diagrammatic form a median longitudinal section through an individual of the same age as figs. 8 and 9.

plication, their protoplasm being finely granular and staining readily with colouring matters. The backward growth of the vestibule occurs first in the regions at the two sides of the anal cone (cf. fig. 3), but soon extends to the median portion behind the cone (fig. 9), so that this part of the vestibule grows towards the free end of the fixed larva, during the rotation of the alimentary canal, as a single actively extending diverticulum, in which the primary differentiation of median and lateral regions is no longer marked.

Fig. 6 will serve to explain more clearly the relations of the oral grooves and neighbouring structures at a stage very slightly earlier than that of figs. 8 and 9. The section passes in a direction corresponding to the line K L in fig. 16, and consequently involves the apex of the epistome, the lateral folds, and the oral grooves. The anal cone, visible in fig. 5, is, of course, not involved by the section, which in other respects differs from the former figure mainly in the facts that the diameter of this portion of the vestibule has become lessened, and that by the partial rotation of the alimentary canal the apex of the epistome has come nearly into contact with the posterior wall of the vestibule (the manner in which this happens will be understood by comparing fig. 1 with fig. 16), the form of the lateral folds being at the same time altered (cf. fig. 6 with fig. 5). By this change of position of epistome and lateral folds, the oral and anal sections of the vestibule communicate merely by a comparatively small round aperture. The oral grooves are no longer continuous posteriorly with the anal portion of the vestibule, although on the left side of the section at least, a trace of the former continuity is distinguishable. During later stages the growth of epistome and lateral folds completely separates the oral from the anal division of the vestibule, the aperture *a. v. v.* in fig. 6 being gradually constricted until it finally disappears.

At the stage of figs. 8 and 9 a considerable amount of histolysis is taking place. This process affects specially the stomach, the epistome, the anal cone, and the ventral portion of the vestibule. In the case of the stomach, portions of the

epithelial cells and some of their nuclei pass bodily into the lumen of the organ (cf. figs. 8 and 9), where they are found quite free at later stages. The more projecting parts of the epistome and of the anal cone lose most of their component cells. The cilia of the latter become indistinct, the cell-substance itself obviously degenerating (fig. 9). Ultimately ciliated portions of the cells are thrown off into the vestibule (figs. 9 and 12), in which they can be discovered until a very late stage in the metamorphosis. They no doubt leave the vestibule either by the mouth or by the (adult) vestibular aperture, when the latter is formed.

The histolysis of the ventral portion of the vestibule (fig. 9, *v. v.*) similarly results in the passage of fragments of cells into its own cavity.

This process is again illustrated by fig. 12, a section passing in the plane of the line E F in fig. 9. The permanent vestibule is in this section (cf. fig. 16) completely separated from the degenerating portion, its lumen, like that of the latter, containing fragments of degenerating cells.

The ventral division of the vestibule (*v. v.*) in fig. 9 occupies the position of the future stalk, and in later stages its cavity becomes more and more reduced until it finally atrophies. During this process, the cells previously found in its lumen disappear. In sections parallel to the plane of attachment the cavity (just before its atrophy) appears as a fine tube surrounded by a series of elongated cells radiating from it towards the body wall. It is very tempting to assume that these cells are phagocytes, engaged in the destruction of the vestibule. After the atrophy of the latter, its place is occupied by numerous "globules" (fig. 10), which will themselves be replaced by ordinary connective-tissue corpuscles (fig. 13).

The same assertion may be made of other parts of the "primary body cavity," which is at the stage of fig. 9 almost completely filled with "globules," resulting from the histolysis of the brain, the sucker, the tissue at the base of the epistome and anal cone, and other larval structures. When the primary individual is mature the "globules" have disappeared, and are

replaced by a gelatinous matrix, in which lie connective-tissue corpuscles. Are we not justified in assuming that the "globules" are the active agents in the histolysis, and that they are in fact typical phagocytes?

During the histolysis of portions of the anal cone, the latter structure itself becomes much depressed. This feature of the metamorphosis, although already obvious in fig. 9, may be further illustrated by means of fig. 7, a section passing in a plane corresponding to the line I J in fig. 16.

Owing to the further depression (occurring at a slightly later stage) of the anal cone, the marked bilateral arrangement of this part of the vestibule is, in part at least, lost. At the stage of figs. 8 and 9, as can be easily seen from these figures themselves, the posterior portion of the vestibule is no longer reduced in the median plane to a small slit between anal cone and vestibular wall (as in fig. 1), but is, in this position also, a spacious cavity lined by a columnar epithelium (fig. 9).

After the anal cone has reached the condition of the latter figure the vestibule, in sections parallel to the long axis of the stomach, will usually appear bounded posteriorly by a simple uniformly curved wall, whilst its œsophageal side is floored by the degenerating tissue of the epistome (fig. 7). In later stages, however, the well-developed epithelium of the sides of the vestibule extends inwards, so that the cavity is then entirely bounded by its permanent, partially regenerated epithelium.

In the next stage represented very considerable changes have occurred, whereby the alimentary canal has taken up a position not unlike that which it will ultimately retain. Fig. 10 represents an actual section which passes in the median longitudinal plane of a larva at this stage. Whereas in fig. 16 the axis of the stomach is but slightly inclined to the surface of attachment, in the present instance it has assumed a position almost at right angles to this plane, and the concavity of the gut is now directed towards the primitively posterior end of the fixed larva. In the course of this rotation of the alimentary canal the vestibule, owing to atrophy of one

at least of the portions described in the last stage, has become somewhat simplified. All the more ventral regions (situated in the neighbourhood of the surface of attachment) have completely disappeared, and in their place is found a mass of cells filling a cylindrical stalk, which obviously corresponds to that of the adult Pedicellina. The anal division of the vestibule has continued its backward growth and now lies almost at the free end of the young animal. At about this stage it acquires a secondary opening to the exterior on the side corresponding to the posterior surface of the larva. This opening is formed by a simple concrescence between the vestibular epithelium and the external ectoderm of the body, accompanied by a linear perforation formed at the point of junction of these two distinct portions of ectoderm. My sections have given me no indication of the occurrence of a "labial invagination" (Barrois, q. v.) placing the above portion of the vestibule in connection with the exterior.

The character of the vestibular aperture, immediately after its formation, may be seen from fig. 11, a section passing in a plane corresponding to G H in fig. 10. The vestibular aperture, at the sides of which tentacles (*t.*) are already developing, is shown, by an examination of the remaining sections of the series, to have the form of a slit elongated in the direction of the median plane of the animal. Immediately before the formation of the aperture the vestibular epithelium would appear, in a section of this kind, quite unconnected with the external ectoderm, but already extending towards it in the form of a median groove, similar in appearance to the portion *g. v.* in fig. 11.

The mouth in fig. 10 has, at first sight, the appearance of being closed. By a comparison, however, of fig. 10 with fig. 16, it would seem that the apex of the epistome is really represented (in the former) by the ectoderm closing the (permanent) mouth, and it is thus probable that the commencement of the digestive tube in fig. 10 (*v. or.*) is a part of the oral division of the vestibule. This impression is strongly confirmed by a section (not figured) similar to, but later than,

fig. 9. In the individual referred to, the stalk portion of the vestibule is still present, but is small, and is connected with the œsophagus very much as in the diagram fig. 16; i. e. at some distance from the point where the apex of the epistome ultimately meets the vestibular wall.

In somewhat later stages the permanent mouth is formed by the perforation of the septum between the two portions of the vestibule in fig. 10, and probably in the position of the aperture *a. v. v.* in fig. 16.

In living individuals of the same age could usually be discovered a small projection of the surface of the body in the region marked *?s.* in fig. 10. This represents the larval "sucker," which, as Barrois has correctly stated, disappears during the metamorphosis. The region of the "dorsal organ" or brain of the larva is doubtless indicated by the marked angle on the left side of the stalk of the individual just referred to. None of the previous histological peculiarities of the organ remain at this stage, and it is in fact already almost impossible to distinguish with certainty its position.

It appears to me that Barrois has suggested the real explanation of the metamorphosis of Pedicellina, although he has confined himself to one or two short statements, which are given without any indication of the manner in which they are to be interpreted. I quote below one or two passages from Barrois' note so many times referred to (3), the given quotations reproducing, so far as I am aware, the whole of Barrois' explanation of this complicated subject.

(i) "La première position" [corresponding, from the description, with my own fig. 10] "représente un état tout à fait analogue au Loxosoma, avec anus en haut et œsophage en bas."

(ii) "L'inférieure" [portion du vestibule] "qui porte la couronne, et dont les éléments viennent former la glande du pied."

(iii) "Les deux organes énigmatiques de l'exoderme" [i. e. sucker and dorsal organ] . . . . "ne sont, suivant moi, que des organes provisoires; tous deux sont rejetés sur la face

dorsale, où ils finissent par disparaître, peu à peu. Sans doute il faut voir, dans les deux soies décrites par Salensky sur la face dorsale du Loxosoma crassicauda, le reste de l'organe des sens antérieur" [i. e. the dorsal organ] "qui, d'après mes recherches, vient occuper cette place."

I have already (4) explained my reasons for the belief that the dorsal organ at any rate, and perhaps the sucker, are important organs, which throw considerable light upon the mor phology of the Polyzoa, so that I cannot accept Barrois' conclusion that these structures have no particular significance.

It is obvious that, however accurate Barrois' conclusions (quoted above) may be, they need further explanation. The similarity between larva and adult in the Entoprocta, even in the position of the buds in Loxosoma, is so striking that some means of comparing the two stages is necessary. I therefore suggest the following explanation of the relation between larva and adult.

It does not seem to me that Caldwell's theory of the surfaces of the Polyzoa receives any support from the metamorphosis of Pedicellina. The short line between mouth and anus remains unchanged throughout the metamorphosis, and in order to prove that it is not ventral, it still remains necessary to show that the dorsal organ of the larva is not a brain, and that the larval surfaces do not correspond with those of a Trochosphere.

Figs. 17—19 (Pl. XXII) are diagrams representing a possible explanation of the metamorphosis of the Entoprocta, but although founded on the history of Pedicellina, Loxosoma is the form which is actually (hypothetically) represented.

Fig. 17 explains a possible conception of one of the earlier stages in the acquirement of the sessile habit by the free-swimming Polyzoon ancestors. The form is, however, to all intents and purposes, a Loxosoma larva, with brain, sub-œsophageal ganglion (not discovered in Pedicellina until a stage later than fig. 10), and a pair of buds, one of which is shown. I believe there are no authentic instances of the fixa-

tion of a Polyzoon larva by any other than its oral surface, and it may therefore be assumed that this method of fixation was acquired at a very early stage in the phylogeny of the group. Let us suppose, however, that this "Archi-Loxosoma," on fixing itself by the edge of its vestibule, left an aperture (for the entrance of food), surrounded by the ciliated ring (*vide* fig. 17), leading from the exterior into the otherwise closed vestibule, and situated behind the anus.

Subsequent development may be imagined to give rise to a form like fig. 18, in which the vestibular opening is an elongated slit, extending along the whole of the region formerly occupied by the posterior side, and still surrounded by the ciliated ring. The mouth, in order to obtain its food as conveniently as possible, now faces the posterior side (of the former stage), and this has entailed a rotation of the entire alimentary canal, in the manner shown in fig. 18.

By the growth of the proximal end of the Polyzoon, the mouth would be thrust away from the point of support, and the animal might thereby obtain an advantage in procuring food by means of its ciliary currents. But during this process, the proximal portions of the ciliated ring would become far less efficient for obtaining food than the distal portions, and would tend to atrophy. The final result would be the acquirement of a form like fig. 19, representing in a very slightly diagrammatic form, an adult Loxosoma. The ciliated ring is here represented as consisting of two disconnected portions, corresponding (1) to the ring of tentacles; (2) to the foot-gland (cf. the second of Barrois' conclusions quoted on p. 248). The foot-gland has remained practically as an open groove, a series of ciliated tentacles having been developed round the margin of the permanent vestibule.

The position of the buds in the larval Loxosoma appears at first sight fatal to the above hypothesis. That this larva does actually develop buds normally can hardly be doubted, since I have shown not only that these structures are developed twenty-four hours after hatching (which might, however, be an abnormal circumstance, due to the want of proper conditions

for fixation), but also that ectodermic thickenings, the commencements of the buds, are to be detected some time before the embryo is ready to leave the maternal vestibule, the possibility of the development having been influenced by abnormal conditions being here out of the question.

In figs. 17, 18, and 19, the position of the dorsal organ is represented as not having been much altered during the rotation of the alimentary canal, which has, so to speak, been pulled through the loop formed by the dorsal organ and the somewhat hypothetical subœsophageal ganglion. Assuming for the moment this position for the dorsal organ, we find that throughout the metamorphosis the buds retain their original situation (in Loxosoma) between the dorsal organ and the ciliated ring, and that their position with regard to the œsophagus is practically the same as that which characterised them at their first appearance.

Is there, however, any reason for believing that the position of the dorsal organ is correctly indicated in the diagrams? It seems to me that this question must be answered in the affirmative. In the first place, the degenerating dorsal organ of Pedicellina does in reality occupy this position, and in the second place (*vide* No. 3 of Barrois' conclusions on p. 248), the circumœsophageal commissures may be represented by the strong ganglionated nerves passing from the ganglion to the "posterior sense-organs" in L. crassicauda, as originally described by Salensky (see also No. 4, Pl. xix, fig. 1). Should the metamorphosis of Loxosoma be proved to bear out this suggestion of Barrois', we must assume either that the whole brain has atrophied, or that the adult possesses at most a small portion of the brain at the ends of the two widely separated œsophageal commissures.

With regard to the actual metamorphosis of Pedicellina, I have to point out that I have not succeeded in demonstrating the presence either of œsophageal commissures or of a subœsophageal ganglion. The latter structure becomes distinct only at a stage later than fig. 10, and it then has the position which characterises the adult ganglion.

No. 1 of Barrois' conclusions quoted on p. 248, appears to me perfectly just. It is impossible in fact not to be struck with the great resemblance between the solitary Pedicellina shown in fig. 10 and an adult Loxosoma, and this similarity is quite conspicuous even at much later stages. The obliquity of the lophophore in Loxosoma is hence, on the view already explained, another of the archaic features of this genus, the lophophore having still a marked inclination to the "anterior" side of the animal (fig. 19).

It is unfortunate that the metamorphosis of Loxosoma, which possesses a foot-gland, should be unknown, but we are able to make certain inferences from the phenomena of budding. Both vestibule and foot-gland originate as longitudinal groove-like invaginations of the ectoderm of the "anterior" face of the bud. Fig. 15 is a reproduction of a drawing from Oscar Schmidt, in which the foot-gland is represented as originating from the two proximal cells of the ectoderm of the "anterior" side of the bud, and in which it is further seen that these cells are not in the least marked off from those which are taking part in the formation of the vestibule. The relations of lophophore and foot-gland in this figure are indeed exactly those of the ciliated ring in the diagram (fig. 18).

The Metamorphosis of Pedicellina viewed in its relation to the above Hypothesis.

I have no reason to believe that the position of the ciliated ring shown in fig 1 is in any way altered during the subsequent metamorphosis. This structure in all probability degenerates in situ.

The ciliary apparatus of an ordinary Trochosphere is not, however, constituted entirely by the præoral circlet. In the neighbourhood of the latter there occurs in Polygordius, e. g., (cf. Hatschek, No. 2) a series of smaller cilia forming a postoral circlet, whilst a third part of the apparatus is constituted by "a ciliated groove running between the two ciliated rings, and prolonging itself into the ciliated mouth." This

last portion is obviously represented in Pedicellina by the ciliated oral grooves, continuous, as in Polygordius, with the mouth. The relations of these grooves during the metamorphosis appear to me to deserve further consideration.

We have found that the median postanal portion of the vestibule is continuous with the oral grooves, of which it may, indeed, be said to form a part. Acccording to Hatschek (1) it is, like other portions of the vestibule, lined by ciliated cells.

If we are justified in assuming that the oral groove—a part of the typical Trochospheral ciliary apparatus—extends, potentially at least, from the mouth completely round the vestibule to the postanal region, it seems to me that considerable light is thrown on the metamorphosis. The morphological position of the oral groove will be in no way altered during the rotation of the alimentary canal, and in fig. 16 it will continue to pass from the mouth round the ab-anal side of the altered lateral folds to the median post-anal portion of the vestibule, even though it is no longer distinguishable in the persisting division of the latter structure. In figs. 16 and 6 we observe, however, the commencement of a separation of the oral groove into two parts—one continuous with, and becoming indistinguishable from, the "oral" section of the vestibule (*v. or.* in fig. 16), and the other potentially passing from the free apex of the epistome in fig. 16 to the end of the reference line *m. v.* in the same figure. The position of this latter portion will be the median line passing from *a. v. v.* to *m. v.* Owing to the fact that it is situated behind the anal cone it is, of course, unpaired (cf. fig. 5), and it appears to me that its situation may be very fairly considered to be represented by the linear groove which in fig. 11 has formed the permanent vestibular aperture. From the margins of this groove are developed the tentacles, which, if the above reasoning is legitimate, are formed from the region of the oral groove.

The fact that the tentacles of the adult lophophore of the oral side are on the ab-anal side of the mouth appears to me

to prove that the lophophore itself is developed from a morphologically præoral portion of the oral groove.

The relation between the velum proper and the oral cilia has become, in the Entoprocta, considerably complicated by the formation of a fold of integument (vestibular wall), carrying the former to some distance from the latter. When the Pedicellina larva attaches itself, the distance between the two structures becomes increased. The velar portion maintains its position at fixation, and soon atrophies; the oral groove, on the contrary, growing away from the degenerated velum. Even during the phylogenetic history of the process we may suppose that the velum atrophied at fixation. This is par excellence a locomotive structure, and would be useless in an attached condition. The oral cilia would, however, continue (in the hypothetical stage of fig. 18) to convey food to the mouth, and the cells bearing them would, after a time, become prolonged into tentacles, by which their range of activity would be extended.

During the abbreviated metamorphosis of Pedicellina it has hence resulted (if the above be true) that the velum takes no part in the change of position involved in the passage to the adult condition.

Summarizing the above, I may express my conviction (1) that the metamorphosis of Pedicellina is a simple modification of a more archaic process, due to abbreviation of development, (2) that the oral groove persists in part as the adult lophophore, (3) that the vestibule closes at fixation, and undergoes the whole of its alterations in the interior of the larva, opening secondarily only when the adult condition is practically attained.

The adult form is reached by the elongation of the stalk of fig. 10, and by the replacement of its contained "globules" by characteristic connective-tissue and muscle-cells; by the formation of a stolon and a diaphragm, and by various alterations in the calyx. The more important of these consist in the complete (or almost complete) loss of the obliquity of the lophophore, in the development of the permanent ganglion

and generative organs (if these are formed in the primary individual, as is probably the case) and in the complete formation of the vestibular aperture and tentacles. I have made no special observations on most of the above points, although on the important question of the origin of the colony from the primary individual, I am able to throw some light.

In the first place, it may be stated that adult colonies are by no means restricted to one growing point, as stated by Hatschek (1). Of very common occurrence is the development of two growing points, one at each end of the unbranched stolon: I have noticed this even before the formation of a single secondary calyx. A third growing point may be developed as a lateral branch of the main stolon; the amount of branching is, however, always slight in P. echinata, and apparently in all cases the œsophagus of each calyx is on the side directed to the growing point to which this calyx properly belongs, as already indicated by Hatschek.

The formation of the stolon is shown in fig. 13, a longitudinal section of the stalk of a completely developed but still solitary individual. The young stolon, which is cut medianly, is developed on the œsophageal side of the Pedicellina. The base of the stalk (which is alone represented) consists of a thick cuticle, underneath which occurs a layer of ectoderm, surrounding a gelatinous matrix in which lie connective-tissue and muscle-cells. The section, however,—an extremely good preparation—is contradictory to the theory of Hatschek, according to which the apex of the stolon is provided with a hypoblastic vesicle derived from the dorsal organ, and engaged in the formation of the mid-gut of the secondary calyces. I may at once state that I have entirely failed to convince myself of the occurrence of any such vesicle, at any period, in the stolon, and I am forced to believe that Hatschek has been mistaken in assuming its existence. Neither in sections nor in entire specimens (whether living or treated with reagents) could I discover the slightest evidence of the presence of Hatschek's vesicle, although I have investigated both adult and young stolons in this connection.

It appears to me probable that the growing point of the stolon of Pedicellina (*vide* fig. 13) consists solely of an ectodermic layer secreting a cuticle and of a mass of indifferent mesodermic connective-tissue cells, embedded in a structureless jelly. If this is the case, the only organ derived from the hypoblast of the embryo would appear to be the mesenteron of the primary individual, all other parts of the colony being devoid of any derivatives of hypoblast cells.

This conclusion can hardly be avoided unless we assume that some of the stellate cells of fig. 16 are really hypoblastic in nature, although indistinguishable from the mesoderm cells in their appearance. Owing to the nature of the process by which the dorsal organ degenerates, it is impossible to assert that some of its cells do not become amœboid wandering cells which migrate into the growing point. It can, however, be safely stated that no hypoblastic vesicle is formed from the degenerating dorsal organ. It may further be pointed out that the conclusion arrived at on a previous occasion as to the nervous (epiblastic) nature of the dorsal organ, in Pedicellina as in Loxosoma, is in opposition to the view that this structure plays any part in the budding.

The well-known fact that calyces of Pedicellina may fall from their stalks, which thereupon develop new calyces, appears to me in direct contradiction to Hatschek's view of the budding. The loss of the calyces is probably a normal, periodically occurring process, which is perhaps to be regarded as a means of rejuvenescence, and which is at least analogous to the formation of the "brown bodies" in the Ectoprocta. It is exceedingly easy to discover individuals in healthy colonies in which the calyx has been lost, and a new "bud" (easily recognised by its small size and immature condition) is being developed just below the scar. Specimens kept in captivity seem invariably to lose their calyces if the quantity of water is not very large, the calyx falling off at the "diaphragm." This structure, which is merely a constriction at the base of the calyx, filled by a row of flat cells, is perhaps a special arrangement by which the calyx can break away from the stalk, without

injury to the latter. I have been unable to show that calyces which have thus left their stalks are able to become the starting-points of fresh colonies. The specimens under observation have invariably died after a day or two, even if kept in a tide-pool.

Calyces formed at the scars produced in the manner above indicated, seem to me (from superficial examination of entire specimens) to develop in exactly the same manner as those produced at the true growing point. The occurrence of this phenomenon is undoubtedly adverse to Hatschek's theory of budding; the whole of the stomach falls away with the calyx, whilst the existence of a plug of cells filling up the diaphragm appears to preclude the possibility of the migration of any cells derived from the stomach to the proximal side of the diaphragm. Unless, indeed, it is assumed that some of the "connective-tissue" cells of the stalks as well as of the stolon are endodermic in nature, it must be concluded that none of the cells of the bud are descendants of any of the cells belonging to the embryonic hypoblast.

With regard to the further history of the budding (whether at the growing point or at the apex of an old stalk) I have very little to say. The free end of the stolon (or stalk) before long develops an ectodermic invagination (fig. 14) destined to give rise to the lophophore and, according to my view, to the whole of the alimentary canal of the bud. The latter is from the first continuous with the lophophoral rudiment, and in other sections of the series to which fig. 14 belongs, the stomach and vestibular cavity are separated from one another by means of a septum. The latter does not, however, cut off the whole of the deepest part of the invagination, but, since it is not developed in the position of the œsophagus the vestibule and stomach remain continuous with one another (as in fig. 14). By the formation of a diaphragm and by other processes already described by Hatschek, the bud attains its adult condition. The continuation of the stolon is formed by a lateral outgrowth from that region in the young bud which afterwards becomes the base of its stalk, precisely as in fig. 13 with the exception of the fact that the new growing point is formed long before the

bud is itself mature. It is worthy of remark that the young vestibular invagination does not occur accurately at the apex of the stolon, but on the side of the apex turned towards the growing point. In this respect it exactly agrees with the position of the vestibular invagination formed near the apex of a stalk which has lost its calyx, and again with that of the incompletely rotated vestibule in intermediate stages of the metamorphosis. It may indeed be said that the young vestibule of all the buds is inclined towards the growing point, and that in all cases it subsequently undergoes a rotation in the same direction (but to a less marked degree) as that occurring at the metamorphosis.

The history of the Pedicellina-larva appears to me to point to the existence of a fixation-period in Loxosoma also. In this case, the buds observed by me in the larva of L. Leptoclini would probably have to undergo a change of position, during the metamorphosis, similar to that represented in figs. 17—19. I am inclined to believe that the degeneration of the larval stomach observed in the same species, after a free life of one or two days, was abnormal, and was due to the absence of the conditions necessary for fixation.

On the Nature of the "Brown Bodies" of the Ectoprocta.

The above statements with regard to the life-history of the Entoprocta may, perhaps, give some indication of the manner in which the "brown bodies" of the Ectoprocta have originated. There can probably be no longer any doubt whatever that these structures are degenerated polypides, which are subsequently replaced by new ones budded off from the walls of the zoœcia.

In the metamorphosis of Pedicellina the purely larval organs degenerate and form a mass of cells, which subsequently become connective-tissue cells. The degeneration is here slight, and has not yet acquired sufficient importance to give rise to a characteristic "brown body."

Whilst in the adult Loxosoma nothing comparable to the formation of "brown bodies" is known, the adult Pedicellina has developed a special arrangement—the constriction at the base of the calyx—by which the latter may be lost without material injury to the remainder of the colony.

In the adult Ectoprocta there seems to be the same necessity for the rejuvenescence of some of the organs, but here the occurrence of a thick ectocyst, usually intimately connected with that of neighbouring individuals, in general prevents the loss of any part of the body wall, as in Pedicellina. In some of the stoloniferous Ctenostomata, however, the entire zoœcium is deciduous.

But even in Pedicellina one may almost speak of a "zoœcium" in the same sense as in the Ectoprocta. It is a well-known fact that septa occur at intervals across the stolon of Pedicellina, and in most cases are developed in such a manner that a piece of the stolon, connected with the base of each stalk, is cut off from the remainder of the stolon by a pair of symmetrically-placed septa. There are thus typically two septa between the bases of each two stalks, and stalk-bearing and stalkless sections of the stolon alternate regularly with one another.

It is thus possible to consider stalk plus portion of stolon connected with it, the representative of a zoœcium. The distal end of the zoœcium is from time to time segmented off, carrying with it the whole of the alimentary apparatus, whilst a new polypide is developed within the remaining portion by a process of budding. By the formation of a new constriction the distal part of the zoœcium—the calyx—becomes again differentiated from the proximal part—the stalk.

In the Ectoprocta the occurrence of the same process is usually obviously impossible, and the polypide alone degenerates, forming a "brown body" which subsequently passes into the new stomach, and is ejected by the anus. The occurrence of this circumstance is already foreshadowed in two particulars in Pedicellina. We find, in the first place, that a new polypide is actually budded off by the ectoderm of the zoœcium at or

before the loss of the calyx; and, in the second place, that the tissues have already acquired, at the metamorphosis, the power of disposing of degenerated structures.

In the Ectoprocta one may hence suppose that, owing to the inconvenience of losing a portion of the zoœcium at each rejuvenescence, the new polypide is budded off near the preceding one, instead of from an entirely different part of the zoœcium, as in Pedicellina (below the diaphragm). The degenerating alimentary canal and other structures are then worked up by the "Parenchymgewebe" (Vigelius), which has inherited this kind of power from the larval tissues, into the condition of a "brown body," which passes into the new stomach, and reaches the exterior by means of the anus.

In the development of the Ectoprocta an archenteron is formed, in a large number of cases at least. The embryo is, however, richly supplied with yolk; it develops within the interior of the parent, and its alimentary canal is hence, in many cases, functionless.

At its metamorphosis this larva possesses no functional alimentary canal, and must hence form a new one. But since in its previous phylogenetic history our Polyzoon has acquired the power of developing new "polypides" from various parts of its ectoderm, a fresh gut could without difficulty be formed within the body wall of the metamorphosed larva; since the latter is now in the same condition as an adult zoœcium whose polypide has just become a "brown body."

This, indeed, is what actually happens. The larva passes at once into the condition of a zoœcium containing a "brown body," the remains of its larval organs. The complicated metamorphosis of Pedicellina has been given up, the larval structures now degenerating by the method employed during the atrophy of the polypides in adult individuals, and finally leaving the zoœcium by passing as the first "brown body" into the alimentary tract of the primary polypide, and thence to the exterior.

The metamorphosing Ectoproctan larva is probably in the same condition (irrespective of the difference pointed out

in the methods by which the alimentary canal is lost in the two cases) as the primary individual of a Pedicellina colony would be immediately after the loss of its calyx, supposing that it had not meanwhile developed a stolon and secondary calyces.

Unless I am mistaken in my views with regard to the metamorphosis of Pedicellina, it appears to me necessary to conclude that in the Entoprocta the ventral line of the body extends from *a. v.*^{2} in figs. 10 and 19, down the right sides of the figures, as far as *a. v.*^{1} The median dorsal line will in consequence be represented by the entire left sides from *a. v.*^{1} to *a. v.*^{2} These surfaces are most clearly expressed in the young Loxosoma bud, in which the whole of the surface turned away from the parent (characterised by the possession of vestibule and foot-gland) is ventral, whilst the opposite surface of the bud is, conversely, dorsal.

I hope to be able before long to publish some account of the development and metamorphosis of the Ectoprocta. Till that time I prefer to withhold any further expression of opinion with regard to the surfaces and relations of the larvæ of this group of the Polyzoa.

List of Papers Referred to.

1. B. Hatschek.—"Embryonalentwicklung und Knospung der Pedicellina echinata," 'Zeits. f. wiss. Zool.,' Bd. xxix, 1877, S. 502.
2. B. Hatschek.—"Studien zur Entwicklungsgeschichte der Anneliden," 'Arb. a. d. Zool. Inst. zu Wien,' Bd. i, 1878, S. 277.
3. J. Barrois.—"Métamorphose de la Pédicelline." 'Comptes rendus de l'Acad. des Sci.,' T. xcii, 1881, p. 1527.
4. S. F. Harmer.—"On the Structure and Development of Loxosoma," 'Quart. Journ. Mic. Sci.,' vol. xxv, 1885, p. 261.

EXPLANATION OF PLATES XXI & XXII,

Illustrating Mr. S. F. Harmer's Paper on "The Life-history of Pedicellina."

Reference Letters.

an. Anus. *an. c.* Anal cone. *a. v.*[1] and *a. v.*[2] Hypothetical morphologically anterior and posterior ends, respectively, of the vestibular aperture. *a. v. v.* Aperture between oral and anal divisions of vestibule (in position of permanent mouth). *b.* Bud. *br.* Brain (= "dorsal organ"). *c. c.* Fragments of ciliated cells. *c. p.* Ciliated pit of brain. *c. r.* Ciliated ring. *d. s.* Dorsal sense-organ (of Loxosoma). *epst.* Epistome. *f. br.* Fibrous part of brain. *f. g.* Foot-gland. *ga.* Ganglion of adult. *g. p.* Growing point of stolon. *g. v.* Median groove of permanent vestibule, ultimately becoming the vestibular aperture (in position of part of oral groove of larva ?). *int.* Intestine. *l. f.* Lateral fold of vestibular wall. *l. v.* Lateral portions of anal division of vestibule. *m.* Mouth. *mes.* Mesoderm. *m. v.* Median postanal portion of the anal division of the vestibule. *œ.* Œsophagus. *o. g.* Oral groove. *rec.* Rectum. *s.* Sucker. *st.* Stomach. *t.* Tentacle. *v.* Vestibule. *v. a.* Its aperture. *v. an.* "Anal" division of vestibule. *v. or.* "Oral" division. *v. v.* Ventral division. *x.* Large-celled tissue at base of epistome and anal cone.

PLATE XXI.

Pedicellina echinata.

Fig. 1.—Median longitudinal section of a larva quite recently fixed (on Coralline).

Fig. 2.—Obliquely longitudinal section (in the plane C D in figs 3 and 4[1]) of a similar larva.

Fig. 3.—Horizontal section of a slightly older larva, passing through brain (= dorsal organ), œsophagus, epistome, and anal cone.

Fig. 4.—Obliquely transverse section (in the plane A B in fig. 1), at a stage very soon after fixation.

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[1] In describing one section as passing in a plane indicated in the figure of another, it is to be understood that the details in the two individuals do not always exactly correspond. This is due, partly to a difference in age between the two larvæ figured, and partly to variations in the position of the internal structures, owing to varying conditions of muscular contraction.

Fig. 5.—Horizontal section, at an early stage in the metamorphosis, passing through the tip of the epistome, the lateral folds and oral grooves, and the apex of the anal cone.

Figs. 6 and 7.—Two sections of a considerably older individual, passing respectively in the planes K L and I J in Fig. 16.

Figs. 8 and 9.—Two sections of an individual of the age of Fig. 16, passing in an obliquely longitudinal direction. Fig. 8 cuts the mouth and one of the lateral portions of the permanent vestibule, Fig. 9 passing through the rectum and the degenerating vestibule of the stalk. In another section of the same series the two parts of the vestibule are continuous, exactly as in the diagram, Fig. 16.

Fig. 10.—Median longitudinal section of an advanced, but still solitary, individual.

Fig. 11.—Horizontal section (in the plane G H in Fig. 10) through a similar specimen.

Fig. 12.—Section of an individual of the age of Figs. 8 and 9, passing in the plane E F in the latter figure.

Fig. 13.—Median longitudinal section through the stalk of a solitary individual with commencing primary stolon. The arrow indicates the position of the oral side of the calyx.

Fig. 14.—Obliquely transverse section of a young bud, developed at the growing point.

PLATE XXII.

Fig. 15.—Young bud of Loxosoma, from the ventral side. Copied from O. Schmidt, 'Arch. f. mik. Anat.,' Bd. xii, 1876, Pl. III, fig. 17.

Fig. 16.—Diagrammatic longitudinal section of a metamorphosing Pedicellina at the stage of Figs. 8, 9, &c.

Figs. 17—19.—Diagrams illustrating the supposed morphological nature of the metamorphosis of the Entoprocta. A full explanation is given in the text.

Dr. Dohrn's Inquiries into the Evolution of Organs in the Chordata.

By

J. T. Cunningham, B.A., F.R.S.E.

Seven years elapsed from the publication of the 'Ursprung der Wirbelthiere' before the appearance of the first of Dohrn's 'Studien zur Urgeschichte des Wirbelthierkörpes,' that on the mouth of Teleosteans. As he points out in a short preface to that paper the three chief peculiar articles of faith in his previous essay, in comparison with the views current at the time, were that the ancestors of Vertebrates closely resembled Annelids, that the principle of change of function was the safest guide in tracing morphological histories, and that the extent to which degeneration might proceed was unlimited.

In the attempt to reconstruct the Vertebrate ancestor, Dohrn has concentrated his attention almost exclusively on the actual structure and development of the organs of existing Vertebrates, convinced that a great deal of what was generally believed concerning the relation of the organs was inaccurate, and that no light could be thrown on the question by hasty conclusions drawn from superficial resemblances of the organs of Vertebrate and other embryos, until the organisation of the Vertebrates themselves was more thoroughly investigated.

The following is a list of the studies with the dates of publication :

1882. I. "Der Mund der Knochenfische."
II. "Die Entstehung und Bedeutung der Hypophysis bei den Teleostiern."
1883. III. "Die Entstehung und Bedeutung der Hypophysis bei Petromyzon Planeri."

1884. IV. "Die Entwicklung und Differenzirung der Kiemenbogen der Selachier."
V. "Zur Entstehung und Differenzirung der Visceralbogen bei Petromyzon Planeri."
VI. "Die paarigen und unpaaren Flossen der Selachier."
1885. VII. "Entstehung und Differenzirung des Zungenbein und Kiefer Apparates der Selachier."
VIII. "Die Thyreoidea bei Petromyzon, Amphioxus, und den Tunicaten."
IX. "Die Bedeutung der Unpaaren Flosse für die Beurtheilung der genealogischen Stellung der Tunicaten und des Amphioxus, und die Reste der Beckenflosse bei Petromyzon."
X. "Zur Phylogenese des Wirbelthierauges."

Ancestral Mouth.—In the first of these studies reference is made to the question of the position of the ancestral mouth, which in the 'Ursprung der Wirbelthiere' was located between the crura cerebelli in the fourth ventricle. Professor Fritsch and Mr. Sanders argued that this was an untenable supposition, because it would be impossible to accept the consequence of it, namely, that all the cerebral nerves belonged to a supra-œsophageal ganglion. Dohrn acknowledges the justice of the objection, and provisionally abandons the quest of the ancestral mouth. He has never since resumed the inquiry. He deals with investigations of the development of the actual mouth, the results of which confirm his view that the aperture represents a united pair of gill-clefts. In embryos of Teleosteans he found that there was no stomodæum, and that the mouth arose as a pair of enteric outgrowths which at first opened to the exterior, one on each side, the apertures only subsequently meeting in the middle ventral line.

Hypophysis of Teleosteans and Petromyzon.

The hypophysis also in Teleosteans, according to the second paper of the series, does not arise from an ectodermal oral invagination or stomodæum, but from a pair of endodermal evaginations in front of those which form the mouth. The organ therefore represents a pair of præoral gill-clefts (i. e. it is derived in the Teleosteans from the endodermal parts of

such a pair) which in the actual development of Teleosteans never acquire an opening to the exterior. In a postscript to this paper Dohrn mentions Hatschek's results concerning the origin of the ciliated pit in Amphioxus. This pit is the left of a pair of anterior evaginations of the endoderm, which opens to the exterior while the other remains closed. According to Dohrn these two diverticula are homologous with the hypophysis in the Teleostean, and the opening in Amphioxus is the persistent branchial opening. The ciliated pit of the Ascidians is also homologous with that of Amphioxus. According to Bateson the proboscis cavity with its pore in Balanoglossus is homologous with the ciliated pit in Amphioxus, but whether the body cavity of the proboscis in Balanoglossus can be derived from a pair of gill-clefts is a question which seems to threaten to do away with the possibility of the diagnosis of organs according to their embryological origin.

The hypophysis in Petromyzon has a unique history in the individual, and this forms the subject of the third member of the series. The examination of the embryos of Petromyzon was undertaken by Dohrn in order to prove that the fundamental difference generally supposed to exist between the branchial cartilages of Selachians and of Petromyzon was entirely imaginary, but the discussion of this subject is postponed till the hypophysis has been considered. Scott[1] had stated that the hypophysis of the Lamprey arose as an ectodermal invagination connected with the nasal pit.

Balfour had doubted this result, but Dohrn entirely confirmed it, except that he found the hypophysial invagination to be at first separate, lying between the commencing mouth and nasal cavity, and that he pointed out that the whole long nasal duct of the adult which runs back beneath the brain is as much part of the hypophysis as the follicular organ formed from its inner extremity.[2] The nasal duct is in fact a fused pair of ectodermal

[1] 'Morph. Jahrb.,' vii.

[2] It seems extremely probable, although I am not aware that it has been suggested before, that the nasal duct which in Myxine opens into the pharynx, is homologous with the so-called nasal duct of Petromyzon. If this be so, of

pits originally belonging to the pair of gill-clefts which has been transformed into the hypophysis. The function of the nasal duct in the adult is apparently to draw in water in order that it may reach the olfactory organ and then expel it; it is probably, to use an undignified word, a sniffing organ, necessitated by the disconnection of the mouth from the function of respiration. This new function of the hypophysial gill-cleft could easily be derived from its original one.

Visceral Arches of Elasmobranchs.

In order to demonstrate the fallacy of the argument that the external branchial cartilages of Selachians were the representatives of a primitive "external" branchial skeleton retained in the existing Cyclostomata, it would have been sufficient, says the beginning of the fourth study, to describe the development of these two cartilages (two to each arch) in Selachians and compare it with the quite different history of the branchial skeleton in the Lamprey. But it seemed advisable to give a complete account of the development of the Elasmobranch gill-arch, as previous results were fragmentary.

It is to be understood that a typical arch such as the first, second, or third branchial, is under consideration, not the hyoid or the posterior, which are either modified or reduced. In a horizontal section of the arch towards its middle the cavity of the arch surrounded by its epithelial cells (head-cavity of

course the connection between the pharynx and the nasal pits in Myxine is formed by the hypophysis and not by a nostril properly so named. The hypophysial invagination in the embryo of Petromyzon comes into very close relation with the pharynx as well as with the infundibulum, and on the hypothesis which I have supported in my paper on Kupffer's vesicle, &c., that the infundibulum represents the original mouth, it is easy to understand how a separation between infundibulum and pharynx might occur in either of two ways, by leaving the hypophysis connected only with the infundibulum as in Petromyzon, or by leaving the communication between hypophysis and pharynx still open as in Myxine. In other Vertebrates again the hypophysial invagination has been absorbed into the stomodæum, and reaches from thence to the infundibulum, but has not retained a connection with the pharynx. These speculations can of course only be tested by examination of the development of Myxine.

Balfour and his school) is seen in the centre dividing the section into an anterior and posterior half. This cavity is continuous below with the pericardium. The artery of the arch is on the posterior side of the cavity, or as it is better to call it, from the destination of its walls, of the muscle tube of the arch. The branchial processes grow out first on the posterior side, and along their base appears a vein which opens dorsally into the artery. Similarly on the anterior side appear branchial processes with an anterior vein, also opening into the artery. The two veins become connected by two horizontal commissures. In the adult the posterior vein becomes disconnected from the anterior and unites with the anterior vein of the arch behind it.

The cartilaginous arch arises as a condensation of mesoderm cells posterior to the muscle-plate. Between the upper and lower venous commissures, where the muscle-tube is already diminished in thickness, condensation of mesoderm cells takes place also on the anterior side, and the two condensed masses uniting, eliminate the muscle-tube between them. This separation of the muscle-tube does not take place dorsally and ventrally, because the cartilaginous arch bends inwards in those regions. A central part of the muscle-tube is thus separated and lies on the inner side of the arch; it becomes the adductor arcus visceralis. Both Gegenbauer and Vetter believe the adductor mandibulæ to be homodynamous with the adductor arcus, but this is an error, the former is homodynamous with the whole musculature of one (or more) arch. The external middle portions of the tubes form the musculi interbranchiales; the dorsal, externally the constrictor superficialis, internally the interarcuales. Other muscles come from the ventral portions. The coracohyoid is a true body muscle, and has nothing to do with visceral arches.

The cartilage already described, the middle portion first developed, forms the two middle internodes of the adult arch. Above these dorsally is the basale, below the copulare. The cartilage separates the adductor from the interarcualis above, from the coracobranchialis below. The cartilage is internal to

the artery; and the artery is at first posterior to the muscle-tube. The branchial cartilaginous rays arise as condensations of mesoderm cells separate from the arch, and between the artery and posterior vein. The so-called external cartilages are simply the most dorsal and the most ventral of the series of rays altered somewhat in position, and therefore have no similarity with the arches in Petromyzon, which are true arches.

Branchial lamellæ are never developed on the anterior side of the hyoid arch, or of the spiracular arch. The external filaments of the embryo arise as simple elongations of the posterior lamellæ of each arch, the anterior not elongating at all. A curious suggestion is made concerning the function of these elongated filaments, namely, that they serve to absorb yolk; how the yolk gets into them could not be discovered, but yolk is present in the filaments and in their veins, in the posterior branchial vein, and the efferent arteries, never in the branchial artery or in the heart.

Thymus of Elasmobranchs.

At the time when the external filaments have attained to about half their length, but when the branchial rays are not differentiated, a proliferation of epithelium takes place in the upper angle of the first gill-cleft, forming a kind of bud. Similar buds are formed in the four posterior gill-clefts, but the fifth bud disappears again entirely in the Sharks, but persists in the Rays. These buds form the thymus of the adult. The cause of the separation of these portions of the branchial epithelium is the shortening of the clefts. The upper portion of the original clefts is obliterated by a coalescence of the arches, accompanied by processes of growth which alter the original position of the terminal rays of each series, and so produce the extra-branchial cartilages. The epithelial nodules of the thymus after they have sunk into the mesoderm become associated with mesodermic cells, a process which ought not to excite surprise, since the epithelium in question originally no doubt formed branchial laminæ into which mesoderm extended. The bending of the arches above described is

connected with the formation of the united portions of the musculus constrictor superficialis, but the original cause of the whole process is to be explained only after further investigations have been described. Ecker first definitely described the thymus of fishes in his article "Blood-vessel Glands," in Wagner's 'Dictionary of Physiology,' Bd. iv, but could find no such organ in the Sturgeon, in Cyclostomata, or in Teleosteans. In a foot-note Dohrn points out that the thymus of Teleosteans exists in the position already accurately defined by Leydig in his 'Anat. histol. Untersuchungen über Fische und Reptilien.' In this note also emphatic contradiction is made of Gegenbaur's generally accepted view that the pseudobranchia of Teleosteans is the reduced gill of the hyoid arch, and therefore not homologous with the pseudobranchia or spiracular gill of Elasmobranchs. Dohrn maintains that Johann Müller was quite right in asserting that the pseudobranchia of Teleosteans was homologous with the spiracular gill of Elasmobranchs, and that Balfour, who has been followed by Hoffmann, was mistaken in supposing that in the Teleosteans the choroid gland represents the spiracular gill. Stieda found that the thymus of mammals arose from only one gill-cleft, the last, or last but one; Dohrn states that the carotid gland may possibly represent a rudimentary thymus derived from another cleft.

Branchial Skeleton and Arches of Petromyzon.

After showing that the extra-branchial cartilages of Elasmobranchs are really displaced gill rays, the next point in arguing that the branchial skeleton of Petromyzon is composed of true branchial arches, is to demonstrate the development of this skeleton, and this is the object of the fifth paper. It is known from the researches of Scott and Balfour that the first trace of the visceral arches appears in the form of head-cavities, rounded cell-tubes between the diverticula of the gut, which afterwards form the gill-clefts. The question of correspondence between the head-cavities and the dorsal myotomes is left for a future period. There is a difference between the embryonic gill arches of Petromyzon and those

of Elasmobranchs in the position of the original vessel of the arch. This vessel in the latter forms lies near the outer border of the arch; in Petromyzon it lies as near as possible to the inner surface. The arch elongates and becomes flattened antero-posteriorly; the muscle-tube undergoes a corresponding compression. The cartilaginous arch arises anterior to the muscle-tube, but soon divides this tube in the middle of the arch completely, separating an adductor on the inner side from a constrictor on the outer, as in Selachians. The cells of the anterior wall of the muscle-tube have a remarkable peculiarity. They persist, in embryonic form, as long tubes, which run the whole length of the arch, and show a transverse striation only on the exterior. All the muscles run the whole length of the arch and unite, dorsally as well as ventrally, with those of the other side; the important point about this is that if the cartilaginous rods were to disappear the condition would be the same as that which actually exists in Myxine. The chief difference between the gill laminæ of the adult Petromyzon and those of Selachians is that the former are directed towards the exterior, the latter towards the interior, and this difference appears at their first origin in the embryo. It is probable that the adductors serve as inspiratory muscles by lifting up the ventral side of the branchial region, and so expanding the branchial cavities, while the constrictors are expiratory, their contraction driving the water out.

Thus it is shown that the branchial skeleton of Petromyzon is composed of true cartilaginous branchial arches. It is true that these arches in the Cyclostomata are not segmented, nor are they in the Teleostean; and this shows that Petromyzon is derived from a form more premature than the Selachian, in which the segmentation had not yet occurred. The same truth is indicated by the homology of the hypophysis with the nasal duct, an homology which, as Dohrn frankly acknowledges, was first asserted by Goette in his 'Entwicklung der Unke.' Petromyzon must have branched off from a condition in which the hypophysis was still an independent præoral pair of gill-clefts. That the gills of Petromyzon are homologous with

those of Selachians has been suggested by Huxley and P. Fürbringer, and is by Dohrn's results fully established. Myxine is a further modification of Petromyzon, and shows a remnant of the branchial skeleton in the cartilage of its ductus œsophageo-cutaneus. The internal position of the branchial artery in the embryo Petromyzon is simply explicable as a consequence of the displacement of the branchial lamellæ towards the interior, and this change of position has been brought about by the necessity of protecting the gills which arose when the present habits of the animal (either burrowing in mud or attaching itself to other animals) were acquired.[1]

Thus the theory that the branchial cartilages of Petromyzon represent an archaic system not elsewhere present except in the extra branchial cartilages of Selachians falls to the ground, and with it disappear the consequences which Gegenbaur formerly deduced from it. The Cyclostomata had no jaws it was said because their ancestors had no true gill arches from which jaws might be derived, whereas the truth is probably they have lost the jaws through the conversion of the biting

[1] In my paper on Myxine, in the previous number of this Journal, I have described the habits of Myxine from actual observation. There can be no doubt that during far the greater portion of its time the animal lies motionless, buried in mud, with only the extremity of its snout protruding. In this condition the method of respiration, unique among fishes, namely, the constant passage of a current of water through the nostril to the gill-pouches, is the only method possible. Doubtless this method is also the most convenient when the animal is boring into the body of a fish, or when its whole body has penetrated into the flesh of its prey; and it is difficult to say which of its habits, burrowing or boring into its prey, was the prior cause in producing the existing condition of the respiratory organs. I have not yet ascertained whether the respiratory current is maintained by ciliary action, or by internal muscular action, or by both combined. No muscular respiratory movements are visible externally. Ammocœtes, it is true, burrows, although it has a branchial skeleton; and I do not know how the Ammocœtes, when buried, can carry on the method of respiration which is seen in Petromyzon. Petromyzon never burrows, it conceals itself beneath stones and in crevices, but it could not take in water by all its branchial apertures as it does unless it were surrounded by water free from sediment. The comparison of the habits of Petromyzon and Myxine illustrates the diversity of functions performed by

into a sucking mouth. It was said that they had no limbs because the skeleton of a limb was derived from an arch of the branchial skeleton, and no true branchial arches were present; the truth is that the limbs are not derived from branchial arches, as is now generally acknowledged, and there is a rudiment of the pelvic fin in Petromyzon, to be afterwards described.

The Origin of the Fins of Fishes.

The true history of the origin of the limbs of fishes, paired and unpaired fins, as Dohrn reads it, is set forth in the sixth Study. In the original ancestral condition the Vertebrate body was similar in most respects to that of an Annelid. The medullary tube was an open plate, the intestine extended through the whole length of the body to a terminal anus, and on each segment were two pairs of appendages, processes of the body wall provided with processes of the body musculature, in fact, dorsal and ventral parapodia. The nerve plate was, of course, ventral, when the animal was reversed in position and the plate folded into a tube, the two series of ventral parapodia were brought together in the median dorsal line and coalesced both laterally and longitudinally, forming the dorsal fin, which was originally continuous along the whole length of the body. Another change which took place was that a new anus was formed out of the fusion of two gill-slits, and in consequence

one organ, and the contrast between the functions of homologous organs in two forms. An important function of the sucker-mouth of Petromyzon is to adhere to stones in the bed of a river, and without this power the animal would immediately lose control of its own movements, and be carried away at the mercy of the currents in which it habitually lives. This function is entirely wanting in Myxine, whose mouth is not truly a sucker at all, but a boring apparatus. I have never seen a Myxine use its mouth to attach itself, while Petromyzon never leaves its mouth attachment at one place, except to immediately secure it again at another. Yet the mouth of Myxine can take in food without boring, as is demonstrated every day in the North Sea when the fisherman finds on his lines numbers of Myxine which have taken the baited hook far down into the intestine without using their teeth upon the bait at all.

the postanal gut disappeared; the degeneration of the postanal gut is actually repeated in ontogeny. The contraction of the ventral part of the tail thus brought about caused the series of dorsal parapodia behind the anus to coalesce in the same manner as the ventral parapodia, and thus the median anal fin was produced. The præanal dorsal parapodia were never approximated laterally, but partly disappeared, partly coalesced longitudinally to form the existing pelvic and pectoral fins. The fins therefore have nothing to do with gills, either in the way supposed in Gegenbaur's Archipterygium theory, or in the way originally suggested by Dohrn in the 'Ursprung der Wirbelthiere.' In the theory now taught by Dohrn the metameric external gills of Annelids are left out of consideration; the ancestor, it is to be presumed, had none. The facts on which the theory is based, and which are important results of investigation however explained, are as follows:—The musculature of the pectoral fin is derived in embryos of Elasmobranchs (Pristiurus) from a series of muscle buds separated from the ventral end of each myotome. Each bud divides into four pieces, two above and two below. The same is true of the pelvic fin. That these fins cannot be serially homologous with any parts of the gill arches is proved by the fact that the musculature of the gill arches is derived from the head cavities, and these are ventral to the myotomes. So also the gill cartilages are not homodynamous (serially homologous) with the ribs, for the ribs are between the myotomes, the series of which is continued anteriorly above the gill arches. A large number of myotomes contribute to form each fin. Behind the anus on each side muscle buds are given off from the ventral ends of the myotomes; these are serially homologous with those already described, and in all probability, although the transformation was not traced, they form the musculature of the anal fin. The musculature of the dorsal fins arises from buds given off dorsally exactly as those belonging to the paired fins are given off ventrally. The fin rays in the dorsal fins arise as median cartilaginous rays, at first quite unconnected with any other part of the skeleton. One would have expected

to find, if the theory be true, that these rays were originally double; but Dohrn says nothing of this difficulty, attaching the greatest importance to the musculature. It has been objected to Dohrn's theory by myself and Professor Carl Vogt that in Teleostean embryos there is a præanal median fin in addition to the præanal paired fins; to which Dohrn has replied that it has not been proved that this fin has any musculature, and therefore it is probably a new development peculiar to the class in which it occurs.

Morphology of the Mandibular and Hyoid Arches of Selachians.

We come next to a discussion of one of the most complicated chapters in Vertebrate morphology, the question of the mandibular and hyoid arches in Selachians. We will take a rapid survey of the facts as they exist according to Dohrn's investigations, and then consider the deductions he draws from them. In embryos of Pristiurus, Scyllium, Mustelus, Centrina, Torpedo, and Raja the conus arteriosus at its terminal bifurcation forms the hyoid arteries, the arteries of the hyoid arch. From each of these arteries near its origin arises another artery which runs parallel to and anterior to the hyoid artery. Between the bases of these two lies the thyroid gland, and the arteries are to be called the thyroid arteries. The hyoid artery supplies only one series of branchial laminæ, the posterior. There is also but one branchial hyoid vein, the posterior. There is only one venous commissure from the hyoid vein instead of two as in the posterior arches, and this commissure opens into the thyroid artery. The art. thyroidea has hitherto been called the art. mandibularis. The thyroid artery, after receiving the venous commissure, is continued into the spiracular artery. The hyoid vein divides dorsally into two branches, one of which runs back and joins the dorsal aorta system, the other runs forward as the carotis posterior, joins for a short distance behind the hypophysis with the same vein of the other side, then separates running one each side of the hypophysis, the vein of each side receiving a large vein from the spiracular gill.

The musculature of the hyoid arch is peculiar in this respect, that no internal portion of the muscle-tube is segmented off by the cartilage, and accordingly no adductor is formed. The musculi interarcuales are also absent, and there is a complicated system of ligaments fastening the hyomandibular cartilage. The ventral muscles, on the other hand, are similar to those of the posterior arches.

With regard to the cartilage of the hyoid arch, development shows that in the Sharks the upper middle internode, dorsal to the venous commissure forms the hyomandibular, no separate basale or dorsal internode is formed; but, as the hyomandibular carries a number of branchial cartilage rays, and also a dorsal ray, which is homodynamous with the upper extra-branchial (so-called) cartilage of the gill arches, it follows that the hyomandibular contains the basale (dorsal internode) of the hyoid arch.

In the Sharks the first rudiment of the mandibular arch appears at the level where the hyoid vein joins the spiracular artery, but unlike the posterior rudiments it consists from the first of two cartilaginous centres: the under becomes the mandible, the upper the upper jaw, the so-called palato-quadrate. No adductor is formed in the mandibular arch. It has been generally taught that the masticatory muscle of the jaws is the homologue of the adductor, but this is not so; no homologue of the adductor is present.

There are no cartilaginous rays on the mandibular arch. The doctrine, therefore, of Gegenbauer and his followers, that the lower and upper jaw are parts of a single cartilage arch equivalent to a posterior gill arch is unfounded.

In the Rays the development of the cartilages of the hyoid arch is quite different to that described for the Sharks.

There are two cartilage-centres, one near the posterior edge of the arch, the other near the anterior side, behind the spiracular cleft; each cartilage has its own muscle system. The first cartilage is separated into a dorsal and ventral part by the venous commissure, and each part bears gill rays. The second cartilage becomes the hyomandibular, it has its own muscle

system which forms the mus. levator. The conclusion which must be drawn is that the hyomandibular in the Rays is a remnant of an arch anterior to and entirely distinct from the hyoid arch, while in the Sharks the dorsal part of the hyoid arch with its rays is fused with the hyomandibular. According to Gegenbaur the hyomandibular in the Rays represents only the mandibular process of the hyomandibular of the Sharks; if this were true there would be no rays dorsal to the venous commissure in the Rays, whereas the fact is that these dorsal rays exist, but the cartilage they belong to is separate from the hyomandibular. Dohrn finally suggests that the upper jaw is also an independent gill arch, and the mandible another, but for the present leaves the further tracing of the transformations for more profound investigations. He concludes the section on the hyoid arch in the Rays with the remark that he is satisfied to dispel the illusion that we already know what we want to ascertain.

The spiracular cartilage is next taken in hand. Dohrn has investigated its origin in Scyllium canicula and catulus, Pristiurus, Mustelus, Raja and Torpedo. He found it always a single cartilage, and states that there is no foundation for the theory that it is either an enlarged single ray, or a combination of rays. It is probably a portion of an independent arch, but what relation this arch bears to others it is at present impossible to say. The adductor mandibulæ is developed from the whole of the walls of the mandibular head cavity, no portion being separated off as an adductor; only one differentiation of a portion occurs, namely, the formation of the levator maxillæ superioris from the part lying nearest to the spiracle.

When it has been postulated that the hyoid arch is really double and contains two arches fused together, it becomes necessary to inquire what has become of the cleft originally existing between these two arches. Has the cleft disappeared without leaving a trace, or has it merely undergone a metamorphosis? Dohrn answers that the pair of clefts, i. e. the endodermal parts of them, have united in the median ventral line and formed the thyroid gland. This organ arises in the

embryo in the middle line very far forward as an outgrowth of endoderm cells close behind the mouth, and subsequently passes backwards losing its connection with the pharynx. In a note Dohrn promises in a future study to discuss the spiracular cleft of the Selachians and Ganoids, and the pseudobranch of Teleosteans, and to show that between the mandible and the hyoid in Teleostean embryos on each side a deep invagination of the ectoderm occurs, which is to be regarded as the ectodermal part of the cleft represented by the thyroid. (It is probable that this invagination is the same as that observed by other embryologists and diagnosed as the Teleostean representative of the spiracle.) In another note it is stated that evidence will at a future time be adduced to show that in the jaw and hyoid system of Teleosteans five independent visceral arches are combined: 1, upper jaw; 2, lower jaw; 3, spiracular cartilage; 4, hyomandibular; 5, hyoid.

The Thyroid of Petromyzon.

The subject discussed in Study VIII is the thyroid in Petromyzon and its homologue in Amphioxus and the Tunicata. In the larval Ammocœtes the first trace of the thyroid appears at the time when the most anterior branchial diverticula of the endoderm grow out. Its first rudiment is a diverticulum directed downwards and somewhat forwards, close beneath the median part of the first pair of branchial diverticula, which is homologous with the spiracular clefts of Selachians and the pseudobranchiæ of Teleosteans. Between the stomodæum and enteron on each side runs the most anterior branchial artery, homologous with the spiracular artery of the Selachians; it opens into the cephalic aorta of its own side, Petromyzon possessing two cephalic aorta one on each side of the notochord. The growth backwards of the mesoderm of the velum causes the opening of the thyroid diverticulum to be pushed farther back, so that it soon comes to lie at the level of the second pair of branchial sacs, and later between the second and third. A sagittal ingrowth of mesoderm now divides the thyroid anteriorly into two halves. On each side another

pushing in forms the glandular lamella, the uninvaginated part forming the cover-lamella. In the glandular lamella a differentiation takes places into conical masses of gland-cells, the apex of the cone turned to the cavity of the gland, and ordinary ciliated cells. In the advanced larva of Ammocœtes two ciliated grooves run transversely in the wall of the pharynx, in front of the gill-sacs, and converge on the median ventral line to meet in the opening of the thyroid. These grooves Dohrn has ascertained to be derived from the endodermal sacs which represent the spiracular clefts, and which in Ammocœtes never acquire an opening to the exterior.

Now the endostyle or hypobranchial groove of Ascidians, e. g. Cione intestinalis or Salpa, is closely similar in histological structure to the thyroid of Ammocœtes. There is the same differentiation into bulbous agglomerations of gland-cells, and a more even layer of ciliated cells. Moreover, in the Ascidian there is a pair of ciliated grooves immediately behind the mouth, which ventrally converge to the hypobranchial groove, dorsally to the ciliated pit (hypophysis). These grooves of the Ascidian must be homologous with those of Ammocœtes, and must therefore represent in the Ascidian the spiracular clefts. And it follows that Tunicates must be derived from fishes, not vice versâ. The reason suggested for the transformation is that the thyroid and spiracular clefts have been converted into mucous-secreting organs to aid in the conveyance of nourishment to the œsophagus.

In Amphioxus there is not a hypobranchial groove, but a hypobranchial ridge, but this ridge has the same histological character as the thyroid in Ammocœtes and the hypobranchial groove in Ascidians. A homologue of the peripharyngeal ciliated grooves is not mentioned as occurring in Amphioxus, and the development of the hypobranchial ridge has not been studied.

The conclusion drawn from all this is that both Tunicates and Amphioxus are degenerate fishes derived from ancestors more or less similar to the Cyclostomata. A difficulty which arises in considering Dohrn's arguments is that no reason is

given why the spiracular endoderm sac should open into the thyroid endoderm sac, since these were presumably originally separate; the spiracle being anterior to the hyomandibular, the thyroid between hyomandibular and hyoid. Dohrn does not mention this question, being satisfied so far to show that the condition of the ciliated grooves in Tunicates is directly derivable from the condition in Ammocœtes. The derivation of the arrangement in the latter from that in Selachians is not discussed.

Rudiments of Paired Fins in Petromyzon.

In the ninth Study Dohrn returns again to the question of the fins. How, he demands, could an animal of the size and complication of the Cyclostomata obtain for itself organs of such fundamental effect on the whole organisation as pectoral and pelvic fins? The question is perhaps not so convincing as he thinks; for, on his own hypothesis, the neural and ventral parapodia must at one time have arisen, and the theory of the evolution of organs is not at present in such a state as to make it any more easy to understand how these organs arose than how limbs could arise in the Cyclostome, unless, indeed, it were postulated that the segmented vertebrate ancestor, with its dorsal and ventral parapodia, was a creation into whose previous origin it were impious to inquire. But what is more to the point is that, although Gegenbaur believed no rudiment of fins could be discovered in the Cyclostomata, Dohrn has discovered in Ammocœtes rudiments of muscle-buds similar to those which in other fishes form the muscles of the unpaired fins. These buds, however, remain as indifferent cells during the Ammocœtes stage, and are only differentiated into the fin muscles when the metamorphosis into Petromyzon takes place. The buds are given off ventrally as well as dorsally, and as the dorsal series forms the muscles of the dorsal fin, the præanal ventral ones must at one time have formed muscles of then existing paired fins. Moreover, there is, according to Dohrn, a rudiment of the pelvic fins in Petromyzon, namely, the longitudinal folds bordering the anus. Below

these folds are a pair of muscles, called by Schneider, in his 'Beiträge zur vergl. Anatomie der Wirbelthiere,' the anal fin muscles. According to Dohrn, these muscles serve to protrude the so-called penis of the male Lamprey. Dohrn raises the question of the possibility of copulation in the Lamprey, a possibility which does not really exist, for in the female there is a protrusible tube at the abdominal pore, which is shorter but otherwise exactly similar to that of the male. Dohrn suggests that the anal fin muscles of Schneider are homologous with the muscles of the pelvic fin in other fishes (Selachians especially).

Origin of the Vertebrate Paired Eyes.

The most recent study deals with the embryology and phylogeny of the Vertebrate eye. It was obvious to previous embryologists that the nervous part of the eye was originally in the wall of the brain. Lankester suggested that the ancestor was at this time transparent, while Balfour believed that though the tissues may have been transparent, the original cause of the outgrowth of the optic vesicle was the covering of the original superficial eye by the formation of the medullary tube. But the starting-point of Dohrn's inquiry is the development of the eye-muscles. Balfour indicated briefly the origin of these muscles from the most anterior head-cavity. Marshall (this Journal, vol. xxi) ascertained that only the rectus internus superior, inferior, and obliquus inferior arose from the præ-mandibular cavity, while the obliquus superior arose from the mandibular, the rectus externus from the hyoid. But Marshall believed that the dorsal parts of the head cavities from which the eye-muscles were formed were homologous with myotomes, and not with the ventral cœlom in the trunk. Dohrn does not agree with this, and holds that the dorsal parts, like the ventral, are not homologous with the myotomes in the trunk, but only with the ventral walls of the body cavity. As a consequence of this it follows that the eye-muscles are true muscles of visceral arches, and must have been originally branchial muscles. The reason why branchial

muscles came into relation with the eye is that the light reached the latter, when the medullary tube began to close, through the ectodermal pit of a præoral gill-cleft. This ectodermal branchial pit is now the lens of the eye, whose peculiar mode of formation is thus explained. The vascular part of the same gill arch is retained in the choroid gland of Teleosteans, which receives its blood supply from the pseudobranchial vein, and the arteria centralis retinæ, which is the efferent artery of the lens branchia. This hypothesis explains the vessels of the campanula Halleri, of the pecten of Reptiles and Birds, the embryonic lens vessels of Mammals, as remnants of the blood-vessels of the branchia represented by the lens. Leaving the eye, Dohrn next goes on to support his view that almost the whole of the head except the brain represents visceral or ventral structures, just as the tail contains only dorsal structures, and asserts his belief that attempts to estimate the number of myotomes in the head are all in vain. In his opinion the cerebral nerves have lost those branches which innervated myotomes and their derivatives, and have, in consequence of the extraordinary enlargement and complication of the ventral region, increased to a corresponding degree their visceral branches, at the same time having undergone great alterations in distribution on account of the changes of relative position among the gill arches. Thus, the attempts of Van Wighe and others to diagnose dorsal branches of the cranial nerves are founded in mistaken views. A ramus dorsalis of a spinal nerve never innervates a mucous tube, any more than the ramus dorsalis, so called, of a cranial nerve innervates myotomes and muscles of a dorsal fin. Again, Dohrn points out how necessary it is to understand more accurately the anatomy and development of the vertebrate organs before constructing complete and simple schemes which reduce the head to a number of myotomes as formerly to a certain number of vertebræ. A great anatomist once said that if he wished to read romances he knew better specimens than histories of creation wherewith to amuse himself, à propos of which Dohrn points out that if phylogenies are to be compared with romances it is as

well to remember that the most sensational are not always the best works of art.

We have thus given a summary of Dohrn's results and indicated the point of view from which he regards the problem of vertebrate phylogeny. The speculations formulated in the 'Ursprung der Wirbelthiere' have been in some cases supported in others overthrown by his later researches, but he still holds strongly to the fundamental thesis that the original ancestor was a segmented animal more or less similar to an Annelid, and that the organisation of Cyclostomata, Amphioxus, and Tunicata can only be explained by profound degeneration. Whatever the fate of his various theories may be in the future of morphology, it is certain that his studies form a massive contribution to the really scientific study of organogeny, and that his independent attitude and stimulating suggestiveness of thought are worthy of his favorite motto, "Was fruchtbar ist allein ist wahr."

REVIEW.

Patten on the Eyes of Molluscs and Arthropods.

In the last number of the 'Mittheilungen' of the Zoological Station of Naples appears an extensive article by Dr. William Patten on the "Eyes of Molluscs and Arthropods." The article contains the record of a number of observations on the structure of the eye in these animals, which appear to be of considerable value and importance. Accompanying the record of facts is a variety of theoretical and speculative statements, which are so extraordinary as not only to call for special notice, but are even likely to lead some readers to underestimate the value of the observations. Indeed, the attitude taken by this young and inexperienced naturalist in criticising the work of his predecessors, and in the enunciation of astounding general propositions, of the eccentricity and inadmissibility of which he appears to be altogether unconscious, is one which is greatly to be regretted as likely to diminish the weight which would otherwise be attached to his statements of fact, obviously the outcome of industrious investigation.

A large portion of the memoir deals with the eyes of Mollusca, of which we shall not here say anything further. The most important new result recorded in the memoir is that relating to the essential structure of the compound eye of Arthropoda. Dr. Patten appears to have discovered that Grenacher is wrong in supposing that the cells of the crystalline cones are the matrix cells of the corneal lenses. He has found a distinct layer of epidermic matrix cells, which produce the cuticular lenses, and were entirely missed by Grenacher. This new layer is therefore the equivalent of the vitreous layer of the monomeniscous Arthropod eye. The crystalline cone cells are, on the other hand, according to Patten, part of the retinal apparatus, and the rhabdom of Grenacher, which forms a sort of stalk to the group of crystalline cone cells, is really formed by them, and is not a cuticular product of the retinula cells of Grenacher, which surround it, and, according to that observer, produce it. Dr. Patten's observations on these points require confirmation, but appear to be likely to prove correct. As to nerve-endings, his observations are more doubtful, since he has committed himself in a somewhat over-confident manner to a series of speculative generalisations on the subject of nerve-endings, for the formulation of which it is only too obvious that neither his knowledge of facts nor his acquaintance with the work of contemporary histologists qualify him. He objects altogether to the term "nerve-end cell," and holds that all the cells of the Arthropod ommateum are supplied with nerve-fibres, the chief of which are those which, according

to him, form a meshwork in the crystalline cone cells, being derived from an axial nerve-fibre, which runs up the rhabdom in order to spread itself out in those cells. It certainly cannot be at once admitted that the fibres which Patten has thus traced in so many directions are nerve-fibres, though possibly they are so. On the other hand, contrary to his assertions in reference to the Arthropod eye, Patten lays down the law in a dogmatic fashion in regard to the Molluscan hypodermis. "The nerves," he says, "must terminate between the cells, and probably extend to their very outer ends." The "must" of the foregoing assertion depends on the correctness of a speculative account of the phylogenetic development of a nervous system, for many of the details of which Dr. Patten has no conclusive grounds to urge. At present, it may be remarked, histologists have been led, by the observations of Ranvier and others, to admit that nerve-fibres do in some regions terminate between the cells of the epidermis of Vertebrata, but it is also very generally held that nerve-fibres of organs of special sense terminate in the substance of special nerve-end cells.

Dr. Patten's observations are possibly correct, but he does not strengthen the confidence likely to be placed in them by dogmatism of the kind in which he indulges. Our knowledge of the relation of nerve-fibres to nerve-end cells is admittedly very unsatisfactory, and will require observations over and above those of Dr. Patten to put it on a satisfactory footing.

Such being the main facts of importance which Dr. Patten seeks to establish, we may pass to a brief notice of some of his more astonishing theoretical statements. In the course of the extensive memoir (over 200 pages) which Dr. Patten has devoted to this subject, it is very seldom that we find a continuous straightforward and intelligible account of the facts, with a sober discussion of probabilities as to matters in which his own observations are in conflict with those of other observers, or are incomplete. Dr. Patten is continually introducing into his record, with an unbecoming assumption of wisdom and authority, speculations or statements of a theoretical nature, which are so extravagant and betray so much ignorance as to make the reader regret very heartily that they have been allowed to disfigure a treatise which must on other grounds command attention. For instance:—1. On p. 625 the description of the eye of Penæus is introduced with the following utterance:—"The great impetus that modern zoological science has received from comparative anatomy has not been due so much to more subtle or able comparisons as to a more perfect knowledge of the structure of single forms." How there is to be comparative anatomy without comparison, or how comparison is to proceed without an increased knowledge of the single forms compared, is not explained by Dr. Patten. The sentence, so far as it means anything, appears to be a negation of the value of scientific morphology altogether. This, however, is a trifle compared with what follows,

and we quote it merely to show Dr. Patten's appreciation of the scope and tendency of morphological research.

2. A few lines below the passage above quoted we find the following dictum. "We must expect a certain amount of structural uniformity in those organs which have to carry by the same means the same forms of energy to similar perceptive centres." This seems to be almost a truism; if the "same means" are employed for such a purpose we certainly must expect uniformity. But what does our author mean by "carrying the same forms of energy to similar perceptive centres?" He is speaking of the eye; what is the form of energy which he imagines to be carried by means of the eye and optic nerves to a perceptive centre? A perusal of his final chapter explains this paradoxical allusion. Our readers will hardly credit the statement in the first instance, but it is actually true that Dr. Patten supposes that the energy of sunlight is carried with quantitative significance by nerves from the eye to nerve centres. He writes (p. 712): "In plants this sun energy is used in the chlorophyll grains, for in them the production of organic matter takes place. But in animals it is probable that the pigment granules are only the receivers of energy—the heliophags, as we shall call them—while this energy is transmitted by nerve-fibres to centres where it is consumed in the production of protoplasmic compounds." This astounding theory of "heliophags" is only part of a general theory of "dynamophagy," which is developed at great length by Dr. Patten in his final chapter.

"Living bodies," he says, "are distinguished by their power to absorb matter and energy, and from them produce high compounds by whose disintegration force is liberated as motion. This sequence of events is vitality. . . . . We have only to deal with the second of these processes, the absorption of energy or **dynamophagy**, and more especially with the absorption of solar energy or **heliophagy**." Eyes then are primarily not organs of sight but heliophags, organs for the absorption of solar energy, and only secondarily acquire a sensory significance! Similarly auditory organs are declared to be absorbers of the energy of sound vibrations, whilst "the energy of coarser vibrations, of pressure, contact, or movement" is "absorbed" by tactile hairs and "that of gases, solutions or chemical compounds," by means of taste-cells!

It is thus coolly proposed by Dr. Patten to revolutionise all the established conclusions of modern physiology in regard to the nervous system, of which conclusions he, it is only fair to say, appears to be entirely ignorant. He actually imagines that the energy received from external bodies is *quantitatively* transmitted from the surface of an animal by its nerves to the nerve-centres and there made use of. It is hardly necessary to point out that such a notion is simply preposterous, and that to speak of "the absorption of energy" as he does, betrays not only a fundamental ignorance of physiology but also of physics. The energy of the nervous

system and of the animal body generally, is, it is scarcely necessary to say, taken into the body in the form of potential energy of food-stuffs, and exists there as the potential energy of the proteids or higher chemical combinations which constitute protoplasm. All that the sense organs do in the way of bringing the 'actual' energy external to the animal body into relation with the nerve-centres, is to furnish special trains of explosive substance (i.e. of substances whose potential is suddenly convertible into actual energy), so that energy of various forms external to the body is able to initiate at appropriate points, and by means of special apparatus the conversion within the body of potential into actual energy, the amount of which has no relation whatever to the amount of the incident energy by which the explosion was started. Precisely as the energy liberated in a gun barrel is not the energy of the fall of the hammer which explodes the detonator, nor proportional to it, so is the energy of the animal body entirely distinct from the energy which sets its various sense organs in operation. The sense organs of the animal body may be compared to the detonating apparatus; and Dr. Patten might as well tell us that the purpose of a gun's trigger is to absorb energy and transmit it to the ball, whilst ignoring altogether the gunpowder, as to talk about sense organs being "dynamophags" and eyes being "absorbers" of the "beneficial effects of the sunlight."

3. In elaborating his doctrine Dr. Patten commits himself to many erronous statements, which show how little he is qualified to deal with the subject. We may note a few of these. On p. 709 Dr. Patten writes of the "animal pigment, especially that of *colourless* plastids." Animal pigment is declared to be "a living substance!" It is further stated, without the slightest attempt to support so startling a conclusion, that the "pigment granules of animal tissues are modified chlorophyll granules!" Dr. Patten not only expresses new ideas but also has invented a new chemical terminology. He writes of "waste products, such as carbonic acid gas, sulphides, ammoniates and ureates." His knowledge of chlorophyll and of the steps by which animal pigment granules are to be derived from chlorophyll granules may be judged of by the following: "Chlorophyll, as is well known, is extremely unstable and soluble in many fluids, even in water."

4. The statement that "it is well known that pigment, like chlorophyll, is dependent for its existence upon the sunlight," is totally at variance with fact. Instances of the formation of chlorophyll in plants which are excluded from sunlight are known, and still more numerous instances of animals which develop brilliant pigment although living in what is relatively to ordinary daylight, darkness. No doubt in the race, pigment must have a direct dependence on the access of sunlight; in the absence of light it cannot be of service to the organism. But there is no evidence to show either that chlorophyll or pigment are dependent for their existence upon sunlight.

5. In green plants, according to Patten, "chlorophyll is without doubt the substance affected by sunlight," and "the only rational supposition is that pigment is the substance in animals directly affected by the sunlight." It is somewhat impertinent of Dr. Patten to accuse those who may not assent to his crude theories of entertaining irrational suppositions. Most physiologists will remember that there are not a few simple experiments which demonstrate that protoplasm devoid of pigment is affected by sunlight and by its visible as opposed to its thermal factors. For instance, Engelmann has shown that the colourless Protozoon Pelomyxa contracts when exposed suddenly to sunlight, and the retina of albinoes is "directly affected" by sunlight.

6. It is a matter for regret that Dr. Patten has not made himself acquainted with the facts as to the action of light on protoplasm. One of the most important lines of inquiry in the minute study of the eyes of Arthropods, Molluscs, and other Invertebrates, is to be found in an exact determination of the presence or absence of pigment in the nerve-end cells and of the distribution of pigment granules in those cells. The question is a difficult one to investigate, because the observer generally is compelled to dissolve the pigment present in an ommatæum before a satisfactory study of the cells can be made. Dr. Patten, in the more valuable portion of his memoir containing the record of his observation, has not given so much attention to this matter as we could wish. It is remarkable that whilst he indulges in such "tall talk" with regard to pigment and heliophagy and the fundamental relation of pigment to this newly discovered function, yet he himself professes (we do not throw doubt on his observation) to have traced the chief optic nerve-fibres of the Arthropod polymeniscous eye to the colourless transparent cells of the crystal cones. It is evidently a subject which does not trouble him much since he quite recklessly attributes to other authorities on Arthropod eyes, statements with regard to the presence or absence of pigment in nerve-end cells which are the reverse of those made by the gentlemen in question. Thus at p. 670 he says: "Let us take for instance one of the lateral eyes of Scorpio and it will be found, according to Graber and Lankester, that the ommateum consists of ommatidia each one composed of five central colourless cells or retinophoræ." The reader who has followed us so far will not be surprised to learn that the particular cells in question were described and figured by Lankester as pigmented and not colourless, and were made by him the text of a discussion as to the significance of pigment in nerve-end cells.

7. It is not, however, of any use to expect accuracy of observation as to the contents of books and contemporary memoirs from Dr. Patten. He is far too much engrossed with laying down new principles of physiology and expounding to a benighted world the results of his philosophic meditations. As he himself says (p. 672), since doctors disagree, he intends to choose his own course, picking

out such facts as suit his theories and denying the existence of those which do not.

It is not to be expected that a writer who openly professes such principles should quote accurately the observations of other people. At the same time this incapacity for accurate observation of books and the neglect to observe at all such books as a text-book of physiology, and one also of physics, must lead Dr. Patten's reader to consider the possibility that his incapacity for correct observation extends also to other matters.

8. To continue our notes. On p. 685 we read: "We must admit that the possibility of regarding the phaosphere found in Euscorpius italicus by Lankester as an aborted nucleus is not so remote as he would have us believe." Whether the phaosphere can possibly be an aborted nucleus or not may be an open question; it is but another instance of Patten's extraordinary inaccuracy when he states that Lankester "would have us believe" anything on the subject. The matter was not discussed by Lankester at all.

9. On p. 717, Dr. Patten declares that he often hears "it said of any pigmented spot that it is not an eye, but simply a meaningless collection of pigment," and also alludes to "those who believe that pigment is a waste product." We trust that it is not in the excellent Zoological Station of Naples, where Dr. Patten has recently been pursuing his studies, that he has heard the above quoted remark. Was it made by a fellow student at Trieste or in the laboratory of Leuckart at Leipzig? In any case it seems to be a pity that Dr. Patten should have repeated these disparaging remarks concerning pigment spots, because no sensible person attaches any importance to them, and it is scarcely worth while to adduce, as Dr. Patten does, the well-known facts which render them unjustifiable.

10. On p. 716 we find it stated that "an organ most perfectly adapted for the condensation and absorption of the greatest amount of (solar) energy is likewise perfectly constructed for the perception of objects." The concentration of light is stated to be the condition essential for the most perfect "heliophagous organ," and it is declared that "the amount of energy absorbed would depend upon the most perfect condensation of light upon a given area." One surely would expect a writer on the theory of eyes to make himself acquainted with the simpler facts known as to the properties of lenses! But it seems that Dr. Patten has not found time to do this. The rays of light concentrated by a lens are, it is hardly necessary to say, merely those rays which fall upon the surface of the lens. Hence if the mere absorption of the energy of these rays is all that is needed, there is no advantage whatever in the provision of a lens. The naked epidermic surface of an area equal to that of a lens would present a perfect instrument for the "absorption" of solar energy, and, indeed, would "absorb" more than can the retina with the lens intervening between it and the surrounding medium. In plants accordingly we find no lenses but a simple

exposure of green surface to the solar rays. On the other hand if, as is the case according to received theories, the process which goes on in the retina is not important as an absorption or (to use a better term) a conversion of energy quantitatively, but only qualitatively, that is to say, in respect of initiating active changes in the nerve-end cells with the subsequent consequences of which the amount of energy converted has not so much to do as has its quality, then we can understand that a lens which disposes the solar rays on the retinal surface in a manner conducive to the localisation of their differing quality, has importance and value.

11. A melancholy instance of the extent to which Dr. Patten acts upon the principle of bending facts to theory, even at the risk of the grossest disrespect to contemporary authors of acknowledged competency, is found in his treatment of Sars's observations on the luminous organs of Euphausia. Patten wishes to consider these organs as eyes, and instances of his hypothetical "heliophags;" accordingly he suggests that Sars was misled by flashings of reflected light when he stated that they gave out light at intervals. In a note at the end of Patten's paper the editor of the 'Mittheilungen' very honestly states that Messrs. Paul Mayer and Giesbrecht have in consequence examined three living specimens of Euphausia, and entirely confirm Sars's observations and refute the unjust insinuations made by Dr. Patten.

12. A similar unwarrantable adhesion to theory, in the face of opposing facts, is seen in Dr. Patten's attempt to evade the consequences of the observations of Lankester and Bourne on the lateral eyes of Scorpions and Limulus, in regard to the latter of which animals they have the confirmatory evidence of Grenacher. Dr. Patten has propounded a theory of the Arthropod eye, to the effect that it is in all cases derived from a vesicle formed by invagination of the epidermis, and consists, therefore, of three layers of cells, viz. the two layers of the flattened vesicle and the epidermic layer which grows in front of it during its nipping off and detachment from the point of invagination. It is probably true that this is the structure and the ancestral history of the ordinary compound eyes of Crustacea and Insects: but there is no justification in the small area of facts observed by Dr. Patten himself for including all Arthropod eyes, all ocelli wherever situated and however constructed under this type. It is not clear why Dr. Patten insists on the universality of his generalisation, applying it to groups of Arthropods which he knows nothing about, and presuming to deny the accuracy of observations which he has not taken the trouble to test. Lankester and Bourne described the ommateum of the lateral eyes of Scorpions as being "monostichous," like the great lateral eyes of Limulus. They figured sections of the lateral eyes of both Euscorpius italicus and of Androctonus funestus. Their sections are in existence, and leave not the slightest doubt as to the accuracy of the statement

that these lateral eyes consist of simple depressions of the epidermis, there being no folding in of the edges of the depression so as to form a vesicle, and consequently no duplication or triplication of the layers. The fact thus established, that there is no vitreous layer in certain Arthropod eyes intervening between the cuticle and the nerve-end cells, naturally enough is an obstacle to Dr. Patten's sweeping generalisation. After citing the observations in question he dismisses them with the cool remark : "For theoretical reasons I am obliged to assume that this layer (the vitreous) is always present."

Were Dr. Patten not dominated by theories, one more extravagant than another, he would not have "assumed" anything about such an important matter, but would simply have taken a Scorpion (common enough at Naples), and cut some sections of its lateral eyes. Dr. Patten, however, openly professes that he has made it his habit in constructing his views on the structure of eyes to "choose his own course, picking out those facts which seem to point in the right direction;" that is to say, which support a favourite theory or amplify a startling generalisation, and ignoring or flatly denying, without troubling to bring them to the only test recognised by loyal students of nature, those which cannot be thus used.

13. Finally, we must point out that, in expressing his opinions, Dr. Patten often shows as great a want of manners as of fundamental knowledge. He objects to the supposition that in more complex eyes some of the pigmentiferous cells are due to intrusive connective tissue which has penetrated between the cells of epidermic origin. One author, he states, "has carried this supposition to an absurd degree." There is nothing "absurd" in the supposition, as Dr. Patten would recognise were he acquainted with the histology of the epidermis. In Lumbricus, Hirudo, and even in some Vertebrates, the occurrence of such intrusive connective tissue is a demonstrable and admitted fact; and in relation to the eye of Arthropoda it appears to have been actually observed taking place, according to Kingsley's recent account of his investigation of the development of the eye of Crangon ('Zoolog. Anzeiger,' No. 234). But in any case it ill becomes a novice to charge his masters and teachers with "absurdity." It should be enough for him to demonstrate an error (if he can) and to employ respectful language in doing so.

Grenacher is subjected by Dr. Patten to even more objectionable treatment. On p. 728, this young American, after citing an opinion published by Grenacher, says: "This he knows is absurd, and cannot be true." The expression is offensive and discreditable.

On the whole we cannot congratulate Dr. Dohrn on his contributor. There are, no doubt, some laborious observations contained in this ill-regulated production; but it is a question whether their value will counterbalance the effect on the author's reputation of the evidence which it bears of his want of both scientific and social education.

The Anatomy of the Madreporarian Coral Fungia.

By

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With Plates XXIII, XXIV and XXV.

During a visit, extending from the middle of September, 1885, to the middle of January, 1886, to the island of Diego Garcia, an atoll lying in S. lat. 7° 13′, E. long. 72° 23′, I was able to collect and preserve a large number of specimens of Fungia dentata, which form the subject of the present memoir.

The Fungiæ were very abundant within the lagoon, especially among the knolls and banks of growing coral on its east side, where, at low spring tides, they could be collected by scores from depths ranging from three to ten feet. They occur singly, more usually in groups of five or six, among the massive Astræids and Madrepores of which the knolls are chiefly composed, usually lying in a hollow or basin, or half hidden beneath the spreading branches of some large Madrepora, and are thus protected from being swept away by the tides which set strongly across the knolls.

Specimens of a diameter of three inches and more were extremely common, but it was very rarely that I could find any of smaller size; the smallest that I was able to procure measures as much as two inches across, and a prolonged search failed to reward me with a single smaller specimen, or

with an example of the nurse-stock.[1] In this I was very much disappointed, for Professor Moseley succeeded in finding a specimen at Tahiti in the course of a few hours' search, whilst I was unsuccessful day after day. Although the Fungiæ at Tahiti lay in only three inches of water, and the search was an easy one, he mentions the great difficulty he had in finding the nurse-mass among the numerous adult forms on the reef; and in my case, where they lay in three feet or more of water, it is possible that I may have overlooked the small nurse-stock and the smaller recently detached Fungiæ; this is the more likely since both the nurse-stocks and the young forms would probably remain hidden beneath the great flat plates of dead Madrepore which form the basis of the mounds of living coral. Yet I can scarcely believe this to be the case, for not only did I search very closely by wading and diving among the corals, but I frequently turned over the above-mentioned flat plates of coral-rock and examined their under surfaces without ever finding a single example, nor did I ever meet with a group of very small forms, nor with anything like the group of nurse-stocks attached to the corallum of an old and dead Fungia, as figured by Stutchbury (39). I am inclined to believe that sexual reproduction followed by asexual reproduction by budding from a nurse-stock takes place in Fungia only at certain seasons of the year, and that it was not in progress during my stay at Diego Garcia. This seems the more probable because I have found no trace either of ova or of spermatozoa in any of the large specimens which I have brought home for examination. Reproduction in Fungia appears to be effected also by budding and by simple fission. In the British Museum there are several examples of the former process, in which indubitable buds can be seen growing from the base of a large Fungia. The buds always arise from

[1] Semper (38) calls the nurse-stock of Fungia a Strobila, but as this name was originally applied to the dividing parent-stock of Aurelia, which is essentially different from the bud-producing parent-stock of Fungia, and since it is objectionable to use the same name for two very different phenomena, I use the word nurse-stock for the fixed parent of Fungia.

the base, and it is not unlikely that they may be formed only when, by some accident, the coral has been overturned. Examples of fission are rare, but I have in my possession a dead corallum which is nearly divided into two separate Fungiæ, and in which the axial fossæ are already completely separated from one another and form mouths excentrically placed on peristomial discs inclined towards one another at a wide angle. There is a similar specimen in the British Museum. In the same collection there is a very good specimen of a nurse-stock brought by H.M.S. Alert from the Seychelles, found on March 5th, 1883. This is a young specimen from which the first bud has not yet been detached, and the soft tissues still extend down over the outside of the corallum to the basal disc; unfortunately the spirit in which it was contained has been allowed to evaporate, and the soft tissues are unfit for examination. But since it was found only two months later than the date of my search at Diego Garcia and in the same seas, it may be taken as an objection to my opinion given above, that there is a special season of sexual followed by asexual reproduction from a nurse-stock in Fungia. It is quite possible that sexual reproduction may be very much economised in these corals and is of rare occurrence, the maintenance of the numbers of a species being ensured firstly by the budding off of an indefinite number of forms from the sexually produced nurse-stock, and secondly, by the simple asexual processes of budding and fission above described. The whole history of the reproduction of these forms is very imperfectly understood, although it presents many problems of the greatest interest. A naturalist travelling in coral-seas should not fail to try and secure some specimens of the nurse-stocks carefully preserved in spirit, as well as specimens of the young free forms recently separated from the parent-stock. To this should be added any observations that may be possible on the relative frequency of the nurse-stocks, on the frequency of budding or fission, and on the rate of growth. I was unable to carry out an extended series of observations on the Fungiæ at Diego Garcia, for the knolls on which they were found lay

in the lagoon at some distance from my hut, and want of space and appliances prevented me from constructing proper aquaria in which to study them at leisure. Such as I tried to keep alive in buckets and tubs full of sea water soon perished, the water rapidly becoming foul in the hot climate unless a constant stream is kept through it. As for placing any individuals of Fungia or masses of any other coral in a particular spot on the beach where they might be readily accessible for study, a short experience showed me the impracticability of the suggestion. Placed on the lagoonward beech in smooth water they were quickly covered with and destroyed by the sand; on the external shores they were at once rolled over and over and destroyed by the great waves which are capable of moving masses weighing 2 cwt. and more, and throwing them up in a sort of low wall all round the island.

The specimens which I brought home for examination were killed with hot corrosive sublimate, and afterwards treated with picric acid and preserved in 70 per cent. spirit. In this way I was able to preserve several specimens with the short stumpy tentacles fully expanded, as is shown in fig. 1.

Although the general features of the corallum of Fungia have been well known for a long time, and have more recently been carefully described by Professor Martin Duncan (5), the arrangement of the soft tissues, and their relation to the corallum has not yet been studied. G. von Koch, it is true, has recently published a few remarks on the subject (23) and gives a figure, but the latter is incorrect in details, and the description merely amounts to a statement that the general anatomy of Fungia corresponds with that of the other Madreporaria; he does not attempt to give a detailed description of the internal structure. As any attempt to remodel the classification of the Madreporaria must depend on an intimate knowledge of the relation of the soft parts to the corallum, I shall give in the following pages as detailed a description of the anatomy as circumstances will permit.

The family Fungidæ was established by Dana in 1846. In his splendidly illustrated work on the 'Zoophytes of the

Wilkes' Exploring Expedition,' he gives descriptions of several species, with drawings of the hard parts and the living animals. To the latter I shall have occasion to refer further on. The family thus established was made the subject of a memoir by Milne-Edwards and Haime, in which many new species were described, and the characteristic features of the corallum were noted. The same authors give a full description of the family in the 'Histoire des Coralliaires,' but confine themselves to the study of the corallum throughout. Professor Martin Duncan has lately published a memoir on the same family, dealing especially with the hard structures, and to his account I have little or nothing to add. The following description of the corallum is taken principally from his paper (5).

The corallum is simple and discoidal, the base usually rather concave, and the upper surface convex. The theca is distinct and confined to the basal surface; it is continuous in the central part of the disc, but in its more peripheral parts it is perforated by numerous apertures, which lead through it into the interseptal loculi. The septa are numerous, arranged in seven cycles in the moderately large forms, and are continuous; the free margins of the septa are dentate. The theca is ornamented with radiating rows of spines, each row corresponding in position with a septum above, and representing a costa. The axial fossa is elongate and shallow. The columella is trabeculate and rudimentary. Special structures named synapticula are characteristic of the Fungidæ; they consist of nearly vertical or curved rows of bars, bridging over the space between and connecting the lower portions of two contiguous septa. By them the lower parts of the interseptal loculi are divided up into nearly vertical channels, bounded on two sides by synapticula, and on the other two by the septa. Excellent figures of the synapticula are given by Professor Duncan.

The flat discoid shape of Fungia is not a characteristic of the genus, but occurs in other groups of the Madreporaria, e.g. Deltocyathus among the Turbinolidæ, Stephanophyllia and Leptopenus among the Eupsammidæ. The flat shape is a secondary effect produced by the mode of

growth, for in its youngest stage the corallum of the nurse-stock of Fungia is cup-shaped and resembles a Caryophyllia, having a distinct lateral theca, and a basal disc by which it is attached. In the course of subsequent growth the peristome expands laterally, so that the nurse-stock already shows traces of the discoid shape before any young forms are set free; this is very well seen in the specimen dredged by the "Alert," referred to above. The separation of the young Fungia from the nurse-stock takes place at a short distance below the edge of the peristome, so that only a small portion of the lateral theca of the nurse-stock passes into the free form. As growth proceeds the peripheral ends of the septa are the seats of the greatest activity in the deposition of calcareous matter, each septum at once growing outwards and sending off calcareous processes from its lower edge, which meet and fuse with those of adjoining septa to form the perforate theca. Thus, the increase in size of the coral proceeds almost entirely in a horizontal direction, bringing about the discoid shape of the adult. The corallum of a young Fungia just set free from the nurse-stalk has a circular opening beneath, which leads into the interseptal loculi and marks the point of former attachment; this hole is soon filled up by the deposition of calcareous tissue, which remains as a small boss in the centre of the base of the disc, marking the space which represents the basal disc of the attached coral. The remainder of the under surface is a thecal structure, the more central imperforate part of which is probably that portion of the lateral theca of the nurse-stalk above the line of detachment, the outer and perforate part being derived from a fusion of the lower ends of the septa, and not intimately connected with the synapticula, as I think after a careful examination of the fresh corallum, though on this point I am at variance with Professor Duncan. The series of figures 3—8 show how the discoid shape is derived from the cup-shaped coral by lateral growth. The theca of Fungia, although entirely confined to the basal surface, and perforated by numerous apertures leading into the interseptal loculi, is quite homologous with the theca of other Madreporaria, and bears similar

relations to the mesenteries and the cœlenteron, as I shall describe further on. It is important to notice that the theca is formed, in the course of outward growth, from the fused ends of contiguous septa, as is stated by G. von Koch to be the case in the lateral thecæ of other cup-shaped Madreporaria.

I shall use throughout the same terminology as Fowler in in his admirable paper on "Coral Anatomy" (9); but since the flattened form of Fungia makes it a little difficult to distinguish "base" from "basal disc" in a general description, I shall make use of the terms "oral surface" and "aboral surface," the former including the mouth and peristome, the latter the theca and basal disc.

Drawings of living Fungiæ have been given by Eschscholtz, Quoy and Gaimard, and Dana. The first of these gives a tolerably correct figure, but only four cycles of tentacles are represented, the more peripheral cycles not being noticed. Quoy and Gaimard (33) figure two species of Fungia under the name of Fongies à grosses tentacules (Fungia crassitentaculata). These are remarkable for the great length of the tentacles, which are represented as scattered irregularly over the disc. Milne-Edwards and Haime, not noticing the regular arrangement of the tentacles in Eschscholtz's figure, say: "Toute la partie supérieure du corps de l'animal, correspondante à la partie lamellifere du polypier, est garnie des tentacules épais qui ne sont pas groupés en forme de couronne comme chez la plupart des Zoanthaires." Dana's figure of Fungia lacera in the 'Zoophytes of the Wilkes' Exploring Expedition' is reproduced in his book on 'Corals and Coral Islands,' and he says in the latter work: "The tentacles are scattered over the disc instead of being arranged in regular circles. It is evident from the figure that the apparent circles, where there is more than one, in Actiniæ, arise from the crowding of the series of tentacles together, and also that the inner row of tentacles in polyps is the older. It will be noticed also that each of the tentacles stands where a new ridge or calcareous septum in the coral begins." That the

circles of tentacles in Actiniæ do not arise in the manner here suggested is sufficiently proved by the researches of Lacaze Duthiers and the Hertwigs, but I have not seen it contradicted of Fungia that the tentacles are scattered irregularly over the disc. Yet so far is this from being the case, that on first taking the living animal out of the sea I was immediately struck with the arrangement of the tentacles in definite cycles. Fig. 1 is a drawing of F. dentata, somewhat contracted by spirit, but with the short stumpy tentacles still expanded. Their arrangement will be at once understood by a comparison of the drawing with the diagram (fig. 2).

Each tentacle is placed, not, as Dana says, on the innermost extremity of each septum, but on a slight elevation of the upper edge of each septum near its innermost extremity; from the point of attachment of the tentacle the septum is continued obliquely downwards and inwards towards the axial fossa, none but the last two cycles ending at any great distance from it. Since the tentacles correspond exactly in position with the septa, what is stated of the arrangement of the one holds equally good of the other.

There are twelve primary septa, of which ten reach the mouth, two being rather shorter than the others and placed opposite each end of the long axis of the mouth. The tentacles placed on the inner extremities of these septa overhang the mouth, but are small and degenerate.

Both Lacaze Duthiers (7) and von Koch (21) describe twelve septa as rising simultaneously in the first cycle, making the prime number of septa twelve instead of six. According to the latter six alternate septa grow faster than the others, giving the appearance of two cycles of six each; this is apparently not the case in Fungia whose twelve septa of the first order are, with the exception above mentioned, of equal size.

There are twelve septa of the second order alternating with those of the first; they reach very nearly to the mouth and are all of equal length; the tentacles corresponding with them form, with those of the first cycle, a ring surrounding the mouth.

There are twelve pairs of septa of the third order. Each pair of this order embraces a septum of the first order. The fourth order contains twenty-four pairs of septa, each pair embracing alternately a septum of the first and a septum of the second order.

The fifth and sixth orders also contain twenty-four pairs of septa each. Those of the fifth order are the longer, and each pair embraces a septum of the first and second orders alternately; those of the sixth order are much shorter, and each pair embraces a septum of the third order.

These last two orders, the fifth and sixth, very possibly represent only one complete cycle of septa and tentacles. But since the difference in the length of the septa shows a difference in their time of origin I have thought it better to keep them separate.

The seventh order contains ninety-six pairs of septa, with their corresponding tentacles. The septa are extremely short and rudimentary; the tentacles are minute and placed close to the circumference of the disc.

The sequence of the septa is 1, 7, 5, 7, 4, 7, 6, 7, 3, 7, 6, 7, 4, 7, 5, 7, 2, 7, 5, 7, 4, 7, 6, 7, 3, 7, 6, 7, 4, 7, 5, 7, 1, in each system.

The tentacles are arranged in six tolerably definite circles at different distances from the mouth, as may be seen in figs. 1 and 2. The arrangement both of tentacles and septa is very regular and easily made out at each end of the long axis of the mouth, but it becomes more irregular and obscure at the sides. Thus, contrary to previous statements, Fungia is perfectly regular, and agrees with other Madreporaria in the arrangement of tentacles and septa. The tentacles, which are small and club shaped in most species, are correctly figured by Dana. The remaining external features do not call for special notice and may be understood by reference to fig. 1.

To examine the internal structure, I decalcified some specimens in nitric acid solution, and afterwards stained and cut sections from them in the usual way. Another specimen I half decalcified and dissected, and further made use of the

method invented by G. von Koch, to study the relations of the hard and soft parts in situ. This method, which is described in the 'Proceedings of the Zoological Society,' 1880, p. 41, yields valuable results with smaller corals, but requires considerable experience, and is not wholly satisfactory when so large a coral as Fungia is dealt with.

Von Koch, in a short note on the anatomy of Fungia and other Madreporaria, published in the beginning of this year, rightly says that its structure is essentially the same as that of other Madreporaria, but he makes no mention of the peculiarities which obtain from the relation of the soft parts to the synapticula, and his diagram is incorrect in some particulars (23).

The Mesenteries.—It is obvious that, since the lower parts of the interseptal loculi are broken up by synapticula, there must be some corresponding modification of structure in the mesenteries, if the latter structures are present in Fungia. Professor Duncan was so much struck with this in his studies on the corallum that he was led to express a doubt whether mesenteries could exist at all (5). Transverse sections show that mesenteries do exist, and that in all their essential characters they have the arrangement typical of Hexactinian Actinaria. They are arranged in pairs, each pair being distinguished by the arrangement of its longitudinal muscle-fibres, which are placed on the adjacent faces of the two mesenteries composing the pair, except in the case of the two pairs of directive mesenteries, one pair at each end of the long axis of the mouth, in which the longitudinal muscle-fibres are placed on the reverse sides. The space included between each pair of mesenteries is an entocœle (Fowler, 9) and in each entocœle there is a septum. There are seven orders of mesenteries in the Fungia which I am describing, corresponding to the seven orders of septa.

The primary and secondary mesenteries are attached to the stomodæum, and in their upper parts traverse the whole space from the mouth to the periphery of the disc. The tertiaries are not attached to the stomodæum, but reach nearly to it.

The remaining orders are of constantly decreasing length, the septenaries being very minute. All the mesenteries are firmly attached by their upper border to the peristome.

In the upper portions of the interseptal loculi there are no synapticula; here the mesenteries are free to radiate across the whole space between the mouth and the periphery of the disc, and show in this part the ordinary structure, viz. a central structureless supporting lamina, the Mesoglœa,[1] bearing a layer of endoderm on each face. On one face are borne the longitudinal, or retractor muscles, bundles of stout muscular fibres developed from the endoderm, supported by and appearing as if intimately connected with, supporting offsets of the mesoglœa. On the opposite side to the longitudinal muscles are the transverse muscle-fibres, much more feebly developed than the former, and having a general arrangement at right angles to them; they are not easy to see in Fungia.

In the lower portions of the interseptal loculi the continuity of the mesenteries is broken by the synapticula, and they present special modifications of structure which can only be understood by a careful comparison of figs. 10, 13, and 15. Fig. 10 represents a mesentery of the third order dissected out from a decalcified specimen, and viewed from the side. The upper portion is seen to be complete, and exhibits the characteristic arrangement of muscle-fibres, but it will be noticed the longitudinal muscles are gathered into more distinct bundles than is usually the case in Actinaria. Each face is covered with a layer of endoderm, as may be seen by reference to figs. 13 and 15. The lower portion of the mesentery is necessarily discontinuous owing to the intervention of the synapticula. Here the mesoglœa is seen to be continued into a number of strong bands or ligaments to which the separate bundles of longitudinal muscles are attached, these ligaments passing down through the intersynapticular spaces to be fastened,

[1] I have coined this term as a substitute for the term "mesoderm" in the Cœlenterata, and use it as the equivalent of the "Stützlamelle" or "Gallertsubstanz" of German authors, for reasons which are stated at the end of the paper.

according to their position, either to the aboral body wall, or to thickened lines of the mesoglœa of the skeletotrophic investment shortly to be described. In sections these ligaments may be seen as thickenings of the tissue surrounding each intersynapticular passage, more rarely as complete partitions dividing such a chamber in two. The endoderm covering each face of the mesentery becomes continuous, where the latter is divided by the synapticulum, with the endoderm of the skeletotrophic investment. The theca being perforate in all but its most central portions, the ligaments of the mesenteries pass through the perforations and are continued outside the theca, to be fastened to the aboral body wall (see fig. 15). The theca or aboral surface of Fungia is completely covered with soft tissues which do not closely invest the corallum, but are separated from the latter by a portion of the cœlenteron. It is important to observe that this extra-thecal portion of the cœlenteron is, partially at least, divided into chambers by mesenteries in the manner described above.

The free edges of the primary and secondary mesenteries below their insertion into the stomodæum, and the free edges of the remaining mesenteries in their entire length, are furnished with the thickenings known as mesenterial filaments. In the primaries and secondaries the mesenterial filaments are very thick; their epithelial cells are very long, attenuated, and crowded close together, showing an abundance of deeply-staining nuclei. Nematocysts are scanty; but I was able to distinguish a number of cells, which seemed to be of the same character as the gland-cells described by the Hertwigs in Actinia and Sagartia (15). In the other mesenteries the filaments are not so thick, and gland-cells are less abundant, nematocysts more so; in other respects their structure resembles that of the filaments of the primaries and secondaries. At the lower end of the free border of each mesentery is a bundle of much-coiled filaments, forming the structure known as an acontium.[1] These structures in Fungia are strictly

[1] Professor Moseley has pointed out to me that the name acontium is used by Gosse and the Hertwigs to describe only those structures at the base

comparable with the mesenterial filaments, of which they appear to be a continuation. The lower free edge of the mesentery appears to be prolonged into a long lamellar offset, which is much plicated, and surrounded along its free edge with the thickening which forms the main body of the acontium. This thickening has a similar histological structure to that of a mesenterial filament, differing from it only in the larger size of the epithelial cells of which it is composed, in the abundance of nematocysts, and the corresponding poverty of gland-cells. Fowler describes acontia of similar structure in Flabellum patagonichum, and states that they are protruded through cinclides in the peristome (9, p. 14). Although I handled some hundreds of living Fungia I never saw the acontia protruded, either through the cinclides or through the mouth, but in some species of Mæandrina, acontia of exactly similar structure to those above described were protruded through large cinclides on the peristome when the animal was irritated.

According to Gosse (11) and the Hertwigs the acontia in Sagartia have the form of bunches of long filaments developed at the lower free edge of each mesentery, each filament being ribbon shaped, with one of its borders much thickened. Such acontia are protruded through cinclides in the body wall. The acontia observed by Fowler and myself are protruded in Flabellum and Mæandrina through cinclides in the peristome, and appear to be more simple in structure, consisting of a much plicated off-set or prolongation of the lower free border of the mesentery, the edge of which is thickened in continuation with the thickening which forms the mesenterial filament above,

of the mesentery which are in the form of a bunch of filaments. I have followed Fowler in extending the name to that mass of contorted filaments, which is generally known as the contorted mesenterial filaments. This I have done because (1) they differ in histological detail from the filament on the upper part of the mesentery in exactly the same manner that Gosse's acontia differ. (2) They may be protruded through cinclides. (3) They are clearly a less differentiated condition of Gosse's acontia, but not morphologically distinct from them. To those who adhere to Gosse's original definition, the name as I use it in the text, would be incorrect.

but differing from this in histological detail. The more complicated and effective acontia of Sagartia are probably developed from a simpler form, such as this.

In Fungia the mesoglœa appears to break up dendritically in the swelling of the acontium, instead of ending in a T-shaped swelling, as in other forms (*vide* fig. 12).

The acontia of the tertiary and succeeding mesenteries lie coiled up in the exocœles at the bases of those mesenteries, and in section appear to fill up the greater part of those spaces (*vide* figs. 13 and 15).

Since the septa are large compared with the interseptal loculi, and since the septa are always in the entocœles, it follows that the two mesenteries forming a pair are pushed apart from one another, whilst the adjacent mesenteries of contiguous pairs lie close together. So much is this the case, that when a decalcified animal is cut across transversely the adjacent mesenteries of contiguous pairs appear from their position to form pairs, and in old specimens they may become fused together at a little distance from the periphery, as is shown in fig. 11.

The Cœlenteron.—The cœlenteron is represented by the axial space lying below the stomodæum, the peripheral chambers known as exocœles and endocœles, and the extra-thecal space lying on the aboral surface between the theca and the external body wall.

The axial portion of the cœlenteron is not definitely circumscribed. Above, it opens freely into the stomodæal invagination; below, it is limited by the investments of the trabecular columella; at the sides it is partially limited by the thickened borders of the mesenteries above described. So far as it can be considered as a definite cavity it is no doubt the cavity in which digestion is chiefly effected, the process being carried out by the secretions of the gland-cells of the mesenterial filaments.

The relations of the peripheral parts of the cœlenteron are difficult to understand in this, as in all other Madreporarian corals, but are further complicated in Fungia by the presence of synapticula. The cœlenteron is composed of all those

chambers which are lined with endoderm, and if a cast be made of all those chambers it will represent the space occupied by the cœlenteron. Such a cast I have attempted to represent in fig. 16. The peripheral chambers of the cœlenteron are divided by the mesenteries into exocœles and entocœles; in those corals in which, as in Fungia, all the septa are entocœlic, the entocœles are almost obliterated by the septa which rise up within them, but morphologically lie wholly outside them, since every part of the corallum is invested with its proper layers [viz. a layer of cells lying next to the calcareous substance from which the latter is secreted (the calycoblasts of von Heider), a very thin layer of mesoglœa, and a layer of endoderm], and is thus separated from the cœlenteron by the three layers of tissue which limit every part of the body. Thus, in a cast of the cœlenteron, the latter is seen to be broken up into wedges by the spaces which are occupied by the septa (*vide* fig. 16), and in Fungia these wedges are further perforated by the apertures through which pass the synapticula connecting adjacent septa.

Further than this, outside the theca (which is basal and also perforate in Fungia), there lies a portion of the cœlenteron, communicating with the intra-thecal chambers by canals which pass through the perforations in the theca, and, like the intra-thecal chambers, divided by the continuations of the mesenteries into exocœles and entocœles (*vide* figs. 15 and 16, *cœl.*). These complicated relations cannot, I conceive, admit of rational explanation unless the theory of von Koch be admitted, namely, that the corallum is derived primitively from the basal ectoderm, and that the theca is formed by the fused peripheral parts of the septa, which in fusing divide the mesenteries, and leave a portion of the cœlenteron external to the theca. From his account of the development of Astroides calycularis (21) it appears that the skeleton first makes its appearance as a ring of calcareous nodules situated between the ectoderm of the basal disc and the surface of attachment. As development proceeds radial folds of the ectoderm and mesoglœa (mesoderm) are formed, beneath which are

lines of calcareous crystals; these are the first rudiments of the septa. At a later stage the septa form proportionally high plates, over which the ectoderm is bent in the form of a fold, the septa begin to branch at their peripheral ends, and eventually these branches meet and fuse with one another to form the theca, which cuts the mesenteries in two portions and isolates the more peripheral part of the cœlenteron from the more central, the former being limited externally by the soft body wall, which at first extends down to the base of the theca. One might almost speak of the corallum as being pushed in from below, all the three body layers being invaginated to receive it. Eventually the ectoderm which is bent over the corallum, having the sole function of secreting calcareous matter, comes to be represented by that layer of cells lying between the mesoglœa and the corallum, to which von Heider has given the name of calycoblasts. In old specimens the external body wall becomes atrophied around the lower part of the calyx, where it is physiologically replaced by the theca, but it still holds its place as an investment of the upper part of the calyx (Randplatte of von Heider) (14). The young nurse-stock of Fungia, so long as it remains cup shaped, has all the characters of a Caryophyllia, and may be compared strictly with the young Astroides. Stutchbury (39) says of it, "So long as the young Fungia retains the form of a Caryophyllia it is entirely enveloped by the soft parts of the animal, but as the upper disc of the coral spreads out and assumes its characteristic form, the pedicle is left naked and the soft parts extend only to the line where the separation afterwards takes place." In the "Alert" specimen in the British Museum the soft parts still extend to the base of the nurse-stalk, although the upper disc has begun to widen out. When the young Fungia separates from the nurse-stock a clean scar is left at the point of detachment, through which there is for a short time free communication to the interior. But the deposition of calcareous matter round the central ends of the septa soon blocks up this passage, and immediately afterwards the soft parts covering the theca (which is now nearly confined to the

basal surface as in the adult) meet in the centre and fuse together, so that the primitively external corallum is now entirely covered over by soft tissues, and one can only predicate its origin from the fact that it is everywhere covered with three investing tissues, the ectoderm, now represented by the calycoblastic layer, the mesoglœa, and the endoderm, to which I have above referred under the name of the skeletotrophic investments. That part of the cœlenteron which in Fungia lies external to the theca on the aboral surface, is the same morphologically as the extra-thecal part of the cœlenteron described as existing around the upper part of the calyx in other corals by von Koch, von Heider, and Fowler. For further information on this interesting subject I must refer the reader to the published works of these three authors.

The Stomodæum.—This is extremely short in Fungia. I was unable to trace the existence of gonidial grooves (siphonoglyphes) at its ends, though they no doubt exist. When alive the animal constantly closes the middle portion of its mouth, leaving small apertures at the extreme ends through which currents of water pass in and out. I did not determine whether these currents are constant in direction.

Histology.—This is simple in character and does not differ in any essential from the Actinian type.

The ectoderm of the peristome is composed of long columnar epithelial cells, whose inner ends are drawn out into fine processes which rest on the mesoglœa; each ectoderm cell has a distinct oval nucleus which stains deeply in borax carmine. Numerous smaller interstitial cells lie between the processes of the inner ends of the epithelial cells. Large nematocysts are embedded in the ectoderm; they are especially abundant on the ectoderm of the tentacles, but excepting for this the histology of the tentacles is quite similar to that of the rest of the peristome. I could not distinguish more than one kind of nematocyst in Fungia. In every case the lower part of the thread is armed with a spiral line of spines; when inverted the terminal end of the thread is coiled obliquely round the basal portion. The ectoderm of the aboral surface differs slightly

from that of the peristome in that the epithelial cells composing it are less columnar and more cubical, and it is scantily provided with nematocysts.

Between the corallum and the mesoglœa there is invariably a layer of rounded, granular, soft-looking cells which do not stain easily; their nucleus is tolerably large and stains but faintly in borax carmine. From their position these are clearly equivalent to ectoderm cells; they are the calycoblasts (*vide* fig. 17, *cy.*). They are simple rounded cells, as described by their discoverer, von Heider; I could find no trace of striation in them as Sclater did in Stephanotrochus, nor does their shape agree with his account.

Between the ectoderm and endoderm of the body wall, and between the two layers of endoderm which form each septum, lies a sheet of homogeneous tissue called by German authors "Stützlamelle," by Englishmen "mesoderm," or sometimes "the supporting lamina." I have called it the Mesoglœa for reasons which are more conveniently given at the end of this paper. I could find no trace of structure in this layer in Fungia, though it is possible that the use of proper reagents in the fresh condition might have disclosed a fibrillar structure. It stains slightly with hæmatoxylin, not at all with borax carmine.

The endoderm is composed throughout of a single layer of cubical cells with a tolerably large nucleus and a nucleolus. Presumably these cells bear cilia in the living animal. In many parts of the body, but particularly in the region of the insertion of the mesenteries, the endoderm is crowded with masses of rounded nucleated cells of peculiar appearance; at first sight they might easily be mistaken for endoderm cells forming a layer several cells deep. When treated with iodine they give a blue colouration, so that there can be no doubt that they are symbiotic algæ, which occur so plentifully in the endoderm of many Actiniæ. I was unable to find any trace of gonads in the specimens which I examined.

The study of the anatomy of Fungia justifies the position which has always been assigned to it, between the perforate and imperforate Madreporarians. The theca, it has been

shown, is perforate in its more peripheral portions, imperforate in its central portion, and as age increases the imperforate area increases largely. The canals passing through the perforate portion, and putting the intra-thecal in communication with the extra-thecal cœlenteron are, no doubt, homologous with the system of canals described by Fowler in Rhodopsammia parallela. I can offer no explanation of the origin and significance of the synapticula. Physiologically they seem to serve as stays or buttresses, giving solidity and coherence to the corallum.

The most important result of my researches seems to me to be the strong evidence furnished in favour of von Koch's theory of the formation of the skeleton in the Madreporaria, the evidence in favour of the existence of extra-thecal cœlenteron being, as I think, particularly conclusive.

The Mesoglœa, Mesoderm, or Stützlamelle in Cœlenterata.

Throughout my paper I have used the name Mesoglœa for the (structureless) supporting membrane which separates the ectoderm from the endoderm in Fungia, as in all the Cœlenterata.

The names given to this layer by German authors are Stützlamelle, Zwischensubstanz, Gallertschichte, or Mesoderm. Among English authors the use of the name mesoderm has become general in describing it. Whilst the exact significance of this layer in the Cœlenterata and its homology with the mesoblast of the higher Metazoa are, to say the least of it, far from being settled, it seems to me that the use of the name mesoderm is highly productive of confusion and error.

The names ectoderm and endoderm, meaning simply outer and inner skin, were first given by Allmann to the outer and inner cell layers of the Cœlenterata (G. J. Allmann, "On the Anatomy and Physiology of Cordylophora," 'Phil. Trans.,' cxliii, 1853), and had they always retained this their original signification there could have been no objection to the use of

the name mesoderm for the median layer of the Cœlenterate body. But, as the Hertwigs have pointed out very clearly, from the time that the primary germinal layers, the epiblast and hypoblast, of the higher Metazoa were first compared and homologised with the ectoderm and endoderm of the Cœlenterata, there has been an increasing tendency to use the names ectoderm and endoderm as the equivalents of epiblast and hypoblast; and this is especially the case among German authors, with whom the use of the names epiblast, mesoblast, hypoblast for the germ layers of the embryo, has not found general acceptance. It followed that the name mesoderm came to be used in the same sense, or very nearly so, as mesoblast, instances being numerous among German authors, and not infrequent even among English authors, where the mesoderm of the germ or embryo is spoken of. The difficulty arising from the identical use of these two names was appreciated by the Hertwigs and by F. E. Schulze, who treated the subject at some length, each in their own way. F. E. Schulze asks the very pertinent question whether the name mesoderm can only be used in those cases in which a special layer of cells arises early, that is, before the development of tissues and organs, as a special germ layer; or whether one can speak of a mesoderm when a differentiation of a special middle layer of tissue from the outer or inner epithelial layer arises later and without the formation of a special germ layer. He concludes by drawing a distinction between triple-walled animals, such as the Cœlenterata, and those which have three germinal layers (viz. the higher Metazoa (Triploblastica) but not the Cœlenterata), admitting at the same time that the Cœlenterates have not a mesoderm in the sense of a distinct layer of cells derived from either or both of the two primary germ layers before the latter show any differentiation into tissues or organs. He speaks of them as being "dreischichtige" but "zweiblättrige." The Hertwigs, in dealing with the difficulty, proposed to limit the use of the words ectoblast and entoblast (i. e. epiblast and hypoblast) to the germinal layers of the embryo, and to use the names ectoderm and endoderm

to denote the outer and inner limiting layers of the adult body, whilst the name mesoderm should include all those tissues which lie between the two limiting layers above mentioned. This nomenclature is very objectionable, and indeed has not met with very general acceptance. If limited to the Cœlenterata it would be sufficiently expressive and consistent, but when applied to the higher groups of the Metazoa it becomes utterly impossible of application. To begin with, it is hard to draw any sharp line between the external and internal limiting membranes in the higher Metazoa; in forms where the stomodæum and proctodæum are derived from epiblastic invaginations, and form no inconsiderable part of the digestive tract, the confusion becomes complete. In the Isopoda, for instance, nearly the whole of the digestive tract is formed from the stomodæum and proctodæum; thus, according to the Hertwigs, this clearly epiblastic internal limiting layer would be called endoderm. In the Vertebrate phylum, also, the adult nervous system, clearly derived as it is from the epiblast, would, because it lies between the two limiting layers, come under the name of mesoderm. Moreover, it is an altogether unscientific and confusing method to classify tissues by their position in the adult rather than by their derivation from the primitive germ layers.

The words ectoderm, mesoderm, endoderm, have become so universally used as the equivalents of epiblast, mesoblast, hypoblast, that there is very little hope of their being now limited to the group of Cœlenterata to which they were originally applied, and this being the case we ought to consider how far the median supporting lamella of that phylum, the Stützlamelle or Gallertschichte of German authors, is homologous with the mesoblast of the higher Metazoa before applying the name mesoderm to it. If it is not homologous, but is of a different nature, then some other name than mesoderm should be found for it, otherwise it will be confused with the true mesoblast.

This opens two questions (1) What do we mean when we speak of a true mesoblast? (2) What are the characters of the

median lamella, and what is its origin in the Cœlenterate phylum?

This is not the place to enter into a discussion of the whole subject of the origin and significance of the germinal layers, which the first question introduces, but it may shortly be stated, without going very far wrong, that by mesoblast is meant a layer of undifferentiated cells, developed in the embryo before the differentiation of other organs or tissues, from either one or the other or both of the primary germ layers, the epiblast and hypoblast. By mesoderm, or its adjective mesodermic, are meant all such tissues in the adult as are clearly derived from the mesoblast. This is not the sense in which I should like to use the term, but a sense which has become inevitable from the usage of other authors.

To this idea of mesoblast recent theories on the origin of metameric segmentation have added another highly important signification, and one which is of especial importance to the present question. In the majority of the higher Metazoa (Triploblastica) the mesoblast is understood, in part, to denote the limiting layer of the cœlom. The Platyhelminthes offer a difficulty to this conception in that they are not known to possess a true cœlom, and it is a question whether they ancestrally possessed one, or whether they are the surviving representatives of the triploblastic Metazoon in which the cœlom was not developed. From the analogy of the Discophora, and from other considerations, I am inclined to think it probable that future researches will prove that all the Triploblastica are ancestrally Cœlomata, the presence of mesoblast implying the (ancestral) presence of a cœlom.[1] However this may be, in

[1] In the embryo of Leptoplana, the cells which will form the mesoblast are marked out very early, before the hypoblast and epiblast are definitely established. But it is noticeable that the mesoblast cells are split off from the four large cells which afterwards form the hypoblast, the epiblast having been already marked out by four smaller cells, which eventually increase in number and surround the mesoblast and hypoblast. I think that in this case the mesoblast may fairly be said to have a hypoblastic origin. I can see no objection to the view that this may be a very much abbreviated development, derived from a type in which the mesoblast arose as (hypoblastic) outgrowths

all those forms in which a cœlom is recognised the division of the mesoblast into splanchnopleure and somatopleure, and its relation to the cœlom in limiting it, must enter largely into our conception of what is meant by the term mesoblast.

The origin of the mesoblast is very various; for information on this point I must refer the reader to Balfour's 'Comparative Embryology,' vol. ii, p. 290, where a tabular account of its various modes of origin is given. From this table it will be seen that while instances of a mixed derivation of the mesoblast are not common, a purely epiblastic derivation is still more uncommon, occurring in fact only in the larva of Desor, Bonellia, and perhaps in Lumbricus trapezoides. A purely hypoblastic derivation is of frequent occurrence. It is generally admitted that part of the mesoblast, at any rate, was primitively derived from the epiblast; that in many forms all traces of this derivation are lost has been explained by Lankester (26 and 27) by his theory of precocious segregation. On the other hand there is much evidence in favour of the view that the cœlom is derived from archenteric diverticula, and that the limiting walls of the cœlom are in consequence derived from hypoblast. This is clearly the case in several groups; in others there is reason to believe that the origin of the mesoblast as ingrowths from the lips of the blastopore is an abbreviation of development, and that in the ancestors of the groups in which this occurs the mesoblast took its rise from the walls of outgrowths of the archenteron.

It is assumed that the triploblastic Metazoa took their origin from the diploblastic Metazoa, as the Cœlenterates have been called (I leave the Dicyemidæ and Orthonectidæ out of the question). The Cœlenterate, represented by an Actinia, already in the elongation of its mouth and the arrangement of its mesenteries, shows a tendency to bilateral symmetry. It is supposed that this tendency is further increased, that the radial symmetry of the peripheral chambers is replaced by a bilateral symmetry, metamerically repeated along the long axis formed of the archenteron. If this were admitted it would admit the Turbellaria among the Cœlomata.

by the mouth; and, finally, it is supposed that these chambers are the equivalents of the paired archenteric diverticula seen in the embryo of Amphioxus, outgrowths which are eventually nipped off to form the mesoblastic somites, the walls of which constitute the mesoblast, the cavities the cœlom. If the facts adduced in support of this theory are not numerous enough to warrant our giving unqualified consent to it, there is at least a great deal to be said in its favour. What is important to the present purpose is, that if it be accepted as a probability, and if further it be admitted as a general statement that, throughout the Triploblastica this is the origin of the cœlom, then by far the greater part of what we understand by mesoblast in the Triploblastica is homologous, not with the supporting lamina, the Stützlamelle, of the Cœlenterata, but with the endoderm lining the cavities of the entocœles and exocœles.

If we seek for an explanation of the supporting lamina in its origin we do not get a very satisfactory answer. Kowalevsky (25) describes the development of a jelly-like interstitial tissue between the cells of the inner layer of the thickened ectoderm of the larvæ of certain Alcyonarians. The inner cells eventually lose their primitive shape, become star shaped or spindle shaped, and are separated from one another by an interstitial, jelly-like substance. The outer ectoderm cells form a plaster epithelium, which bounds the external surface of the animal. In this case there is no doubt that the interstitial tissue, usually called the mesoderm of the adult, is derived from the epiblast. We have not so exact an account of the development of the supporting lamina in any other group of the Cœlenterata. Fol (10) describes the appearance of a clear transparent jelly between the two primary layers in Geryonia, but is unable to state which layer it is derived from. Claus (2) is no more explicit on the same subject in his work on Charybdœa marsupialis. Metschnikoff ('Studien über die Entwicklung der Medusen und Siphonophoren') speaks of a similar jelly-like substance making its appearance, but he does not say how. Chun gives no further account of the origin of the jelly-like substance in Ctenophora; but, according to a recent paper

oy Metschnikoff (29), mesoblast cells are marked off in the embryo of Callianira bialata before any tissues are developed, but after the complete separation of ectoderm from endoderm. He says, further, that the case is the same in Beroe and Cydippe. From the account given the segmentation appears to be very peculiar. If the formation of mesoderm is correctly described it would mark off the Ctenophora very sharply from the remainder of the Cœlenterata. Metschnikoff, however, for reasons which are not quite clear to me, refuses to this layer the name of mesoderm.

No account is given of the origin of the supporting lamina in the Hydrozoa, nor in the Actinaria.

The only certain knowledge, then, that we have about the origin of the jelly-like layer is that in the Alcyonarians Sympodium coralloides and Clavularia crassa the interstitial substance is derived from the epiblast, and the cells in it are epiblast cells. Thus the origin of the jelly-like supporting lamina of Cœlenterata gives no direct evidence of its homology with the mesoblast of the Triploblastica, but rather the contrary, for the latter is, as we have seen, rather connected with the hypoblast than with the epiblast.

The characters of the supporting lamina in the Cœlenterata are as follows:

In the Hydromedusæ it is a fine, apparently structureless membrane, interposed between ectoderm and endoderm. When treated with suitable reagents it exhibits a fibrillar arrangement; it contains no cells.

In the Siphonophora it is a structureless jelly-like substance.

In the Scyphomedusæ (Charybdæa) the jelly-like substance is abundant, forming the bulk of the umbrella; it contains no cells, but has a fibrillar arrangement.

In the Discomedusæ (Aurelia) the gelatinous matrix contains a number of oval or star-shaped cells, anastomosing with one another, and mainly derived from the hypoblast.

In the Ctenophora it contains muscular stellate cells, mostly of epiblastic origin, though some are stated by Chun to come from the hypoblast.

According to Metschnikoff, in certain forms these cells are marked out early in the embryo.

In Alcyonaria cells in which the calcareous spicules forming the skeleton are developed lie embedded in a gelatinous matrix.

In Actinaria and Madreporaria the supporting lamina is fibrillar, and contains a few connective-tissue cells. Sometimes muscular fibres are embedded in it (Hertwigs, 15).

It is obvious that in none of these cases (except the doubtful case of the Ctenophora, as described by Metschnikoff) is there anything like a true mesoblast, in the sense of a cellular layer marked out early in the embryo. But there is a third layer of tissue in the body, interposed between the ectoderm and endoderm, which in some cases does and in others does not contain cells, but the bulk of which in all cases is a gelatinous matrix. This third layer assumes immense development in some forms, e. g. the Discomedusæ and Alcyonaria, so that it is wholly misleading to call Cœlenterates two-layered animals. They are certainly three-layered—anyone can see that by cutting a section across any one of them—but the question is, Can they possibly be called triploblastic? Can they be said to possess a third germinal layer—a mesoblast?

It is sometimes argued that the mesoblast is, after all, nothing more than a layer of cells developed from one or both of the two primary layers; that the middle layer of the Cœlenterata contains cells in many instances; that these cells differ from the mesoblast cells of other forms only in the date of their taking up their position in the third layer, the former being separated off from the primary layers in the embryo, the latter in the adult; that this difference in time is not essential; and that therefore the cell-containing middle layer in the Alcyonaria, for example, has as much right to be called a mesoblast as that of any other animal.

I cannot but think that this style of argument leads to a want of precision of ideas, and to a vagueness in the definition of the thing signified. In a great number of forms the middle layer contains no cellular element; it is a nearly structureless

gelatinous matter, poured in, as it were, between the ectoderm and endoderm to serve as a support for those tissues and to give coherence and consistency to the body of the animal. Where no cells are present (Hydromedusæ, Charybdœa), a third cell layer, a mesoblast, obviously cannot be spoken of. In other forms we find cells derived from one or other of the primary layers wandering into the gelatinous substance after the formation of the latter, and retaining a constant position there (Discomedusæ, Actinaria). These cells ought to be considered epiblastic or hypoblastic according to their origin, just as much as the central nervous system of the Vertebrata, entirely surrounded by mesoblastic structures, is considered as part of the epiblast.

In the Alcyonaria the separation of the epiblast cells which are destined to become the skeletogenous cells takes place contemporaneously with the secretion of the gelatinous matrix in which they are embedded. None the more is the layer thus formed entitled to be called a germinal layer, or even a separate cell layer, though a step has been made towards the latter. The cells forming the skeleton are clearly epiblastic in origin, are derived from the epiblast after its demarcation, and are properly considered as its derivatives. The Alcyonarian skeleton is really of epiblastic origin.

In a further stage the cells which, in Ctenophora, are destined to become stellate muscular cells embedded in the gelatinous matrix of the supporting lamina, are, according to Metschnikoff, marked out early in the embryo, at a period when the endoderm is scarcely covered in by the ectoderm. This is nearly the same thing as the formation of embryonic mesoblast, and foreshadows it, but the ultimate history of the cells ought to preclude our calling them mesoblastic.

I do not wish to assert that the supporting lamina of Cœlenterata is not represented in the mesoblast of Cœlomata; it is highly probable that it is. The Alcyonaria and Ctenophora are good examples of the tendency which muscular and connective-tissue cells, primitively belonging to the external and internal limiting layers, have to separate themselves from their

original position and to become more deeply situated. When such cells form a layer situated between epiblast and hypoblast they constitute a third layer, a mesoblast. But in point of fact we have no positive evidence that such a simple third layer exists without the ancestral coexistence of a cœlom. I have already given reasons for believing that such a simple mesoblast does not obtain in the Platyhelminthes. The nearest approach to it is in the Ctenophora, and in them the stellate cells are homologous, not with the whole of the mesoblast of the Cœlomata, but only with a part of it, viz. that part which may be supposed to have originated independently of the cœlom, but of the origin of which the traces are, in the majority of cases, suppressed.

The part is not the whole, nor should the name denoting the whole be given to the part, for which reason I object to giving the name mesoblast, or its equivalent mesoderm, to the supporting lamina of Cœlenterata. I have proposed for it the name Mesoglœa, a name which was suggested to me by Professor Lankester in the course of a conversation on this subject, and which corresponds exactly to the Gallertlage of German authors. Its meaning, "middle jelly," has particular reference to the Medusæ, of whose bodies it forms the greater part.

Before concluding this paper I have to express my obligations to Professor Moseley, who kindly permitted me to use the Oxford laboratory during my studies, and assisted me with much valuable advice. Also to my friends Mr. Hatchett Jackson, and Mr. W. Baldwin Spencer of Oxford, who helped me in many ways.

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DESCRIPTION OF PLATES XXIII, XXIV, & XXV.

Illustrating Mr. Gilbert C. Bourne's Paper on "The Anatomy of the Madreporarian Coral Fungia."

Fig. 1. Ad naturam.—General view of Fungia dentata, showing the arrangement of the tentacles and their relation to the septa.

Fig. 2.—Diagram in illustration to Fig. 1. *m.* Mouth. *t.* Tentacles. 1, 2, 3, 4, 5, 6. Septa (or tentacles) of the first, second, third order, &c. Primary septa coloured red, secondary blue, tertiary green, quaternary yellow, quinary, &c., black.

Figs. 3—8.—Diagrammatic, from Stutchbury, Semper, and Moseley.

Fig. 3 is a young nurse-stock of Fungia immediately after fixation. *p.* Peristome. *th.* Theca. *b.* Base.

Fig. 4. The same, in which the peristome has commenced to widen out and assume its characteristic form.

Fig. 5. Nurse-stock of Fungia; an absorption of calcareous matter has taken place along the line *f*, where the young Fungia will separate from the nurse-stock.

Fig. 6. Young Fungia shortly after separation from the nurse-stock. The peristome has grown greatly in excess of the theca.

Fig. 7. The same more advanced, showing the increasing size of the peristome.

Fig. 8. The adult Fungia.

Fig. 9.—Diagram showing a pair of mesenteries and their relation to the investing tissues of the corallum. The mesentery to the left is seen to be divided by a synapticulum into a central and a peripheral portion. *ec.* Ectoderm. *m.* Mesoglœa of the body wall. *ed.* Endoderm of the body wall. *m'*. Mesoglœa of the mesenteries. *m''*. Mesoglœa of the skeletotrophic tissues. *ed'*. Endoderm of the mesenteries. *ed''*. Endoderm of the skeletotrophic tissues. *ms.* Muscles. *cy.* Calycoblasts. *cd.* Nematocysts. *m.f.* Mesenterial filaments. *sb. a.* Symbiotic algæ. *syn.* Perforation through which a synapticulum passes.

Fig. 10. Ad naturam.—A mesentery of Fungia dentata. *ac.* Acontia. *v. m. s.* Longitudinal muscle-fibres, the remainder of the lettering as before. The shaded part represents that part of the mesentery which is formed of mesoderm and its overlying muscular fibres. The dotted lines show the tubes (parts of the cœlenteron) bounded by endoderm of the skeletotrophic investment which run down between the synapticula, to the mesoglœa of which thickened prolongations of the mesoglœa of the mesenteries are attached, as shown in *x*.

Fig. 11. Ad naturam.—Two contiguous mesenteries not belonging to the same pair, from an adult Fungia, showing their tendency to fuse together in old specimens. Lettering as before.

Fig. 12. Ad naturam.—Section through an acontium, showing the dendritic branching of the mesoderm in the acontium, the large nematocysts *cd.*, and the gland-cells *gd.*

Fig. 13.—Diagrammatic horizontal section through the corallum and soft parts of Fungia dentata. The corallum is shaded, the mesoglœa is represented by a black line. The calycoblasts are omitted for simplicity's sake. The septa are seen to lie in the entocœles, the cœlenteron being broken up by the synapticula into a number of parallel tubes, in each of which are seen the mesoglœa thickenings *x*, which give attachment to the vertical muscle-fibres. The acontia of the lower orders of mesenteries are seen coiled up in the cœlenteron. *mt.* Mesenteries. *syn.* Synapticula.

Fig. 14. Ad naturam.—Section through the body wall of F. dentata. *ed.* Endoderm. *m.* Mesoglœa. *ec.* Ectoderm. *n.* Interstitial cells.

Fig. 15.—Diagrammatic vertical section through the peripheral part of F. dentata. *ec. p.* Ectoderm of the peristome. *ec. b.* Ectoderm of the base. The shading and lettering as in Fig. 13. *th.* Theca. *ex.* Exocœle. *ent.* Entocœle external to theca.

Fig. 16.—Diagram illustrating the relations of the cœlenteron to the corallum. The drawing may be considered as a cast of all the cavities lined by endoderm. At *sp.* are shown the spaces occupied by the septa, by which the body is broken up into a number of wedge-shaped masses. The rows of parallel elongate perforations show the position of the synapticula. At *cœl.* is seen that part of the body cavity which lies outside the perforate theca. *th.* Perforations for theca. *syn.* Perforations for synapticula.

Fig. 17.—A portion of the skeletotrophic investment highly magnified. *ed.* Endoderm. *m.* Mesoglœa. *cy.* Calycoblasts

On Some Points in the Development of Petromyzon fluviatilis.[1]

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With Plates XXVI, XXVII, XXVIII, and XXIX.

The development of the Lamprey has occupied the attention of many embryologists during the last fifty years. Of these we owe the most complete accounts of the changes through which the egg passes to Max Schultze, Owsjannikow, Calberla, Scott, Balfour, and Dohrn. I have recently worked through the development of Petromyzon again, and worked out the origin of several organs which have hitherto been incompletely known. In many of the most important points my researches confirm those of the earlier observers, and to these I have only referred at such length as would make the account intelligible; in others, such as the persistence of the blastopore, the origin of the ventral mesoblast, &c., I differ from previous descriptions; and some points, such as the development of the heart, of the parts of the brain and cranial nerves, are worked out for the first time.

The material for this article was obtained by artificially

[1] The differences between Petromyzon planeri and fluviatilis are so slight, and the intermediate forms so common, that I am disposed to follow Anton Schneider, and to consider them as varieties of the same species. This species may conveniently retain the name fluviatilis, as opposed to the larger form Petromyzon marinus.

fertilising the eggs of the ripe female Lampern, hatching the larvæ out, and rearing them in confinement. The breeding time is during the latter half of April and the beginning of May.

The generative products of both male and female were squeezed into glass vessels containing fresh water, and the contents slightly stirred. The eggs at once adhered to the bottom and sides of the vessel, and were left undisturbed for three or four hours. The water was then poured off and a fresh supply added. This was kept thoroughly aerated by means of Semper's aerating apparatus. The number of eggs fertilised were about 70 per cent. of the total, though some hatches were much more successful than others. The rate of segmentation and development also varied greatly, being influenced by the temperature and manner of aeration. The unfertilised eggs very soon could be distinguished from the fertilized; they developed great cavities or craters and were soon attacked by fungi. The fungus, however, rarely affected the developing eggs.

The spermatozoa have elongated heads, pointed at their free end, but thicker at the end from which the tail arises (fig. 1). Their length is from 35 to 40 micro. mm., of which the head forms 3 micro. mm. They move actively about in the water, until they come into contact with an ovum. They enter the egg through a micropyle, and Calberla states that the head only enters the protoplasm of the ovum, the tail remaining fixed in the micropyle, thus hindering the entrance of other spermatozoa.

The eggs are almost spherical, with a diameter of about a millimetre. On contact with water the outer cell-membrane swells up and forms a gelatinous coating, by means of which the eggs adhere to the bottom and sides of the vessel. This gelatinous envelope is of considerable thickness; it ultimately disappears shortly before the embryo is hatched. Sections through unfertilised eggs show the protoplasm crowded with oval yolk granules, which stain deeply. These yolk granules vary in size, and this is very evident in the segmenting eggs,

where the yolk granules in the more quickly dividing upper pole are much smaller than those in the more inert lower pole. An attempt has been made to show that those parts of the unsegmented egg containing the smaller granules is destined to form the epiblastic parts of the embryo (16).[1] This view seems to me to need confirmation. The small size of the yolk granules in the epiblast might be due to the more rapid division of these cells, causing a more rapid consumption of the food-yolk.

The unusually deep staining which the yolk granules assume very materially increases the difficulty of observation. Especially in the earlier stages of development the cell limits and nuclei were rendered obscure by the masses of deeply stained yolk granules.

As previous observers have stated, there are two polar bodies extruded one after the other. After fertilization the egg contracts, leaving a cavity between it and the egg membrane.

The first furrow appears about the fourth hour; it appears first in the upper pole and spreads round the egg on each side. Calberla states that the micropyle becomes at first oval, then slit like, and finally passes over into the primary furrow. I have not been able to observe this process in my eggs. He further states that the first furrow divides the egg into two unequal parts, a large epiblastic and a small hypoblastic; the smaller of these divides subsequently more rapidly than the latter. Thus, according to him, the first furrow would correspond with the first equatorial one in the Frog's ovum. Scott, although he had no fresh material to work with, was able to correct this, and, as the latter suggests, Calberla was probably misled by cases of abnormal segmentation. Many of the eggs which apparently had not been fertilized divided by one, two, and sometimes three furrows, and when this took place the furrows were nearly always abnormal in position.

The second furrow is vertical and at right angles to the first, and also appears first in the upper pole. The third is

[1] The figures in brackets refer to the list of papers at the end.

equatorial, but nearer the upper than the lower pole. After its appearance the epiblastic half is separated from the hypoblastic or yolk-bearing half (fig. 2).

The external phenomena of segmentation have been accurately described by Max Schultze, with the exception of the next stage. After the first equatorial furrow he describes two more in the same plane, but in my eggs the equatorial furrow was followed by two vertical lines, which appear at first in the upper pole exactly as they do in the Frog's ovum (fig. 3). These are followed by two more equatorial furrows which divide the egg into thirty-two segments. After this the segments of the epiblastic pole divide more rapidly than those of the lower.

Fig. 5 represents a transverse section through an egg thirty-six hours after fertilisation. In this stage it is a blastosphere, with a segmentation cavity enclosed by a single layer of cells except along the line where the epiblastic and hypoblastic cells join. Here the layer is two cells thick. The nuclei of the large cells appear small, but it must be recollected that the amount of protoplasm is very small compared to the yolk. The latter has been omitted for the sake of clearness. Fig. 6 is taken from an egg twelve hours later. Here both the roof and floor of the segmentation cavity are many cells thick. A similar stage is found in the Frog's ovum, but there is this difference between the two. In the Frog's egg the whole of the roof of the segmentation cavity forms epiblast; in the Lamprey it is only the outermost layer. The following stages are accompanied by a thinning out of the roof of the segmentation cavity, and are represented in figs. 7 and 8. On this point my observations tend to confirm those of Calberla, and are opposed to those of Schultze, who found a many-layered roof to the segmentation cavity just before invagination. The thinning out appears to be brought about by the inner cells of the roof passing round to the sides and floor of the segmentation cavity. Just before the invagination which forms the gastrula the roof of the segmentation cavity consists of a single layer of cells; the segmentation cavity is

large and occupies the whole of the upper hemisphere, whilst the lower hemisphere is solid and consists of larger cells, which we may speak of as yolk-cells. The most external layer of these consists of rather columnar cells. These latter cells soon become smaller than the inner yolk-cells, and about the time of invagination the whole egg is enclosed by a layer of small columnar cells, the epiblast. This is brought about by the conversion of the outermost row of yolk-cells into small columnar cells. As Balfour has shown, this takes place latest in the region of the blastopore.

The invagination which forms the mesenteron commences about 130 hours after fertilisation; it commences at one side of the equator of the egg, in the region where the single layer of epiblast cells passes into the yolk-cells (fig. 9). The invagination at first has a wide-arched slit-like opening, but this soon narrows into a small circular pore (fig. 4). The segmentation cavity is gradually obliterated by the invaginated cells. These from the first enclose a cavity, the mesenteron. In this respect the formation of the gastrula is like that of Amphioxus, and differs from that of the Amphibia, where the mesenteron appears later as a splitting underneath the invaginated cells. The presence of a large amount of food-yolk causes the invaginated cells to be pushed dorsalwards. The mesenteron extends as a tubular cavity about two thirds round the embryo. Its dorsal wall is composed of columnar cells resembling those of the general epiblast; the cells forming the floor have the same characters as the yolk-cells (fig. 12). The dorsal side of the mesenteron lies in immediate contact with the under surface of the epiblast throughout its entire length. In this respect again the Lamprey differs from the Frog, where the invaginated hypoblast cuts off a mass of cells on its dorsal side, which subsequently forms the mesoblast.

The mesoblast now appears by the differentiation of two bands of these yolk-cells, which lie in the angles formed by the mesenteron and the epiblast (fig. 12). This differentiation commences in front and is continued backward. The two bands of mesoblast are separated dorsally by the juxtaposition

of the dorsal wall of the mesenteron and the epiblast, and ventrally by the hypoblastic yolk-cells which are in contact with the epiblast over two thirds of the embryo. Subsequently, but at a much later date, the mesoblast is completed ventrally by the downgrowth on each side of these mesoblastic plates. This takes place at a comparatively early stage in the head and that part of the trunk lying in front of the liver. In the posterior part, which remains swollen with yolk, the ventral completion of the mesoblast is delayed.

The first formation of the mesoblastic plates appears to take place by a differentiation of the hypoblastic yolk-cells in situ, and not from invaginated cells (figs. 12 and 13). The subsequent downward growth is brought about by the cells proliferating along the free ventral edge of the mesoblast, these cells then growing ventralwards, pushing their way between the yolk-cells and epiblast (fig. 11).

This account of the origin of the mesoblast differs from that given by Scott. He describes the mesoblast as arising from two sources—(1) cells which are derived from the invagination of the blastoderm, (2) the outermost layer of the hypoblastic yolk-cells, which, according to Scott, split off from the remainder, and form a ventral sheet which completes the mesoblast in that side of the body. The mesoblast in the head is derived only from the first source, as by the time it is completed ventrally the head is raised above the yolk-containing parts.

Shortly before the development of the head fold raises the head from the yolk-bearing part of the embryo, the neural plate becomes evident in the exterior. It extends as a low ridge from the anterior lip of the blastopore to just in front of the blind anterior end of the mesenteron, over two thirds of the circumference of the embryo.

The blastopore is always visible at the posterior end of the neural plate. Schultze has given a very complete set of figures of the exterior of the embryo. As his figures show, with the elongation of the embryo the anterior end curves round and overlaps the posterior, thus obscuring the blastopore. Fig. 10

is a section taken through the blastopore and the head soon after the head is raised above the general level of the egg.

From his observations of the embryo as a whole, Schultze came to the conclusion that the blastopore persisted and gave rise to the anus, and he was supported in this view by Calberla. Later observers, however, who have studied the development of the Lamprey by means of sections, have maintained Benecke's view that the blastopore disappears. Scott describes the neural canal enclosing the blastopore and figures the neurenteric canal thus formed. He describes the formation of the anus, from a protuberance of the alimentary canal which approaches the epidermis and breaks through about the twentieth day. Balfour also states that the blastopore closes and does not form the permanent anus.

My observations of the embryo as an opaque object lead me to the belief that the blastopore remained open. In this I have been confirmed by sections taken through a series of embryos preserved at intervals of a few hours. Primarily the blastopore lies at the posterior dorsal end of the embryo (fig. 4), but by the growth of the dorsal surface and the formation of the tail it comes to occupy a position in the ventral surface. What was the anterior lip in the first position comes to be the posterior in the latter.

Fig. 4 is a view of the embryo twelve days old, as an opaque object, showing the blastopore at the posterior end of the neural ridge. Fig. 16 is an oblique section through an embryo about two days older, showing the nervous cord just separated from the skin, and the notochord both continuing behind the blastopore.

Scott was of opinion that the lumen of the invaginated mesenteron persisted only in the fore-gut. Soon after the invagination is completed this part of the alimentary canal lying in the head and neck becomes raised from the rest of the embryo. It is thus separated off from the yolk-cells, and the hypoblastic cells in this region soon assume a definite columnar appearance, though they continue to contain yolk granules for some days. This region extends to where the liver appears

in older embryos. A similar change in the cells lining the mesenteron takes place at its posterior end. The cells lining the blastopore and extending for some distance into the alimentary canal assume very early a columnar appearance and appear perfectly continuous with the columnar epiblast (figs. 10, 14, and 16.) The cells lining the hind-gut retain the character of the yolk-cells for a long time, but the lumen of the mesenteron in this region never disappears, as Scott and Calberla thought. The lumen of the alimentary canal, with the exception of the mouth, is derived directly from the invagination which forms the gastrula, and no part of it is ever obliterated in the course of development.

A similar persistence of the blastopore to form the anus appears to be common in the Amphibia. It has been shown to occur in the Newt by Miss Johnson, in the Frog by Spencer, and in Alytes by Gasser. Its occurrence in the Cyclostomata seems to point to the fact that it is a primitive feature retained in those eggs whose development is not greatly modified by the presence of a large mass of yolk. Renewed observations in the development of Amphioxus would probably throw some light on this point.

The Central Nervous System.

The early development of the central nervous system has been so fully described by Calberla, Balfour, and Scott, that little is left to be added to their account. But the origin of the neural canal, the relationship of the posterior end of the neural cord to the blastopore, and the later development of the parts of the brain and the cranial nerves present points of interest.

Calberla was the first to show that the central nervous system of the Lamprey arises by a delamination and not by an involution of the epiblast. He described a similar origin for the nervous system of the Teleostei, and Balfour and Parker found the same to be the case in Lepidosteus.

The first trace of the neural plate appears about the eighth day after fertilization, just after the invagination is completed. A

shallow groove is seen running forward from the blastopore, round about two thirds of the embryo and passing a little in front of the blind end of the mesenteron. The groove is a very temporary structure and is soon replaced by a ridge. This arises by the epiblastic cells lining the groove, which are of a columnar shape, budding off cells from their under surface. The result of this is that a keel of cells is formed which forms the neural ridge externally (fig. 12), and internally presses in between the mesoblastic plates. The keel arises solely by the epiblast cells budding off cells in their under surface only. It is much deeper in the anterior third of its course, which region ultimately forms the brain.

The keel in the course of two or three days loses its connection with the epidermis; this occurs at first anteriorly and extends backward, and as Scott has pointed out, it does this of itself and not by an ingrowth of the mesoderm in each side as Calberla described.

Figs. 13, 15, and 16 show the solid neural cord lying above the notochord, which by this time is separated off from the hypoblast. It is important to notice that the neural canal does not arise until after the connection between the neural cord and epidermis is severed. It is about the origin of this neural canal that my observations and those of Calberla and Scott are at variance. They described the epidermic layer of epiblast passing down into the nervous, in such a way that the canal, when it does appear, is lined by this layer. I have not been able to see any trace of this. The cells forming the nervous system appear to me to be all split off from the under surface of the epidermis in the dorsal middle line, and the continuity of the epidermis in this region never seems to be broken by any such invagination as they suggest. Balfour was also doubtful on this point; but in his and Parker's work on the development of Lepidosteus, they state that there is no evidence of the epidermic layer being concerned in the formation of the canal.

The canal seems to arise as a split between the cells in the axis of the solid cord, and not by the absorption of the central

cells, as has been suggested in the case of the Teleostei. It appears at first anteriorly and extends backward, and for some little time the walls of the lumen are by no means sharply defined. Processes from the cells lining the canal project into its cavity and suggest the idea that they have been torn out from between the cells of the other side.

The neural cord remains solid at its posterior end for some time, and here it becomes fused with the surrounding structures in a somewhat remarkable way. It does not fuse round the blastopore as Scott describes, indeed it is not easy, considering its mode of origin, to see how it could; and there is no hollow neurenteric canal. Figs. 14 and 15 represent two sections taken through a larva just after hatching. Fig. 14 is through the region of the blastopore. It shows the neural cord with its canal already formed; beneath this lies the notochord, and beneath this again a solid rod of cells which is continuous with the subnotochordal rod and the dorsal hypoblast. This latter structure is the solid postanal gut. The mesoblastic plates are seen separating off from the hypoblast yolk-cells which occupy the remaining space with the epidermis. Dorsally this is produced to form the dorsal fin. Fig. 15 represents a section through the tail a little posterior to the blastopore. Here the neural cord, notochord, and postanal gut have fused into a rod-like mass of tissue which is ventrally continuous with the hypoblast cells; a few sections posterior to this none of the three embryonic layers are distinguishable except the epidermal portion of the epiblast. A longitudinal median section through the tail is represented in fig. 20. This shows the mass of indifferent tissue which lies in the tail and which by internal differentiation gives rise, as the tail grows, to mesoblastic somites, neural cord and postanal gut. This mass of tissue, which in many respects reminds one of the growing point in a plant, may be called the primitive streak. It is perhaps worth while to point out that it lies at what was originally the anterior lip of the blastopore.

A similar mass of tissue formed by the fusion of the primary layers has been described by Balfour and Parker in

Lepidosteus, Spencer in the Frog, and Miss Johnson in the Newt.

The further development of the central nervous system will be described later after some of the details connected with the mesoblast and hypoblast have been considered.

The Mesoblast.

The origin of this layer from the yolk-cells situated in the angle between the epiblast and the invaginated endoderm has been described above. For some little time the mesoblast remains in the condition of two triangular masses of cells, separated from one another dorsally by the notochord and nervous system, ventrally by the yolk-cells which lie in contact with the ventral epiblast. In the anterior end of the embryo the mesoblast soon unites ventrally by lateral downgrowths; in the trunk, however, which remains crowded with yolk-cells for a week or ten days after hatching, this takes place much later.

Scott has described the formation of the muscle-plates very accurately, and it will therefore be unnecessary to give more than a short résumé in order to make the following account intelligible. About the twelfth or thirteenth day the mesoblastic somites appear by the segmentation of the dorsal part of the lateral mesoblastic plates. These appear at first anteriorly, and the segmentation extends backwards. The most anterior one lies close behind the auditory sac. The ventral unsegmented mesoblast has split into the splanchopleure and the somatopleure on each side, and in the region just behind the posterior gill-cleft these have met ventrally, forming a ventral mesentery, connecting the alimentary canal with the ventral body wall.

The mesoblast somites are shown in fig. 17, which represents a horizontal section through an embryo fourteen days old. They are cubical masses of cells enclosing a small cavity, often entirely obliterated, which represents part of the body cavity. The cells surrounding this are at first uniform in size, and each side is only one cell thick. Like the other cells of

the embryo they contain yolk granules, which are gradually absorbed. In the tail region these mesoblastic somites continue to be segmented off from the primitive streak till five or six days after the larva is hatched.

In transverse sections the mesoblastic somites appear triangular, having a median side against the nervous system and notochord, an external one against the epididymis and a ventral one. Besides these there are the anterior and posterior sides. The cells composing all these, except those of the external layer, develope into longitudinal muscles. Whilst this is taking place the dorsal surface of the embryo has become raised above the general level, so that the embryo in section is no longer round but pear-shaped.

As Stannius, Grenacher, and Langerhans have shown, the muscles of the Lamprey fall into two groups, which differ in structure as well as in their disposition. The first of these form the myomeres, and are derived directly from the mesoblastic somites; the second comprise the muscles of the eye, those belonging to the respiratory system, and those connected with the upper and lower lip and mouth generally. These seem to arise exclusively from the ventral unsegmented parts of the mesoblast, and perhaps, in some cases, from wandering mesoblast cells. The muscles of the heart resemble the latter in many points.

Each myomere in the Lamprey or Ammocœte consists of a number of plates of muscle-substance, lying one on the top of another. Each plate is flat, and more less square in outline. It is bounded anteriorly and posteriorly by the myotomes externally by a connective-tissue layer closely connected with the skin, and internally by a similar layer. Above and below, or dorsally and ventrally, it is in contact with a similar muscle-plate. In some myomeres which have become modified, such as the anterior one which extends far forward over the ear, the shape of the muscle-plate has lost its square outline and become oblong, but in one of the myomeres of the trunk they are almost square in longitudinal section.

From the above description it will be seen that each muscle-

plate or "Kästchen" of Stannius occupies the horizontal space between two myotomes, and that they lie one on another, so that in a horizontal section we see only one, in a transverse or vertical section we see one lying on another like sheets of paper. Each "muscle-plate" contains several nuclei, which stain more deeply than the muscle-substance. It is transversely striated, and faint longitudinal striæ can also be detected; these correspond with fibrillæ, into which the muscle-substance easily breaks up. These latter are especially large, and can be easily recognised in transverse sections near the most external part of the "muscle-plate."

The development of these muscle-plates is as follows:— The outermost layer of cells forming the mesoblastic somite does not appear to be converted into muscles. For a long time it persists as a definite layer of cubical cells with large nuclei lying between the skin and the myomere; this is the case till long after the other cells of the mesoblastic somite have developed into muscles. Finally, this layer seems to disappear, but remains of it can still be distinguished lying just within the skin, even when the myomere has assumed the appearance characteristic of the full-grown Ammocœte. This view that the somatic layer does not take part in the formation of the myomeres, is not in agreement with what Balfour has described in the Elasmobranchs, where both the inner and outer layer become muscular; but, on the other hand, the muscles of the myomeres in Amphioxus appear to be derived from the splanchnic layer only, and the same view is supported by Götte and the Hertwigs.

The remaining cells of the mesoblastic somite begin to grow in between one another, and between each neighbouring somite an intermuscular septum is deposited. The process of growing in between one another is carried on until each cell occupies the whole length from one myotome to the next, and at the same time, each cell becomes somewhat flattened, so that their transverse section, which was at first round, become oval (fig. 24). At the same time longitudinal thickenings occur in the cortical part of the cell, the medullary portion

remaining clear and staining very slightly. The nucleus lies in this medullary portion. The longitudinal thickenings occur at intervals, so that in transverse section the cortex of the cell appears beaded; these fine fibrillæ stain fairly well so that they can easily be distinguished from the medulla. The flattening of the cell goes on until the cell occupies the whole space between two myotomes, not only longitudinally but also transversely (fig. 25). The original nucleus of each cell divides into two or three, so that in each of these plates of muscle-substance two or three nuclei can be seen and an occasional yolk granule, which is, however, soon absorbed. In addition to the longitudinal striation caused by the thread-like thickenings in the cortex, a transverse striation appears. Each plate of muscle-substance remains in this condition, with a clear unstained medulla containing two or three deeply stained, large, flat, oval nuclei (fig. 18), with a well-marked nucleolus; enclosed by a cortex, for about two weeks after hatching. The cortex consists chiefly of its dorsal and ventral walls, and each of these is thickened at regular intervals by the above-mentioned fibrillæ. Each fibrilla runs the whole length of the myomere and is inserted into the intermuscular septa behind and in front. About a fortnight after the young Ammocœte is hatched, the substance of the fibrillæ increases at the expense of the medullary part, and this goes on until each plate of muscle-substance consists exclusively of fibrillar substance. The nuclei have increased in number, but instead of lying loose in medulla they become squeezed in between the fibrillæ, lose their regular shape and can only be recognised as small flattened bodies which stain deeply. The whole plate of muscle-substance now consists of fibrillar substance which stains uniformly with here and there a more deeply stained nucleus (fig. 29). The whole appears homogeneous, the fibrillæ cannot as a rule be recognised, though in some cases they are seen in transverse section as dots. Each "Kästchen" now resembles fundamentally the muscle-plate of the adult Lamprey; and it will be noticed that each is a development of what was a single cell.

The second variety of muscle-fibre met with in the Lamprey seems to be exclusively derived from the ventral unsegmented mesoblastic plate, and from the walls of the head cavities. The muscles with this origin are those which serve to move the lips, the velum and the other structures of the mouth, and certain muscles connected with the gill apparatus, and probably the muscles of the eye. These latter have the same histological structure, but owing to the fact that the eye does not develope until the Lamprey stage, no eye muscles appear till very late in the life of the Ammocœte and I have consequently been unable to follow their development.

The muscle-fibres of this second variety of muscle tissue, consist of long tubular cells, cylindrical in shape, with a medulla of clear substance which does not stain, and a cortex which is thickened at intervals by longitudinal rods. These give the cortex a beaded appearance in transverse section. The medulla contains the nucleus, which stains deeply. This is at first single, but subsequently divides until a row of nuclei occupy the axis of the muscle-fibre, in some cases so closely packed as almost to touch. It will be noticed that these muscle-fibres resemble in the minute structure the first stage in the development of the muscles forming the myomeres. These muscle-fibres are transverse striated.

The fibres of the heart belong to this second variety, and are developed from the same part of the mesoblast. They, however, possess certain peculiarities which will be described after the formation of the heart has been considered.

The Heart.

The first appearance of the body cavity as a space takes place in the region behind the posterior gill-cleft and in front of the liver. The part of the embryo lying in front of this region is at an early stage raised from the posterior half by the backward growth of the head fold, and the embryo lies within the egg-shell bent in half, the angle of the bend being just in that region where the heart is subsequently formed. By this means all those parts in front of the liver are free from the yolk-bearing cells, and the lining cells of the mesenteron all

become columnar. In this anterior region the mesoblast soon unites ventrally. In the posterior region the ventral union of the mesoblast is delayed, the lateral plates of mesoblast lying between the yolk-cells and the epiblast end in a free edge, and until these edges unite, the yolk-cells are in contact with the epidermis ventrally.

In the region between the liver and the last gill-slit the mesoblast splits at about the fifteenth day into a somatic and a splanchnic layer; between the two a well-developed body cavity appears. The former layer lines the body wall, the latter envelopes the alimentary canal. It forms a dorsal mesentery supporting that structure, and a well-marked ventral mesentery of considerable depth connecting the ventral wall of the intestine with the body wall. It is in this ventral mesentery that the heart is developed. The two layers forming the mesentery fuse dorsally and ventrally, but separate from one another in their middle, forming a cavity which is the lumen of the heart (fig. 24). Subsequently both the mesentery connecting the heart with the alimentary canal—the mesocardium—and the ventral one connecting the heart with the ventral body wall, atrophy and the heart lies as a tube unconnected with the surrounding structures (fig. 25).

From the fact mentioned above that the mesoblast behind the heart has not split into somatic and splanchnic layers nor united ventrally, it will be seen that the cavity of the heart communicates posteriorly with the space between the ventral yolk-cells and the epidermis. Such a space would be equivalent to part of the segmentation cavity. Soon after the heart is formed such a space arises, and at once becomes crowded with cells destined to form blood-corpuscles (fig. 26). At first I was inclined to think that these cells were budded off from the yolk-cells, but more careful observation has led me to believe that they originate from the free edge of the lateral plates of the mesoblast, which as I mentioned above are growing down between the yolk-cells and the epiblast. These corpuscles are oval with large nuclei, and they usually contain at first one or two yolk granules which they soon absorb.

The cavity in which the corpuscles lie in great numbers is subsequently shut off by the mesoblast as it grows downwards and becomes the subintestinal vein. It is from the first continuous with the posterior end of the heart, and the corpuscles soon pass from it into that organ. From the first appearance of the heart in the ventral mesentery its walls have been double; the splanchnopleure having split into two layers, of these the outer is at first much the thicker consisting of cubical cells; the inner layer is composed of comparatively flattened cells. The heart at first is a straight tube of the same length as the section of the body cavity in which it lies. Very soon, however, it increases in length, and thus becomes slightly twisted; at the same time two constrictions appear, dividing it into three chambers. The most posterior of these is the sinus venosus; it is directly continuous with the space in which the corpuscles are developing. By this time this space has acquired definite walls by the downgrowth of the mesoblast in this region, and it may now be spoken of as the subintestinal vein.

The liver which developes as a ventral outgrowth of the intestine first makes its appearance in this space, and when the latter gets closed off as a vein, the liver has become a branched gland projecting into it, so that the blood returning from the alimentary canal passes between the tubuli of the liver. Thus, from the very first an hepatic portal system is present. The tubuli of the liver do not appear to have any continuous mesoblastic coating, though here and there a flattened cell can be distinguished in the outside of a tubule.

The venous sinus communicates by a narrow opening with the auricle or second chamber of the heart. This in its turn opens by a similar narrow opening into the ventricle. This latter opening is guarded by a pair of valves, which appear by the tenth day after hatching; they effectually prevent any regurgitation of the blood into the auricle. The walls of the ventricle have undergone a considerable change. From the cells of the inner lining a number of branched muscle-cells have been developed (fig. 36). These cells stretch across the cavity of the ventricle from side to side, and fuse and anas-

tomose with one another in a very complex manner. They contain numerous nuclei, and show a longitudinal striation though not a transverse one. The centre of the ventricle is comparatively free from them, but at the sides they form a spongy reticulum in the meshes of which corpuscles abound.

The ventricle passes anteriorly into the ventral aorta, and at the point where the aorta passes into the solid tissue between the gills there is another pair of valves resembling the auriculo-ventricular ones. The ventral aorta, like the other vessels, arises by a split in the mesoblast which subsequently acquires a definite wall. It passes forward as a single vessel in the ventral median line until it reaches the thyroid gland, and here it splits in two branches. Each branch then passes forward on one side of this body, and ends in the most anterior gill vessel. From the single part of the ventral aorta three pairs of vessels are given off, passing in front of the fifth, sixth, and seventh gill-slits respectively. The posterior wall of the seventh cleft bears no gill filaments, and has no vessel. From each side of the double part of the ventral aorta five vessels are given off, the four posterior of these pass in front of the first, second, third, and fourth gill-slits. The most anterior is the vessel which in the earlier stages passes in front of a gill-slit which subsequently disappears. In the older embryos, when the mouth is fully formed it runs along the base of the velum.

The vessels after traversing the gills unite in the dorsal middle line to form the dorsal aorta; this runs backward to the posterior end of the body, lying just underneath the notochord. From its first appearance it gives off two transverse vessels in the neighbourhood of the pronephros; these supply the glomerulus. Anteriorly it gives off a pair of vessels to supply the upper lip, the carotids. In the older larvæ the aorta gives off a vessel which passes dorsally up one myotome, then along the dorsal surface of the myomere behind it, and hence the blood is collected by a vein which returns it to the posterior cardinal down the next myomere. The larvæ are fairly transparent, and in each myotome these two opposite

currents can be seen, and along the top of each myomere a backwardly directed stream. In the tail the aorta splits, and one branch passes each side of the cloaca; they unite ventrally, and are continued forwards as the subintestinal vein. Before it splits it gives off a vessel which runs back along the base of the notochord to supply the tail; this may be termed the caudal artery. The blood from this is returned by a caudal vein which soon splits into the two posterior cardinal veins. These large veins run forward, one each side of the aorta: the duct of the pronephros runs in their wall. Anteriorly they unite with the anterior cardinals, and form two ducts of Cuvier which open into the sinus venosus. The anterior cardinals bring back blood from the head. The tubuli of the pronephros lie in their cavity, so that the pronephros, like the kidney of the Amphibia, has a double blood supply. The cardinal veins do not appear till after the subintestinal vein, which for some little time is the only vein in the body. Later still a vessel appears in the right side of the intestine, opposite the subintestinal vein in the spinal fold; this, like the last named, passes through the liver. In my latest stages also there is an impaired vessel bringing blood back to the heart from the ventral region of the gills; this is mentioned by Balfour. The blood-corpuscles are of only one kind, large oval disc-like structures, with a well-marked nucleus. The protoplasm scarcely stains, but the nucleus assumes a deep colour.

Owing to the transparency of the larva, the circulation can be watched with great ease. The walls of the vessels at first possess no elasticity, hence great regurgitation takes place, and the blood advances by a series of jerks. The valves at the anterior end of the ventricle and between the auricle and the ventricle prevent this affecting the blood in the heart.

The heart begins to beat long before the cells exhibit any histological differentiation into muscles.

The Pronephros.

The first origin of the larval excretory system is by no means easy to make out, as it arises at a period when the embryo is

crowded with yolk. Scott has described it fully, and in most respects my observations confirm his. As he describes, the first structure to appear is the segmental duct which is at first solid. The cells forming this are derived from the mesoblast cells which lie between the already segmented dorsal part of the mesoderm and the ventral unsegmented portion. These cells form a solid cord lying between the mesoblast and the epiblast; the cord continues to grow backward by a differentiation of the cells in situ. A few hours later a lumen appears in the centre of the cord by the separation of the cells; this soon becomes elliptical in section (fig. 11). It opens into the posterior part of the alimentary canal.

From this account it will be seen that at first the segmental duct is between the mesoblast and epiblast; it, however, soon comes to occupy a deeper position by the growth of the surrounding tissue. So far we have only considered the duct in that part of its course where the body cavity is not yet developed; but in the region of the heart, where the body cavity has already appeared, its origin seems to be somewhat different. The lumen of the segmental duct here becomes continuous with a groove in the parietal peritoneum, lying near the angle where the somatopleure and splanchnopleure diverge. When this groove closes it leaves four or five openings which persist as the openings of the ciliated funnels. This account of the origin of the ciliated funnels agrees with that of Fürbringer, but differs from Scott's, who describes the funnels arising as blind projections of the segmental duct which acquire an opening into the body cavity. Each funnel soon acquires cilia, which extend for some distance down its lumen, and are usually directed downwards towards the tubuli. The funnel is composed of large cubical cells with a large nucleus, at its lip it passes suddenly over into the flat cells of the peritoneal epithelium. At its base it is continuous with a duct which soon becomes elongated and coiled, and ultimately joins the segmental duct. The walls of the tubuli are composed of large clear glandular cells. The posterior end of the segmental duct opens into the cloaca

The segmental duct throughout its course runs in close connection with the post-cardinal vein, lying in contact with it, almost in its wall in the under and inner side. In the anterior region this vein has so grown round the pronephros that the tubuli really lie inside it (fig. 29). The tubuli are covered by a few flattened cells whose presence becomes more obvious about the twenty-fifth day by a deposit of dark brown pigment. The tubuli have thus a venous blood supply. The glomerulus on the other hand is supplied from the aorta. There is only one glomerulus on each side, stretching each side of the alimentary canal and extending through about the same space as the glandular part of the kidney. Each glomerulus is a diverticulum of the peritoneum, which generally becomes sacculated; it receives its blood by a single vessel on each side directly from the aorta.

Since the time of Bowman it has been known that the kidneys of Fishes, Frogs, and Snakes have a double blood supply, the tubuli uriniferi being surrounded by a capillary network of vessels which receive their blood from the renal portal veins, and the glomerulus which is supplied with blood from the aorta by the renal artery. It is an interesting fact to find that a similar blood supply is present from the very first in such a temporary organ as the pronephros of the Lamprey.

In the great majority of cases I found fine ciliated funnels in each pronephros. The whole gland did not extend over a greater space than that occupied by three myomeres, although in some cases the ciliated funnels, which were of some length, overlapped into a fourth myomere, but I was unable to confirm the relationship alleged to exist between the number of ciliated funnels and the number of somites through which the pronephros extended.

The Skeleton.

The skeletons of the oldest larva at my disposition consisted of the notochord derived from the endoderm, and of certain cartilages in the head and branchial region derived from the lateral mesoblast. The origin of the notochord has been completely

described by Calberla, Scott, and others, and I have nothing to add to their account. In the histological differentiation of the chord from a solid string of more or less cubical cells, to the vacuolated cylinder which forms the permanent notochord, there is a stage which is perhaps worth mentioning. In the early stages a transverse section of the chord shows portions of three or four cells, a little later these cells have pushed their way between one another and arranged themselves in such a way that they occupy the whole room inside the sheath of the notochord. Whilst in this condition vacuoles appear in the substance of the cells and for a day or two the notochord presents very much the same structure as the notochord of Amphioxus. This is, however, soon replaced by the vacuolated appearance characteristic of the notochordal tissue of the higher Vertebrata (figs. 18 and 23).

The posterior end of the notochord passes into the indifferent mass of tissue described in the tail. The anterior end is slightly curved downwards apparently by the increased vertical height of the brain. It ends just behind the infundibulum, its end being in contact with the posterior end of the nasal invagination. There is no trace that it has ever passed in front of this point, although in the young stages it reaches relatively almost as far forward as the nervous system. The relation of its anterior end to the brain hence appears to be due to the overgrowth of the nervous system anteriorly.

The cartilage which composes the rest of the skeleton is characterised by the small amount of intercellular substance. This stains very deeply. The cells are large with usually only one nucleus, though sometimes two. I have endeavoured to represent this structure in fig. 19. The branchial bases are the first part of the skeleton to appear. They arise about the twenty-fourth day as straight bars of cartilage lying external and slightly posterior to the branchial vessel. In their relation to the vessel they correspond with the extrabranchial bars of the Tadpole, and the Sharks. The true branchial bars run internal to the branchial vessel.

The bars run behind the gill-slit to which they belong, and

there is no bar in front of the first persistent cleft. They are slightly curved inwards towards the median line in the middle part of their course where they bend round the external opening of the cleft. About the thirtieth day they fuse with one another ventrally and so two rods are formed which lie close together in the posterior half of their course but diverge round the thyroid. About the same time each bar sends forward two processes, one above and the other below the opening of the gill to which it belongs; these ultimately fuse with the posterior edge of the gill bar next in front. The processes of the most anterior bar fuse with each other. Dorsally the last six of the bars also become continuous (fig. 42), and form two longitudinal bars which run parallel and close to the notochord. The most anterior bar does not join this rod but sends a process inwards, serving to support the auditory capsule, which lies just in front of it directly over the first persistent gill-cleft.

The first traces of the basi-cranial skeleton appear on the thirtieth day as two rods of cartilage, the trabeculæ (figs. 40). They lie close against the notochord for their posterior two thirds, anteriorly, however, they diverge and surround the pituitary space. About six days after their first appearance the trabeculæ send out laterally a transverse bar of cartilage which passes out on each side in front of the auditory capsule, lying between the ganglia of the fifth and seventh nerves. Professor Parker has identified this as the rudiments of the pedicle and pterygoid. They lie in the tissue of the bar which is in front of the first gill-cleft which has long ago disappeared.

Immediately beneath the trabeculæ the carotid artery runs forward as an anterior continuation of the dorsal aorta. The trabeculæ have become continuous with the dorsal end of the most anterior branchial bar, which is not united with the longitudinal bar formed from the fused dorsal end of the other six. The connection is very slight but is quite evident in sections. between this and the dorsal end of the second bar some little space exists, the latter when it commences lies at a slightly lower level than the trabeculæ.

The above description represents the condition in my oldest

larva, fifty-two days (fig. 43). The further development of the Lamprey's skull has been described by Professor Parker in his great work on 'The Skeleton of the Massipobranch Fishes.'

The Mesenteron.

The cavity of the alimentary is formed by the invagination of the endoderm described in the first section of this article, when once found it does not disappear again, although in the region of the intestine it may be reduced to a slit by the pressure of the surrounding yolk-cells.

The most anterior section, including the branchial region and that part of the intestine in front of the liver, is now separated from the rest by the raising of the head and neck from the remaining part of the embryo. The lining cells of this portion at once assume a columnar character; the hypoblastic cells in the region of the blastopore, or as it may now be termed the anus, also assume a similar form. But the cells in the middle part of the intestine still retain the features of the yolk-cells, those forming the roof of the enteron being however, rather more columnar than those of the floor and sides.

In the head region almost the whole of the space inside the epiblast is taken up with the brain, which has a great depth, and with the notochord and the alimentary canal, which ends blindly in front. A small band of mesoblast lies on each side of the nervous system and notochord. This segments dorsally into a series of myomeres, the first lying close behind the ear. Ventrally the mesoblast has not grown down between the endoderm, so that along the sides and under surface the hypoblast and epiblast are in contact. The first gill-slit appears, as Scott has described, about the twelfth or thirteenth day, the others arise eduring the next three or four days, the most posterior being the last formed. The gill-slits appear to me to be the result of the ventral downgrowth of mesoblast taking place only at certain places, these forming the gill-bars. Between each downgrowth the hypoblastic lining of the alimentary

canal remains in contact with the epiblast, and here the gill opening subsequently appears about the twenty-second day.

Huxley was the first to point out that the embryo Lamprey possesses eight gill-slits, and his account has been confirmed by Scott and Dohrn, who, however, point out that the first slit remains closed, and does not open to the exterior, as Huxley described. Dohrn has further shown that the first or rudimentary gill-slit becomes converted in the ciliated groove encircling the mouth, which was first described by Anton Schneider in Ammocœtes.

Fig. 27 represents a longitudinal horizontal section of the head of a twenty-one days' old embryo. The eight primitive gill-slits are here shown lined by columnar epithelium, which in the posterior seven is most flattened at those points where the opening will subsequently appear. The corresponding area in the first cleft, however, will be seen to be lined with very high columnar cells. These cells afterwards acquire cilia and come to lie in a deep groove.

The branchial vessels have only appeared in the first gill-bars, but the cells which will be converted into the cartilaginous gill arches have already become distinct (*br. b.*). About the twenty-second day a process begins to grow backward from the middle of each gill-bar into the gill-slit behind. This reduces the slit to a ≺-shaped opening. After the opening to the exterior has been established the gill-bars overlap each other, the passage from the cavity of the mouth to the exterior being directed outwards and backwards. Each gill-bar acquires a few gill filaments, into which the blood courses. The whole is covered by a layer of thick columnar epithelium continuous with that lining the rest of the mouth, except certain small areas, mostly at the end of the short filaments, where the epithelium has become suddenly thin, thus putting the blood into closer communication with the surrounding water.

The columnar glandular-looking cells which line so much of the cavity of the mouth contain a number of very fine gran-

ules, which stain deeply with hæmatoxylin, giving the cell a very characteristic appearance. I have been unable to form any opinion as to the nature or fate of these granules.

The ciliated ring mentioned above is shown in section in fig. 41, *c. g.* It lies close in front of the most anterior gill-bar; ventrally its two halves converge and run back as two parallel grooves to the opening of the thyroid gland in the ventral median line. The grooves here unite, and after receiving the opening of the thyroid they continue as a single groove running in the ventral median line as far as the most posterior gill arch. Dorsally the grooves unite and become continuous with a median dorsal ridge, which is covered by high columnar cells, also ciliated. This ridge extends from the first gill arch to the commencement of the œsophagus. Anton Schneider describes a band of cilia running from this dorsal ridge on each side along each gill arch. This is not present in my oldest larva, but is no doubt formed later.

Dohrn (23) has recently described the development of the thyroid so fully, and his paper is so beautifully illustrated, that it appears to me to be superfluous to describe again the origin of this organ. I can only confirm his results. He deals at length with the homology of the thyroid of Ammocœtes, with the endostyle of Ascidians, and the hypobranchial ridge in Amphioxus. And the homology of the circumoral ciliated ring in Ammocœtes and Ascidians is also pointed out. To these homologies we may add, I think, that of the dorsal ciliated ridge of the young larval Lamprey to the dorsal lamella of Ascidians, and the hyperpharyngeal groove of Amphioxus. It is a curious fact, however, that in the last animal the form of the structure is reversed. We find ventrally a ridge and dorsally a groove, whereas in Ammocœtes and Ascidians we have the ridge dorsal and the groove ventral. In spite of this, I think Dohrn's arguments fully support the homology of the ventral organs, and the same reasoning holds good for the dorsal.

The alimentary canal behind the branchial region may be divided into three sections. Langerhans has termed these

the stomach, mid-gut, and hind-gut, but as the most anterior of these is the narrowest part of the whole intestine, it would perhaps be better to call it œsophagus. This part of the alimentary canal lies entirely in front of the yolk, and is, with the anterior region which subsequently bears the gills, raised from the rest of the egg when the head is folded off. In my later larvæ it is composed of a single layer of very high columnar cells, and is ciliated throughout. Round this is a thin layer of cells, which, I imagine, give rise to the muscular coats. The whole is supported by a dorsal mesentery, each side of which lies the head kidney (fig. 25). The ciliated columnar cells are directly continuous with those covering the dorsal ridge of the branchial region, but not with those of the ventral groove; this later connection must arise subsequently, as Anton Schneider describes it in the fully-grown Ammocœte.

The mid-gut which follows the œsophagus is, in the younger stages, crowded with yolk granules. The cells of the roof soon acquire a columnar shape, whilst the ventral part consists of a mass of cubical cells, each crowded with yolk. By degrees the yolk is absorbed, and the cells assume the same character as those lining the œsophagus. The lumen of the mid-gut is very much larger than that of the œsophagus, the alimentary canal expanding suddenly at the commencement of the former. The absorption of yolk takes place from before backward, so that lumen and walls of the fore part of the mid-gut assume their permanent size and form, whilst the posterior half is choked with yolk. The lining high columnar cells are ciliated and quite continous with those of the œsophagus.

By the time the yolk is all absorbed a longitudinal invagination of the wall of the mid-gut takes place. This occurs anteriorly on the left side, but twisting through a quarter of circle it comes to lie in the ventral side posteriorly. The ridge thus formed reduces the lumen of the alimentary canal from a round to a reniform shape in section. In this ridge or spiral valve runs the subintestinal vein, which has become quite small and has lost its median ventral position. Around

this vessel, filling up the space between the two sides of the spiral valve, is a quantity of fatty tissue. The cilia on the inner face of the spiral valve are very evident.

The lumen of the mid-gut is so large that almost the whole of the body cavity in that region of the Ammocœte is taken up by this part of the intestine; consequently the liver, the only gland opening into the mid-gut, is pushed forward and lies on each side and below the œsophagus. This gland has its origin at a very early stage, about the fourteenth day, as an evagination of the mid-gut, whilst the latter is still crowded with yolk. The diverticulum thus produced grows out in the ventral side of the alimentary canal into that space between the hypoblast and epiblast which was mentioned above as being crowded with blood-corpuscles. This space subsequently becomes enclosed by definite walls by the downgrowth of the mesoblast in this region. It becomes the subintestinal vein which still continues to supply the liver with venous blood. The single diverticulum soon begins to branch, and at an early stage one of the branches becomes differentiated from the others, acquires a large lumen, and forms the gall-bladder. The cells forming the liver are cubical with large nuclei, they do not appear to have a definite outer layer of flattened cells, though occasionally such a cell is present. In the older larvæ the gall-bladder has a great relative size. It lies embedded in the liver on the right side of the œsophagus. The bile-duct runs from it above the mid-gut, bending down to enter the mid-gut in the spiral valve on the left side.

The hind-gut is smaller than the mid-gut, its anterior limit is marked by the termination of the spinal valve, which does not extend into this region. The two segmental ducts open into it just where it turns ventrally to open to the exterior by a median ventral anus. Its walls are in this region slightly puckered. The cells lining it are not so high as in the other parts of the intestine, but more cubical.

Its lumen is from an early stage lined with cells which have lost their yolk, and it is in wide communication with the exterior from the first. This condition seems to be, as Scott

suggests, connected with the openings of the ducts of the pronephros, for this gland is completed and seems capable of functioning long before any food could find its way through the mid-gut, or indeed before the stomodæum has opened.

The stomodæum has a very early origin; it commences on the fifteenth day as an invagination of ectoderm against the blind anterior end of the fore-gut. This gradually deepens and attains a very large size, partly due to great development of the upper lip, which grows forward and downward to constitute the large hooded structure which is so characteristic of the Ammocœte. The greater part of this hood consists of simple muscle-fibres which interlace and cross one another in a diagonal direction. The lower lip does not reach so far forward as the upper (figs. 34 and 35). About the twentieth day the velum begins to appear in the posterior angle of the stomodæum. This structure is formed by two grooves which gradually deepen and cut off a flap of tissue on each side of the middle line. These two grooves, shown in fig. 27, are not very deep. The tissue between them is broken through the next day so that the two lateral folds that remain are covered on their anterior face by epiblast, and on the greater part of their posterior face by hypoblast (fig. 28). Subsequently the mesoblast in these two flaps develope into muscle-fibres, and in the young larva a constant current is kept up by them, passing in at the mouth and out at the gill-clefts. This current is easily demonstrated by the aid of a little Indian ink suspended in the water.

On the twenty-third day two tentacles begin to grow out from the under surface of the upper lip, one each side of the middle line; a little later two more appear on the sides, but placed more posteriorly; later still two more appear behind the level of the last; these are situated at the junction of the lower lip with the upper. Finally, a median tentacle appears in the ventral middle line. This last is far longer than the others and from its base a ridge, which is at first low, but increases in height posteriorly, extends back between the ventral portion of the ciliated ring (figs. 40 and 41). The number of tentacles

is afterwards increased by a pair of new ones arising between each of those already formed. The tentacles subsequently become branched (fig. 39).

With regard to the mesoblast of the head I have little to add to the descriptions of Balfour and Scott. The area over which the gills extend at their first appearance extends to the posterior boundary of the sixth myomere. The most anterior myomere is situated close behind the ear, and the ear lies above the hyobranchial or first persistent gill-cleft. So that at their first appearance the six posterior gill-clefts correspond in their extent with the six anterior myomeres. As the larva grows the gill region appears to elongate with relation to the muscular myomeres, so in my latest larva there are about nine myomeres over the area of the six gills (fig. 43). These anterior myomeres become V-shaped with the open angles directed forwards; turned the opposite way to those of Amphioxus.

The mesoblast between the gills arranges itself into head cavities (fig. 21), and as Balfour and Scott have already shown, there are two head cavities in front of the hyomandibular cleft. These are at first continuous, but with the formation of the stomodæum they separate. One becomes præoral and obviously corresponding with the præmandibular head cavity of Elasmobranchs; the other with the mandibular (fig. 21). The walls of these cavities ultimately form the skeleton of the gill arches, the muscles of which are all of the tubular kind. Owing to the rudimentary condition of the eye in Ammocœtes, no eye-muscles are present and consequently it is impossible to say whether or no they are derived from the walls of the head cavities, but the researches of Stannius and Langerhans have shown that they possess the same histological characters as the muscles of the gills and upper lip.

The Central Nervous System.

The development of the central nervous system has been described above up to the stage when the central canal has

first appeared. The lumen is at first circular in outline, and the walls of the canal of uniform thickness (fig. 11). Ultimately in the region of the body the lumen becomes elongated and slit like (fig. 24); in the anterior end the lumen widens into the variously shaped cavities which form the ventricles of the brain. The cells forming the walls of the canal are primarily more or less cubical, but they soon become spindle shaped, except those which form the roof and the floor of the central canal. These are formed of a single layer of short columnar cells. The canal is in the youngest stages proportionately very much larger than in the later; its size is diminished and its form altered by the thickenings which take place in different parts of the brain.

The white matter first makes its appearance on the eighteenth day as two thin bands, one on each side of the brain and spinal cord (fig. 37). Later these unite in the ventral side and form an anterior commissure. After the appearance of the white matter the ganglion cells lose their spindle-shaped outline and become again circular.

The cranial flexure is very slight; the anterior end of the brain is, however, slightly bent down, and with it the anterior end of the notochord (fig. 23).

About the sixteenth day considerable changes take place in the brain; from the anterior and ventro-lateral angles of the fore-brain two diverticula are given off; these are the optic vesicles (fig. 30). They continue to grow upwards and backwards till their blind end reaches a position behind and above the anterior end of the notochord.

At the blind end of the diverticulum a knob is formed by the outer face proliferating cells, which form a multicellular retinal layer. The posterior face later on developes pigment in its cells. The lens is budded off from the inside of the single layer of epidermis, and lies as a flattened mass of cells close against the retinal layer (fig. 40). The stalk of the primary vesicle becomes solid by its walls coalescing on all sides, and forms the optic nerves. At their origin these nerves form a commissure projecting into the cavity of the fore-brain

on its ventral side; by the twenty-second day this optic chiasma is covered in by a single layer of ganglion cells. It is this body that Dohrn has by mistake figured as the Tuber cinereum (21). The commissure is shown in transverse section in fig. 39; the lumen of the infundibulum is seen below it, the cavity of the fore-brain above.

About the same time that the optic vesicles commence to be given off from the anterior end of the brain a median dorsal evagination also appears. It was mentioned above that in the median line, both dorsally, ventrally, and in front, the central canal is enclosed by a single layer of more or less columnar cells, whilst the lateral walls are thick. This single layer is interrupted ventrally by the formation of the optic chiasma. Dorsally it is produced on the sixteenth day by the evagination in question, which is the rudiment of the pineal gland (fig. 31). The walls of the pineal gland then consist at first of a single layer of cells forming a hollow sac which pushes its way between the brain and the epidermis, spreading out on all sides (fig. 31). At first its lumen is continuous with that of the fore-brain, but ultimately, by the folding of its walls, its cavity is obliterated and the communication with the lumen of the fore-brain is shut off.

The eighteenth day, two days after the first appearance of the optic vesicles and the pineal gland, is the earliest date on which I have been able to recognise the appearance of any division into fore-, mid-, and hind brain. On this day the single layer of cells roofing the central canal becomes folded in the manner indicated in fig. 23. This takes place at about the level of the attachment of the velum, a little in front of the ear. In larva of fifty-two days, this groove has not changed its form, but has become deeper.

The division between the fore- and hind-brain is by no means so well marked; indeed, I have been unable to find any external groove, although it has been described by previous writers. Longitudinal horizontal sections through the brain show, however, that just behind the infundibulum and pineal gland the walls thin out so that the lumen appears diamond

shaped. This thin wall I conclude makes the division between the optic thalami and the crura cerebri.

The hind-brain and mid-brain resemble each other closely in structure, the mid-brain being only a trifle larger. Their cavity, which is at first slit like, becomes triangular by the lateral growth of the roof which pushes the side walls apart dorsally (figs. 40 and 41). This thin roof extends back as far as the second gill-cleft, after which it disappears and the nervous system has the structure represented in fig. 42.

About the forty-fifth day a median longitudinal fold appears in the thin roof; this is the first of the numerous folds found in the roof of the mid- and hind-brain of the adult (fig. 41).

The fore-brain still has its thick side walls, the optic thalami. Just in front of the stalk of the pineal gland a commissure of transverse fibres is found which runs from side to side on about the twenty-third day. This commissure corresponds with the Commissura tenuisima, described by Ahlborn in his exhaustive work on the brain of the adult Lamprey. It also probably corresponds with the commissure found by Balfour in Scyllium situated just in front of the base of the pineal gland. Osborn has recently described a similar commissure in the brain of the Amphibia, Menopoma, Menobranchus, Amphiuma, and Rana, and I have adopted the name he proposes for it, the Superior Commissure. The commissure of the pineal stalk in the Mammalian brain seems to occupy the same relative position. This superior commissure is at first covered with but a few ganglion cells, but these afterwards increase until two bodies are formed, the Ganglia Habenulæ. The left one is very small (fig. 39), but the right is a structure of considerable size, projecting downwards and backwards, and reducing the lumen of the fore-brain to a Y-shaped slit. These bodies have been fully described by Ahlborn in the adult; it is interesting to note that the curious asymmetry they possess is present from their first appearance. No other commissure has made its appearance by the fifty-second day.

The cerebral hemispheres show some signs of appearing as lateral outgrowths in my oldest larvæ, but no trace of paired

lateral ventricles are to be seen. The lateral outgrowths of the hemispheres embrace between them a mass of tissue formed at the back of the olfactory pit, which resembles in every way nerve matter. This structure is shown in figs. 33, 34, and 35, drawn from a series of sections taken through the head of a fifty-two days' larva. This tissue in question appears to consist of ganglion cells. It is traversed by a canal which ends blindly behind and opens by the median nasal pit in front. Posteriorly it is continuous with a sheet of tissue which is described by Dohrn and Scott as giving rise to the pituitary body (fig. 39). Unfortunately my larvæ were not sufficiently old to enable me to determine whether this mass of tissue comes into closer relation with the brain and forms the olfactory lobes, or whether, as seems more probable from what we know of the development of these structures in other animals, it forms only the peripheral portion of the olfactory apparatus.

About the twenty-fifth day some of the ganglion cells in the postero-lateral angle of the grey matter become much larger than the surrounding ones. These cells are particularly frequent in that part of the hind-brain lying between the auditory capsule. They probably develope into the "outer large cells" of Reissner.

With regard to the development of the cranial nerves, I have no observations on the origin of the olfactory nerve, as this apparently does not arise till a much later stage than that attained by my oldest larvæ. The origin of the optic nerve as an outgrowth of the brain has been described above. Owing to the rudimentary condition of the eye, the muscles of that organ are not developed, and consequently the third, fourth, and sixth nerves do not arise till a much later stage. This leaves the fifth, seventh, eighth, ninth, and tenth nerves to be considered.

The origin of these nerves is much obscured by the yolk which crowds the cells of the embryo at the time they first appear. On the seventeenth day the first origin of the ganglia in the fifth and seventh nerve is seen. The ganglia arise as proliferations of the epiblast. By this means a knob of cells

is formed, which arises at about the level of the notochord (fig. 32). This heap of cells arises close behind the lens of the eye, but seems to be distinct from it. It is divided into a larger anterior part, which belongs to the fifth nerve, and a smaller posterior portion, which forms the ganglion of the seventh. The roots of the nerves seem to me—though it is difficult to be certain on this point—to arise as outgrowths from a neural ridge in the lateral surface of the brain; these grow down and fuse with epiblastic thickening. This origin of the roots of the nerves corresponds with that described by Balfour, Marshall, Van Wijhe, and Beard, in the Elasmobranchs, and differs from what occurs in the Amphibia as described by Spencer, where the nerve also is derived from the inner layer of epiblast. As Spencer suggests, this is probably due to the presence of a double layer of epiblast, the epidermic and nervous, in the Amphibia.

By the nineteenth day the ganglion of the fifth nerve has completely separated off from the skin. It has now divided into two portions, which have, however, a common root taking its origin from the hind-brain just in front of the ear. The most anterior part forms a large ganglion on the root of a nerve which runs over the eye (fig. 22). This is the ophthalmic ganglion, and the nerve is the ophthalmic branch of the trigeminus; it probably corresponds with the portio-profunda of the ophthalmicus superficialis of the Elasmobranchs. Immediately behind the ophthalmic ganglion, but quite distinct from it, lies the ganglion of the other half of the fifth nerve. From this a mandibular nerve proceeds to run close behind the mouth, and later a maxillary branch appears præorally. In the angle between these ganglia the eye lies. The nerve connecting the ophthalmic with the main ganglion of the fifth nerve, described by Ahlborn in the adult, is not found at this stage, and both the ganglia are of approximately equal size.

The seventh nerve arises behind the fifth and enters its ganglion, which, when separated off from the epiblast, lies close in front of the ear capsule (fig. 38). In early stages,

whilst the most anterior gill-cleft—spiracle—is still present, the nerve can be seen passing from the ganglia between the rudimentary gill-cleft and the first persistent one—the hyobranchial. Later on the ganglion increases in size, and extends round the under and inner face of the auditory sac towards the ganglion of the ninth nerve, but it never quite reaches it, and the connection between the ganglion of the seventh and of the tenth nerves must be of later origin. Neither does the ganglion of the seventh fuse with that of the fifth, though they are close together, and the root of the seventh does not enter the ear capsule to leave it again, as is the case in the adult. After the appearance of the ciliated ring in the place of the first gill-cleft, the seventh nerve supplies this structure.

A few fibres from the brain enter the recessus labyrinthi of the ear; these arise close to the root of the seventh, and constitute the eighth nerve.

The ganglia of the ninth and tenth nerves would seem to arise from a mass of cells split off from the epiblast close behind the ear. At a little later stage the ninth nerve has its ganglion lying close against the posterior boundary of the ear; the nerve is continued along the posterior wall of the first persistent cleft, the hyobranchial. The ganglion seems to be still connected with the ganglion of the tenth nerve. This is a very large structure; it lies more dorsally than the others and it is in close connection with the mid-brain, having as yet developed no root. Behind it and connected with it lies a ganglion which is situated dorsally above the second persistent gill-cleft; from this chord the main branch of the vagus is continued backward, lying just external to the anterior cardinal vein (fig. 42). In front of each remaining cleft the chord bears a large ganglion, so that, counting the first, there are six distinct ganglia borne on the vagus. I have not been able to trace the fibres of this nerve beyond the last gill-cleft, but my friend Mr. Ransom, of Trinity College, tells me he has traced the vagus into the heart in the adult Petromyzon. Each of the ganglia in the vagus supplies the gill-cleft behind which it lies.

There is no trace of the ramus lateralis of the vagus even in my oldest larvæ.

The ganglion on the ninth nerve lies in front of the first myomere, between that and the ear, whilst that of the vagus lies between the first and second. The first dorsal root of the spinal nerves with its ganglion lies between the third and fourth myomere. Behind this there is a dorsal ganglion lying opposite each myotome.

Sagemehl (17) has described very correctly the origin of the spinal nerves. The dorsal roots with their ganglia arise from a neural ridge which is at first of the same size all along. From this the ganglia begin to grow out about the eighteenth day, intersegmentally, that is opposite the myotomes. The ganglia are in connection with one another for some time by a longitudinal commissure. This commissure appears to consist of the remains of the neural ridge; it ultimately disappears, as in Elasmobranchs. The dorsal nerves, after leaving the ganglia, run into the myotomes and eventually, I believe, reach the skin, though on this point I cannot be quite certain. On the other hand the ventral roots consist of nerve-fibres only, and run straight into the myomeres. They appear, according to Sagemehl, very soon after the first appearance of white matter in the chord, and they never have any connection with the dorsal roots. The resemblance between the distribution of the spinal nerves of this larva with those of Amphioxus as described by Rohon is very striking.

The ear is formed, as Scott has described, from an invagination of the epiblast. This appears very early about the fourteenth day. It soon deepens and becomes completely shut off, consisting then of an oval vesicle with a dorsally placed stalk, the recessus labyrinthi. This last is the remains of the duct leading to the exterior. The ear is in the same condition in my oldest larvæ. No signs of the semicircular canals have appeared. The epithelium lining the vesicle is high and columnar; about the twenty-second day certain patches of the epithelium become higher than the others and the cells develope each a very large cilium which projects into the cavity and

bears a knob at its free end (fig. 41). About the same time a number of small concretions appear in the ear. These form the numerous spherical otoliths.

Summary.

I have now described the structure of the chief organs in my oldest larva, and I propose to conclude this paper by a brief summary of the results obtained.

In the first place the mesoblast is not completed ventrally by a layer of cells split off from the hypoblastic yolk-cells, as Scott has described. But the ventral mesoblast is formed by the downgrowth of the mesoblastic plates, which ultimataly meet and unite in the ventral middle line.

The blastopore does not close up, as later observers have maintained, but, as Max Schultze described thirty years ago, it persists as the anus. There is no neurenteric canal, though a solid strand of tissue proceeds back from the alimentary canal and fuses with an indifferentiated mass of cells, into which the nervous system and mesoblast also pass.

The lumen of the alimentary canal is that of the mesenteron; it does not become obliterated during larval life. In its anterior end the hypoblast remains in connection with the epiblast at certain points, and here the gill-clefts arise; between these the mesoblast grows down and forms the gill-bars. The origin of the ciliated ring and the hypopharyngeal groove and hyperpharyngeal bar are also described, and the ciliated condition of the œsophagus and stomach.

The "muscle-plates," whose structure is so peculiar in the Lamprey, arise each from a single cell of the mesoblastic somites. This increases in size, slides in between the neighbouring cells, and ultimately occupies the whole of the space between two myotomes. Its nucleus divides until each cell contains several nuclei. Striated fibrils then appear and increases till the whole "muscle-plate" consists of little else besides these fibrils, squeezing between them a few nuclei. These "muscle-plates" arise from the segmental half of the mesoblast; the muscles of the gills, lips, and probably of the eye,

have a different structure and arise from the ventral unsegmented part.

The blood-corpuscles arise from the ventral free edges of the mesoblast, before they unite in the ventral middle line, they collect in a large sinus just behind the heart. The heart appears in the ventral mesentery, formed by the union of the lateral mesoblastic plates; at first its lumen is continuous with the sinus just mentioned. This sinus lies between the hypoblastic yolk-cells and the epiblast; it subsequently acquires walls and forms part of the subintestinal vein.

The ciliated funnels of the pronephros are left as apertures by the segmental duct which in its anterior end is formed from a groove. The groove closes up at intervals, leaving four or five openings which become the funnels. They do not arise as blind projections from the duct, which subsequently, acquire ciliated openings. From the first the pronephros has a double blood supply, pure blood from the aorta passing to the glomerulus, and impure blood in the cardinal veins surrounding the tubuli.

The early development of the skeleton is described up to the stage where Professor Parker commenced his researches.

The canal of the central nervous system developes after the neural chord has separated off from the epidermis; it does not appear to be lined by any invaginated epidermis, as Calberla and Scott maintained.

The first sign of differentiation of the parts of the brain is the formation on the sixteenth day of the optic vesicles and pineal gland. The division into fore-, mid-, and hind-brain appears soon after, but the fore- and mid-brain are not separated by any well-marked groove. The first transverse commissure to appear is situated just in front of the stalk of the pineal gland. It forms the superior commissure of Osborn. Afterwards the ganglion cells thicken round it and form the asymmetrical ganglia habenulæ.

The ganglia on the fifth, seventh, ninth, and tenth nerves are derived from epiblastic thickenings. Their roots probably arise as outgrowths from the neural ridge. The ganglion of the

fifth divides into two parts, the ophthalmic and mandibular; these have a common root.

The seventh nerve at its first appearance supplies the first or spiracular gill-cleft; when this is converted into the ciliated ring it continues to be supplied by the seventh nerve.

The connection between the fifth, seventh, and tenth nerve ganglia does not exist and must be of later origin.

The tenth nerve has a large ganglion on its root and bears a ganglion above each of the last six gill-clefts. No trace of the ramus lateralis is to be seen.

The origin of the ganglia on the cranial nerves has no relation to the sense-organs of the skin; these have not appeared even in my oldest larva.

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EXPLANATION OF PLATES XXVI, XXVII, XXVIII, and XXIX,

Illustrating Mr. Arthur E. Shipley's Paper on "Some Points in the Development of Petromyzon fluviatilis."

Reference Letters.

a. Anus. *a. c.* Anterior cardinal. *ao.* Aorta. *au.* Ear. *aur.* Auricle. *b. c.* Body cavity. *bl. c.* Blood-corpuscles. *bp.* Blastopore. *br.*^{1}-*br.*^{8} First to eighth gill-clefts. *br. b.* Skeleton of branchial bars. *br. v.* Vessels of branchial bars. *c.* Cerebral hemispheres. *c. g.* Ciliated groove. *d.f.* Dorsal fin. *d. l.* Dorsal lamella. *d. m.* Dorsal mesentery. *e.* Eye. *e. g.* Egg membrane. *ep.* Epiblast. *f. b.* Fore-brain. *f. g.* Fore-gut. *g.* Groove between mid- and hind-brain. *g. h. l.* Left ganglion habenulæ. *g. h. r.* Right ganglion habenulæ. *gl.* Glomerulus. *g. n.* Ganglion cells at base of olfactory invagination. *h.* Heart. *hd.* Head. *hd. c.* Head-cavities. *h. b.* Hind-brain. *hy.* Hypoblast. *i.* Iter a tertio ad quartum venticulum. *inf.* Infundibulum. *li. t.* Liver tubules. *l. l.* Lower lip. *l. t.* Lamina terminalis. *m.* Mesenteron. *m. b.* Mid-brain. *m. br.* Muscle of branchial bar. *mes.* Unsegmented mesoblast. *mes. som.* Mesoblastic somites. *m.f.* Muscle-fibre of heart. *m.g.* Mid-gut. *m.* Muscle-plate. *my.* Myomere. *n.* Notochord. *na.* Olfactory invagination. *n. r.* Neural ridge. *nu.* Nucleus of muscle-plate. *o. e.* Ciliated epithelium lining nasal invagination. *op. ch.* Optic chiasma. *oph.* Ophthalmic ganglion. *op. th.* Optic thalami. *op. v.* Optic vesicle. *p.g.* Postanal gut. *pin.* Pineal gland. *pit.* Pituitary body. *pr.* Primitive streak. *r. l.* Recessus labyrinthi. *s. c.* Segmentation cavity. *s. cm.* Superior commissure. *s. d.* Segmental duct. *sm.pl.* Somatopleure. *sp. c.* Spinal cord. *sp. gl.* Spinal ganglion. *sp. pl.* Splanchnopleure. *st.* Stomodæum. *s. v.* Sinus venosus. *t.* Tentacles. *th.* Thyroid gland. *tr.* Trabeculæ. *tub.* Tubule of pronephros. *u. l.* Upper lip. *v.* Velum. *v. ao.* Ventral aorta. *ven.* Ventricle. *v.f. b.* Cavity of fore-brain. *v. h. b.* Cavity of hind-brain. *v. r.* Ventral ridge in mouth. *v.v.* Valves of the heart. *y. c.* Yolk-cells. *V. g.* Ganglion of fifth nerve. *V. g. e.* Epiblastic ingrowth to form ganglion of fifth nerve. *VII. g.* Ganglion of seventh nerve. *X. g.* Ganglion of tenth nerve.

PLATE XXVI.

Fig. 1.—Spermatozoa of Petromyzon fluviatilis.

Fig. 2.—Segmenting ovum at the completion of the third or equatorial furrow. *e.g.* Egg membrane.

Fig. 3.—Segmenting ovum, showing the next two vertical furrows which have divided the upper cells and are extending into the lower.

Fig. 4.—Ovum after the invagination is complete, twelve days old, showing the blastopore, *bp.*, at posterior end of the neural ridge, *n. r.*

Fig. 5.—Transverse section through ovum of thirty-six hours. *ep.* Epiblast. *s. c.* Segmentation cavity. *y. c.* Yolk-cells.

Fig. 6.—Transverse section through ovum of forty-eight hours. *s. c.* Segmentation cavity. *ep.* Epiblast. *y. c.* Yolk-cells.

Fig. 7.—Transverse section through ovum of sixty-seven hours.

Fig. 8.—Transverse section through ovum of eighty-six hours, showing epiblast gradually thinning out.

Fig. 9.—Longitudinal section through commencing gastrula, 136 hours. *bp.* Blastopore. *hy.* Hypoblast. *y. c.* Yolk-cells. *m.* Mesenteron. *s. c.* Segmentation cavity.

Fig. 10.—Section through embryo of about the same stage as Fig. 4. *bp.* Blatsopore. *y. c.* Yolk-cells. *hd.* Head.

Fig. 11.—Transverse section through the body of an embryo just before hatching, seventeenth day. *sp. c.* Spinal cord. *n.* Notochord. *m.* Mesenteron. *mes.* Mesoblast. *s. d.* Segmental duct. Zeiss's A, oc. 2, cam. luc.

Fig. 12.—Transverse section through embryo of thirteenth day. *sp. c.* Spinal cord. *n.* Notochord. *mes.* Mesoblast. *m.* Mesenteron. *y. c.* Yolk-cells. Zeiss's A, oc. 2, cam. luc.

Fig. 13.—Transverse section through embryo of fourteen days. Letters as in Fig. 12. Zeiss's A, oc. 2, cam. luc.

Fig. 14.—Transverse section through tail of larva twenty days old. *sp. c.* Spinal cord. *n.* Notochord. *p. g.* Solid postanal gut. *mes.* Mesoblast. *bp.* Blastopore. *d. f.* Dorsal fin. Zeiss's A, oc. 3, cam. luc.

Fig. 15.—Transverse section from the same series as Fig. 14, but posterior to blastopore. *d. f.* Dorsal fin. *mes.* Mesoblast. *pr.* Fused tissue of notochord, spinal cord, and postanal gut, or primitive streak. Zeiss's A, oc. 3, cam. luc.

Fig. 16.—Transverse section of embryo just before hatching, seventeen days, through region of blastopore. *bp.* Blastopore. *sp. c.* Spinal cord. *n.* Notochord. *y. c.* Yolk-cells.

Fig. 17.—Longitudinal section of embryo, showing formation of somites. *n.* Notochord. *mes. som.* Mesoblastic somites. *sp. c.* Spinal cord. *d. f.* dorsal fin.

Fig. 18.—Longitudinal section of embryo just before hatching. *sp. c.* Spinal cord. *my.* Myomere. *sm. pl.* Somatopleuric layer of somite. *sp. pl.* Splanchnopleuric layer. *n.* Notochord. Zeiss's A, oc. 3, cam. luc.

Fig. 19.—A piece of the cartilage of a branchial bar.

Fig. 20.—A longitudinal vertical section through the tail of a larva twenty-

one days old. *a.* Anus. *p. g.* Solid postanal gut. *n.* Notochord. *sp. c.* Spinal cord. *pr.* Primitive streak. *y. c.* Yolk-cells.

Fig. 21.—A longitudinal section through side of head of seventeen days' embryo, showing the first three evaginations to form gill-clefts, and the true head-cavities. *au.* Ear. *hd. c'.* and *hd. c''.* The first and second head-cavity. br^1., br^2., and br^3. The first rudiments of gill-clefts. *br. v.* The vessels of gills. *st.* Stomodæum. Zeiss's A, oc. 3.

PLATE XXVII.

Fig. 22.—A longitudinal section through side of head of a larva twenty-one days old. *au.* Ear. *e.* Eye. br^1., br^2., br^3., br^4. The first to fourth primary gill-clefts. *h. b.* Hind-brain. *oph.* Ophthalmic ganglion. *V. g.* Ganglion in main branch of fifth nerve.

Fig. 23.—A median longitudinal section through the head of a larva twenty-one days old. *pin.* Pineal gland. *op. ch.* Optic chiasma. *inf.* Infundibulum. *n.* Notochord. *st.* Stomodæum. br^2. Second primitive gill-cleft. *th.* Thyroid gland. *na.* Olfactory invagination. *pit.* Pituitary invagination. *m. b.* Mid-brain. *h. b.* Hind-brain. *g.* Groove between mid- and hind-brain. *l. t.* Lamina terminalis.

Fig. 24.—Transverse section through the body of a larva of twenty days. *sp. c.* Spinal cord. *f. g.* Fore-gut. *n.* Notochord. *som. pl.* Somatopleure. *sp. pl.* Splanchnopleuric layers of myomeres. *b. c.* Body cavity. *h.* Heart. *c. f.* Ciliated funnel. *s. d.* Segmental duct. Zeiss's A, oc. 3, cam. luc.

Fig. 25.—Transverse section through trunk of larva about twenty-four days. Letters as in Fig. 24, and *ao.* Aorta. *a. c.* Anterior cardinal. *d. m.* Dorsal mesentery. *sp. gl.* Spinal ganglion. *gl.* Glomerulus. Zeiss's C, oc. 1, cam. luc.

Fig. 26.—Section through embryo, one day before hatching, seventeen days old, cut whilst in the egg-shell. *h* Heart. *sp. pl.* Splanchnopleure. *sm. pl.* Somatopleure. br^7. and br^8. Seventh and eighth gill-clefts. *hd.* Head-cavities behind these. *y. c.* Yolk-cells. *m. g.* Mid-gut. *b. c.* Body cavity. Zeiss's A, oc. 3, cam. luc.

Fig. 27.—Longitudinal horizontal section through a larva about twenty-two days. br^1.—br^8. The eight primary gill-clefts. *br. v.* Vessels of gills. *br. b.* Branchial bars. *f. g.* Fore-gut. *tub.* Tubule of pronephros. *st.* Stomodæum. *v.* Velum. *g. n.* Ganglion cells at base of nasal invagination. *op. ch.* Optic chiasma. *inf.* Infundibulum. *v. f. b.* Cavity of fore-brain. *n.* Notochord. Zeiss's B, oc. 1, cam. luc.

Fig. 28.—Longitudinal horizontal section through larva of thirty-six days. *u. l.* Upper lip. *v.* Velum. *th.* Thyroid gland. *v. ao.* Ventral aorta. *ven.*

Ventricle. *aur.* Auricle. *vv.* Valves. *s. v.* Sinus venosus. *li. t.* Liver tubules. *m. g.* Mid-gut. *br. b.* Branchial bars. *v. r.* Ventral ridge. *my.* Myomere. Zeiss's A, oc. 1, cam. luc.

Fig. 29.—Transverse section through pronephros of larva of forty-seven days. *n.* Notochord. *m. p.* Muscle-plates. *nu.* Nucleus. *ao.* Aorta. *a. c.* Anterior cardinal. *gl.* Glomerulus. *tub.* Tubules. *s. d.* Segmental duct. *bl. c.* Blood-corpuscles. *f. g.* Fore-gut. *d. m.* Dorsal mesentery. Zeiss's D, oc. 1, cam. luc.

Fig. 30.—Transverse section through fore-brain of embryo, seventeen days. *na.* Olfactory epithelium. *op. v.* Optic vesicle. *v. f. b.* Cavity of fore-brain.

Fig. 31.—Transverse section through thalamencephalon of larva of eighteen days. *pin.* Pineal gland. *op. th.* Optic thalmi. *v. f. b.* Cavity of fore-brain. *na.* Olfactory epithelium.

Fig. 32.—Transverse section through region of mid-brain of larva of sixteen days. *st.* Stomodial epithelium. *V. g. e.* Epiblastic origin of ganglion of fifth nerve. *n.* Notochord. *m. b.* Mid-brain.

Figs. 33, 34, and 35.—A series of sections through the anterior end of head of a larva fifty-two days old, to show the ganglia cells at base of olfactory epithelium. *u. l.* Upper lip. *l. l.* Lower lip. *t.* Tentacles. *g. n.* Ganglion cells at base of nasal invagination. *o. e.* Ciliated epithelium lining nasal invagination. *c.* Cerebral hemispheres. *v. f. b.* Cavity of fore-brain.

PLATE XXVIII.

Fig. 36.—Branched muscle-fibres of heart of larva forty-nine days old. *bl. c.* Blood-corpuscles. *m. f.* Muscle-fibre cut across.

Fig. 37.—Transverse section through the hind-brain, showing appearance of white matter and ganglion of fifth nerve. *h. b.* Hind-brain. *st.* Stomodæum. *V. g.* Ganglion of fifth nerve. This section is rather oblique.

Fig. 38.—Transverse section through hind-brain, showing origin of ganglion of seventh nerve from epiblastic ingrowth. *VII. g.* Ganglion of seventh nerve. *au.* Auditory vesicle. *f. g.* Fore-gut.

Fig. 39.—Transverse section through fore-brain of larva forty-nine days old, to show superior commissure. *pin.* Pineal gland. *v. f. b.* Cavity of fore-brain. *s. cm.* Superior commissure. *g. h. l.* Left ganglion habenulæ. *g. h. r.* Right ganglion habenulæ. *op. ch.* Optic chiasma. *pit.* Pituitary body. *inf.* cavity of infundibulum. *u. l.* Upper lip. *l. l.* Lower lip. *t.* Tentacles. Zeiss's C, oc. 1, cam. luc.

Fig. 40.—Transverse section through mid-brain of larva of forty-nine days. *i.* Iter a tertio ad quartum ventriculum. *e.* Eye. *tr.* Trabeculæ. *v. r.* Ventral ridge. Zeiss's C, oc. 1, cam. luc.

Fig. 41.—Transverse section through hind-brain of larva of fifty-two days. *v. h. b.* Cavity of hind-brain. *au.* Ear. *r. l.* Recessus labyrinthi. *VII. g.* Ganglion of seventh nerve. *d. l.* Dorsal lamella. *c. g.* Ciliated groove. *v. r.* Ventral ridge. *v.* Velum. *ao.* Aorta. *br. v.* Branchial vessels. Zeiss's A, oc. 3, cam. luc.

Fig. 42.—Transverse section through region of sixth gill-bar of fifty-two days' larva. *br*^{6}. Sixth gill-bar. *sp. gl.* Spinal ganglion. *ao.* Aorta. *a. c.* Anterior cardinal. *br. v.* Branchial vessels. *ao. v.* Ventral aorta. *X. g.* Ganglion in tenth nerve. *d. l.* Dorsal lamella. *br. b.* Skeleton of branchial bars. *m. br.* Branchial muscles.

PLATE XXIX.

Fig. 43.—Drawing of larva of fifty-two days. The notch in the liver, behind the heart, is due to the large gall-bladder, through whose walls the œsophagus is seen. This drawing was made by Mr. E. Wilson from the living specimen.

The Ammoniacal Decomposition of Urine.

By

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With Plate XXX, figs. 1 and 2.

When freshly voided, healthy urine, as is well known, is a clear, transparent, amber-coloured fluid, with a distinct acid reaction, and a peculiar aromatic odour. If left to itself in an open vessel slight clouds of mucus soon appear which gradually sink to the bottom. After a time the acid reaction is noticed to be slightly increased, and crystals of uric acid and oxalate of lime are deposited. After a longer or shorter interval, dependent on the temperature of the surrounding media, this marked acidity begins to diminish and finally disappears, the urine becomes lighter in colour, a whitish scum forms on the surface, and the well-known ammoniacal odour indicates that it has become alkaline; the uric acid crystals disappear, and whitish granules of urate of ammonia and prismatic crystals of urate of soda take their place, beautiful crystals of phosphate of magnesia and ammonia being subsequently thrown down.

The increase of acidity is called by Scherer the acid fermentation, and is considered by him to be owing to the presence of the vesical mucus. The alkaline change is spoken of as the alkaline or ammoniacal fermentation, and is owing to the decomposition of the urea into carbonate of ammonia.

These so-called fermentative changes are well known, and have long been recognised. So far back as 1682 Van Helmont spoke of the odour of urine as the effect of a putrefactive

ferment, and later on Boerhaave, in a work published in London in 1732, makes direct mention of the presence of ammonia in urine as the result of decomposition.

The source of the ammonia was, however, first clearly understood in 1799, when Cruickshank, Fourcroy, and Vauquelin discovered urea, the two latter observers showing that carbonate of ammonia was the principal product of its distillation, and they further pointed out the relationship between the conversion of urea in solution in water into carbonate of ammonia by heat, and the spontaneous "fermentative" decomposition of urine. With a more accurate knowledge of the composition of urea the reason of its conversion into carbonate of ammonia became clearer, but the discovery of Proust that freshly voided urine could be kept for years in a well-stoppered flask without undergoing any change first led him to conclude that the action of air, especially of its oxygen, was necessary for its decomposition. Later authorities attributed the decomposition to the presence of a ferment, taking its origin in the putrid destruction of the mucus.

Our ideas on the subject were, however, thoroughly changed by the work of Pasteur in 1860. He introduced fresh urine into a glass flask, boiled it for a few minutes, and then effectually closed the flask by fusing its neck. He then found that urine thus treated remained fresh for an indefinite period. If, after the lapse of five or six weeks, he introduced into such urine pieces of asbestos which had been freely exposed to the air, decomposition speedily occurred, giving rise to the ammoniacal smell and the development of numerous organisms, monads, vibriones, bacteria, &c. If, however, the asbestos, previous to its introduction, had been well heated in a blowpipe flame, no change whatever took place in the urine. It was thus clearly shown that the ammoniacal change in urine was directly owing to the introduction of germs from the air, and subsequently Pasteur and Van Tieghem[1] showed that in every fermenting ammoniacal urine the presence of micro-organisms

[1] "Recherches sur la fermentation de l'urée, etc.," 'Comptes rendus,' T. lviii, p. 210—264, 1864.

could be abundantly demonstrated, and to the presence of these the destruction of the urea was to be traced.

The importance of these experiments was at once manifest, not only as giving a clearer explanation of the changes in urine, but also as indicative of the cause in fermentation generally, and in the present day we all recognise the importance of Pasteur's work as being the foundation of our methods of inquiry into the causes of infectious diseases.

Two questions now naturally present themselves for consideration:

1. Whether these organisms, which cause the alkaline fermentation, always gain admission from without, or whether freshly voided urine contains such germs, so that unboiled urine, carefully protected from contact with the air, may still decompose; which would admit of the conclusion that the elements of fermentation do not always arise from without?

2. What particular organism causes the alkaline fermentation, or are several kinds involved?

(1) As regards the entrance of the organism. It has been shown by Cazeneuve[1] and Livon, and Meissner[2] that perfectly fresh urine may be preserved free from any fermentative change by eliminating the possibility of the entrance of air and germs, and Professor Leube, by a series of ingenious experiments, has shown that normal urine, on its exit from the bladder, contains neither fungi nor germs, the development of which would cause decomposition of the urea. Further, by the exposure for a few minutes of nutrient gelatine in shallow glass vessels such as those used in plate cultivations, micro-organisms may be cultivated from the air, which, when isolated, are found to be capable of giving rise to the decomposition of sterilised urine, and which, in form and general characters, are found to be identical with the organisms present in decomposed urine.

(2) Is the ammoniacal change in urine due to the presence of one or more organisms? It is with the object particularly of dealing with this question that I have lately carried on an in-

[1] 'Comptes rendus,' T. lxxxiv, p. 571, 1877.

[2] 'Deutsche Zeitschrift für Chirurgie,' Bd. xiii, p. 344, 1880.

vestigation under the direction of Dr. Klein at the Brown Institution.

I would, however, in the first place call attention to a valuable paper published last year by Professor W. Leube, to which I am indebted for much information, in which he describes at some length a series of experiments undertaken by Dr. E. Graser and himself with the view of determining the particular organisms which produce the alkaline urinary fermentation. He mentions that, as the result of their experiments, they were able to isolate "four well-described varieties" which possessed this property, two of them to a very great extent, and the remaining two only in a feebler sense.

The strongest influence he found to be exerted by small bacilli which he designated the Bacterium ureæ. These bacilli are described as being of a uniform size, ·001 mm. in thickness, of an average length of ·002 mm., with rounded ends.

The second growth of most frequent occurrence is a micrococcus of a globular form, and all of equal size, about ·8 m. (·008 mm.) in diameter. They are occasionally united to form diplococci, or two diplococci may join to form a square. They do not liquefy gelatine.

The two remaining organisms which are said to possess a weaker and less constant action are:

1. Small and thick bacilli of an oval shape with a varying length of 1·2 m. to 1·5 m., their greatest width being always ·7 or ·8 m.

2. Very minute bacilli with a length of from 1·2 to 1·4 m., and a thickness of ·6 m.

With the view of further investigating the life-history of the organisms producing this fermentation, I took a quantity of ordinary normal urine which had been recently voided and divided it into two parts; one part I placed aside in a sterilised beaker to allow of decomposition taking place in the ordinary way; the other part I boiled in a sterilised flask for half an hour. I then filtered it into another sterilised flask, taking the ordinary precautions, and finally decanted it into a number of sterilised test-tubes which were subsequently steamed for

twenty minutes on two successive days in the steam of boiling water; the tubes were then placed in an incubator, and after an interval of three weeks were still found to be sterile without the slightest trace of ammonia being present.

Sterile neutral urine was prepared in the same way.

In starting the cultivation of the organisms I adopted the plan described by Dr. Klein at a recent meeting of the Chemical Society. The fine end of a freshly made capillary pipette was placed in the ammoniacal urine, and a little allowed to ascend in the tube by capillarity; a number of tubes containing nutrient gelatine were then inoculated by passing the pipette through the cotton-wool plug and allowing a droplet of the urine to pass out; the tubes were then placed in water having a temperature of about 40° for the purpose of melting the gelatine; they were then gently shaken so that the droplet which had been introduced should be uniformly distributed, the gelatine being subsequently poured out, with the usual precautions, into the lower of the two dishes used in plate cultivations and allowed to reset. After this had occurred, the glasses were placed on a glass plate, covered with a Bell jar containing a piece of moist blotting paper and maintained at a temperature of 20° C. in an incubator.

By these means after the introduction of the smallest droplet a large number of organisms was obtained, and by the subsequent processes of "fractional cultivation" and "dilution" these were isolated, and the tubes containing the acid and neutral sterile urine inoculated with them with the view of determining the particular organisms producing the ammoniacal change.

By these methods I was able to isolate about twenty different organisms, both bacilli and micrococci, but after repeated experiments I only found one organism—a micrococcus—able to decompose the urea into carbonate of ammonia. It would be tedious and serve no useful purpose to describe each of these organisms, and so I shall confine my remarks to a description of that one which induces the desired change.

If a plate cultivation be made of this micrococcus, and kept

at a temperature of 20° C., in twenty-four hours a number of small points are visible which by an ordinary magnifying glass are seen to have a faint outline, and to be scattered uniformly over the surface; in two days they are very distinct and are seen as circular whitish spots of the size of a fine point. These spots do not increase much in size, and in a few days liquefaction of the gelatine commences.

In tube cultivations, in which the solid gelatine is inoculated by means of a platinum wire inserted for some distance in the depth, the tubes being subsequently placed in an incubator at 20° C., in twenty-four hours the channel of inoculation is visible as a pale whitish streak made up of closely placed minute dots; these in a few days so enlarge that an appearance is presented of more or less parallel lines of small dots, at the same time that the growth spreads over the surface as a whitish film. In about three or four days the first trace of liquefaction is seen with slight depression of the surface; this liquefaction gradually extends downwards from the surface, the liquefied part being thick and uniformly turbid.

The accompanying drawings (Pl. XXX, figs. 1 and 2) show these characteristics, and fig. 2 the amount of liquefaction which had taken place in eighteen days, the tube having been inoculated on the 12th July, and the sketch made on the 30th July.

Microscopically, the micrococci are seen to be mostly single, or diplococci; there are, however, a few short chains and a few small groups of four, five, to eight.

With this organism I inoculated both acid and neutral sterile urine, and in twenty-four to thirty-six hours the ammoniacal change took place. I also inoculated the fluid recommended by von Taksch, consisting of one litre of water, one eighth gramme of acid phosphate of potash, one sixteenth gramme of sulphate of magnesia, and three grammes of urea with a like result.

Therefore, so far as my observations go, the ammoniacal decomposition of urine is brought about by the presence of a micrococcus which differs from that described by Professor W. Leube, inasmuch as it liquefies gelatine. Whether this organism

is identical with the organism known since Pasteur and Cohn ('Zeitsch. f. Biol.,' A. Pfl. ii) as the Micrococcus ureæ I cannot say, because the characters of this latter had—at the time when Pasteur and Cohn investigated them—not been so studied by plate cultivation, &c., as they now are.

I have not been able to detect any other organism having a like effect, although it is possible that there are such possessing this quality in an inferior degree.

DESCRIPTION OF PLATE XXX, figs. 1 and 2,

Illustrating Dr. Wm. Robert Smith's Paper on "The Ammoniacal Decomposition of Urine."

Fig. 1.—Showing dotted appearance of the organism in the depth of gelatine, with surface film, and commencing liquefaction at surface.

Fig. 2.—Showing the amount of liquefaction which had taken place in eighteen days from the date of inoculation of a gelatine tube with the Micrococcus ureæ.

Notes on Echinoderm Morphology, No. X. On the Supposed Presence of Symbiotic Algæ in Antedon rosacea.

By

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With Plate XXX, fig. 3.

The anatomical monograph on Antedon rosacea which has been recently published by Messrs. Vogt and Yung,[1] contains the exposition of a new theory as to the nature of the sacculi, those problematical structures which have hitherto so completely puzzled all the various naturalists who have devoted any attention to the Crinoidea. These vesicular bodies, to which the name sacculi was given by Dr. Carpenter,[2] were regarded by him as possibly sense-organs, by Edward Forbes as ovaries, by Wyville Thomson as calcareous glands, and as possibly excretory organs by Perrier,[3] with whom Ludwig[4] was inclined to agree; while Vogt and Yung describe them as symbiotic algæ.[5]

Colourless, or nearly so during life, they become strongly tinged after death by the red pigment set free from the perisome (antedonin?), and they then appear as the red spots

[1] 'Traité d'Anatomie Comparée Pratique,' Livr. 7, 8, pp. 519—572.

[2] "On the Structure, Physiology, and Development of Antedon (Comatula, Lamk.) rosaceus," 'Proc. Roy. Soc.,' 1876, vol. xxiv, p. 227.

[3] "Recherches sur l'Anatomie et la Régénération des Bras de la Comatula rosacea," 'Arch. de Zool. exp. et gén.,' 1873, T. ii, p. 67.

[4] "Beiträge zur Anatomie der Crinoideen," 'Zeitschr. f. wiss. Zool.,' 1877, Bd. xxviii, p. 305.

[5] Op. cit., p. 570.

which are so abundant at the sides of the ambulacra, especially in the pinnules. They occur in most, if not in all the species of Antedon, but are not always equally visible, being, as a rule, smaller and less darkly coloured in those from considerable depths. Their nature has been a constant puzzle to me ever since I began to make a special study of the Crinoids, eleven years ago; and any observations which would place their nature beyond a doubt would be an immense relief to my mind. Without desiring to express a decided opinion as yet, I find some difficulty in accepting the theory that they are symbiotic algæ, for reasons which will appear in the following pages.

I will first quote the description which is given by the Swiss authors of these structures as they occur in Antedon rosacea.[1]

"Les spores amœboïdes de ces Zooxanthelles immigrent dans les larves, pendant que celles-ci nagent encore dans la mer. M. Goette les a décrites à cette époque comme des cellules contractiles, colorées en jaune, munies de noyaux, et ayant la forme de massues, dont le bout épaissi fait souvent encore saillie au-dessus de l'épiderme, où on les trouve constamment. Ces cellules entrent plus profondément dans les tissus, elles deviennent rondes, développent dans l'intérieur des masses colorées, une ou deux, qui se divisent en granules collés ensemble. Les paquets de granules réunis, ont, suivant M. Perrier, des queues très longues et déliées, ce sont donc de véritables zoospores. Arrivées à cet état, les Zooxanthelles ont encore le mouvement amœboïde dû à leur protoplasme incolore, et sont entourées outre leur contour propre, d'une sorte de capsule formée par les tissus."

The structures which Vogt and Yung call amœboid spores are the pyriform oil-cells of Wyville Thomson,[2] who found them in the larva, "immediately after escaping from the vitelline membrane;" and he further described how "the surface is dotted over with the wider ends of large pyriform lemon-coloured oil-cells immersed perpendicularly in the sarcode." This fact appears to me to be a serious objection to Vogt and Yung's theory that the lemon-coloured oil-cells are the migrating amœboid spores of symbiotic algæ, which must lose

[1] Op. cit., p. 570.

[2] "On the Embryogeny of Antedon rosaceus, Linck (Comatula rosacea of Lamarck)," 'Phil. Trans.,' 1865, pp. 521, 522, pl. xxiv, fig. 5.

no time in making their way into the ectoderm of the recently hatched larva. But unless I am greatly mistaken, these oil-cells are seen in Thomson's figures of the larva while it is still within the vitelline membrane ;[1] and if this should really prove to be the case, it is a fatal argument to one part at least of Vogt and Yung's theory.

Goette[2] does not mention the exact time of their first appearance, but he describes them as follows: "Zu einer gewissen Zeit treten in der sonst noch unveränderten Oberhaut zwischen ihrer Cylinderzellen keulenförmige, tief gelb gefärbte, kernhaltige Zellen auf, deren dickeres Ende nach aussen gekehrt ist und nicht nur die Oberfläche erreicht, sondern bisweilen aus ihr hervortritt." I cannot find, however, that Goette ever spoke of these yellow cells as contractile, as stated by Vogt and Yung; though lower down on the same page he described certain other cells in the epidermis which "sitzen mit dem dickeren kernhaltigen Ende fest an der inneren Cuticula;" and he proceeds to say of them "da sie ferner kontractil sind, so ziehen sie die letztere faltenförmig ein, wodurch die Oberfläche älterer Larven runzelig aussieht." He also gave a figure illustrating this point, in which the yellow cells and the contractile cells are distinguished by separate letters; and I do not know therefore why the former should be called contractile, and described as the amœboid spores of symbiotic algæ by Messrs. Vogt and Yung.

Goette prudently declined to give any opinion as to their nature, but described them as occurring in greatly increased numbers in the largest larvæ which he examined; while they are very abundant in the more advanced Pentacrinoid figured by Wyville Thomson,[3] which has the bases of the arms appearing above the radial axillaries. They are equally abundant in the perisome of the mature Antedon, where they have been

[1] 'Phil. Trans.,' 1865, p. 521, fig. c; pl. xxiv, fig. 4.

[2] "Vergleichende Entwickelungsgeschichte der Comatula mediterranee," 'Archiv f. Mikrosk. Anat.,' Bd. xii, 1877, p. 596, Taf. xxvi, fig. 18.

[3] 'Phil. Trans.,' 1865, pl. xxvii, fig. 1.

described by Perrier,[1] whose excellent account and figures of them seem, for a wonder, to have escaped the notice of Messrs. Vogt and Yung. He writes as follows:

"Immédiatement au-dessous de la couche épithéliale se trouve un tissu particulier formé d'éléments qui semblent relier entre elles la membrane cellulaire qui forme le tégument externe et la membrane qui revêt l'axe calcaire du bras. Ces éléments sont incolores, fusiformes, et les extrémités du fuseau, qui souvent se bifurquent, donnent naissance à des prolongements qui, après s'être plus ou moins divisés, viennent s'attacher à l'une des deux membranes qui relient ces éléments. Dans la partie renflée de ces sortes de cellules étoilées on aperçoit toujours un noyau très-brillant."

The identity of these "éléments étoilées" with Goette's contractile cells is evident from a comparison of the excellent figures given by both authors, and Perrier states expressly that he has not observed them perform amœboid movements or contractions of any kind. He regards them as "surtout des éléments du tissu conjonctif;" and he proceeds to compare them with the yellow oil-cells of Wyville Thomson, "corpuscles jaune clair et très-réfringents . . . qui se retrouvent en plus ou moins grande abondance dans toutes les parties du corps de l'animal." Finding what appear to be several intermediate stages between the two structures, he was led to the conclusion that "les éléments étoilées et les gros éléments jaunes sont morphologiquement de même nature, que ces derniers ne diffèrent des autres que parce que la matière jaune a envahi tout l'intérieur de la cellule et distend ses parois." Now, however, we are told by Vogt and Yung that these yellow cells which Perrier found to be universally distributed in all parts of the mature Antedon rosacea, are really the amœboid spores of symbiotic algæ, and that the sacculi at the sides of the ambulacra and elsewhere are groups of "véritables zoospores" of the same algæ. According to their theory these zoospores are later developmental stages of the yellow cells, Perrier's connective-tissue elements.

The contents of the sacculi were described by Professor Perrier[2] in the following terms:

[1] Loc. cit., pp. 51—53, pl. iii, fig. 11.

[2] Loc. cit., p. 67; pl. ii, fig. 7.

"Chacune des masses en question se prolonge généralement en une sorte de queue très-grêle, assez longue, souvent entortillée, continue avec la membrane propre de la masse d'où elle dépend, et qui paraît complétement anhiste. Dans cette membrane qui forme comme une sorte de sac et sur les parois de laquelle se montre presque toujours une sorte de petit noyau de couleur brune, on trouve un nombre variable de petites sphères incolores, très-réfringentes, ressemblant à des goutelettes d'un liquide transparent, et probablement enveloppées chacune d'une mince membrane qui l'empêche de se confondre avec ses voisines. Ce sont ces petites sphères qui absorbent si rapidement les matières colorantes et qui, sous l'action du sublimé corrosif, prennent très rapidement une couleur brune qui permet d'elles distinguer immédiatement."

When Messrs. Vogt and Yung have clearly demonstrated the mutual relations of the yellow (oil-) cells and the colourless contents of the sacculi, their theory as to the vegetable nature of both structures will have a more certain foundation than it possesses at present.

Meanwhile let us inquire as to what is known concerning the development of the sacculi. Perrier has made a careful study of the mode of regeneration of the arms of Antedon rosacea, and he gives the following description of the changes which take place in the growing bud[1]:—"Pendant que ces changements se produisent dans le cylindre intérieur, certains éléments du cylindre extérieur subissent une modification remarquable; ils se transforment en vésicules rondes remplies de ce liquide ou pour mieux dire de cette matière jaune que nous avons déjà rencontrée dans les éléments du tissu conjonctif des bras. Ainsi, soit dans le développement normal de l'embryon, soit dans la régénération des bras, ces corpuscules jaunes (oil-cells, Wyville Thomson) apparaissent de très-bonne heure, ce qui semble indiquer que leur rôle dans la vie de l'animal n'est pas sans quelque importance." But, according to Vogt and Yung, the yellow cells are the amœboid spores of symbiotic algæ! Perrier states that they appear before the new calcareous elements are visible, just as is the case in the larva; but, according to the same observer,[2] the sacculi of the young arm, "ne se montrent que bien après les premières parties calcaires

[1] Loc. cit., p. 72.

[2] Loc. cit., p. 80.

ont été déposées, et leur développement n'est pas encore complet que les divers articles de la pinnule sont déjà parfaitement distincts;" while he described their development in the following terms: —" D'après ces observations, il semble que la première portion formée est le noyau de chaque vésicule; toutefois, de très-bonne heure, on peut distinguer une délicate membrane autour de ce noyau, membrane qui s'écarte de lui graduellement, et qui préexiste, en conséquence, à la plus grande partie du contenu de la vésicule." It is difficult to believe that the groups of nuclei, which are the first indications of these structures, are really derived from the yellow cells. Their mutual relations, if existing, can hardly have escaped the notice of Perrier in the regenerating arm, or that of Wyville Thomson in the Pentacrinoid larva. The first indication of the sacculi noticed by the latter author[1] in the early larva is a minute vesicle containing a transparent fluid, which is imbedded in the tissue at the base of each of the azygos tentacles; but if this had been anything like the yellow oil-cells, which are abundant all over the Pentacrinoid, Thomson would surely have noticed the fact.

The above, however, are not my only reasons for hesitating to accept this new doctrine of Vogt and Yung's respecting the vegetable nature of the sacculi in Antedon rosacea. This species is the only Crinoid in which I have found these structures in the interior of the body. Although they are very abundant on the pinnules of Antedon Eschrichti and its allied species, I have never found them anywhere else but at the sides of the ambulacra. They are usually more numerous on the arms than on the disc, but less so than on the pinnules, where they alternate very regularly with the groups of tentacles. Thus, for example, I can find none on the disc of an Antedon microdiscus from Cape York, and very few at the sides of the brachial ambulacra; but they are extraordinarily abundant on the pinnules. As a general rule, too, they are more numerous on the outer pinnules than on those nearer the disc, which are distended by the fertile portions of the

[1] 'Phil. Trans.,' 1865, p. 527.

genital glands. But when the glands are short, and do not extend over more than three or four pinnule segments, the distal ends of the genital pinnules are often abundantly provided with sacculi, although there are few or none in their enlarged basal portions. I do not know how this will be explained by the supporters of the algal nature of the sacculi, and their relations in Antedon-species with plated ambulacra present still greater difficulties. I have sometimes failed to find them, even in Antedon, among the various plates surrounding the ambulacra; but, as a general rule, they are fairly well developed in the pinnules, and occupy an extremely constant position with regard to the ambulacral plates. In those species which have the most highly differentiated pinnule-ambulacra the sacculi alternate with the tentacular groups, just as in Antedon rosacea, and the distal edges of the side plates are notched for their reception in the most regular way possible (Fig. 1, *s*). It does not appear probable to me that the calcareous elements of the skeleton would be so regularly modified for the reception of the sacculi if these structures were merely vegetable parasites; and the difficulties inherent in this view of their origin are increased by the fact that the side plates of Hyocrinus, Pentacrinus, and Metacrinus are not notched for the reception of sacculi, which do not occur at all in these genera, even when they are living side by side with Antedons in which sacculi are abundantly distributed at the sides of the pinnule-ambulacra.

Sacculi occur in nearly all the species of Antedon, in Promachocrinus, Atelecrinus, and in three species of Eudiocrinus, though they are absent in the other two. They are sometimes to be found in Rhizocrinus and Bathycrinus, though they are often so small and undeveloped as to be barely recognisable; but I have failed altogether to find them in Actinometra, Holopus, Hyocrinus, Metacrinus, or Pentacrinus,[1] even when these genera have

[1] The structures which I formerly regarded as imperfect sacculi in Pentacrinus, so far as I could judge from the surface characters only, turn out to be of a different nature when seen in thin sections.

been obtained at the same localities as Antedons which have abundant sacculi.

Thus, for example, these red spots or sacculi are largely developed in Antedon abyssorum, A. remota, and in Promachocrinus abyssorum, all from a depth of 1600 fathoms in the Southern Ocean; but I have not seen one on the arms or pinnules of Hyocrinus bethellianus from the same locality; while if they exist at all in Bathycrinus aldrichianus, of the same dredging, they are so scantily developed as to be almost unrecognisable. Here are some similar facts of the same kind. The "Challenger" dredged eleven species of Antedon at a station near the Ki Islands, some with abundant sacculi, others in which they are almost entirely undeveloped; while there is no sign of them at all in four species of Metacrinus and in one of Actinometra from the same dredging, neither do they appear in two species of Pentacrinus or in four of Metacrinus from off the Meangis Islands, though abundant in six Antedon species from the same locality. The "Challenger's" dredgings at Cape York yielded nine species of Actinometra, in which there is no sign of sacculi; but they are enormously abundant on the pinnules of Antedon microdiscus and A. multiradiata, fairly so in A. irregularis, and scanty in A. bidentata—all from the same locality. They are very thick at the sides of the pinnule ambulacra in A. Dubeni and A. carinata, of which latter species over 100 individuals were obtained at Bahia; but there is no trace of them in Actinometra lineata or Act. meridionalis, several examples of which were found living at the same place. I could name many similar cases from the dredgings of the "Blake" in the Caribbean Sea. There are six different stations (near Barbadoes, Montserrat, Martinique, and St. Vincent) where Antedon species occur with abundant sacculi at the sides of the ambulacra, while none are to be found in the species of Actinometra and Pentacrinus which were obtained in the same dredgings.

Antedon tenuis, dredged by the "Challenger" at Station 170, has numerous sacculi on the outer pinnules, just as in the

other seven species of Antedon obtained at this station. They are also present on an example of this same species found at Station 169; but there is no sign of them in Eudiocrinus semperi, from the same station, nor in another individual of this latter type from Station 164, where they are present between the side plates of the ambulacra in Antedon acutiradia, five specimens of which were obtained by the "Challenger."

The above facts seem to me to tell very decidedly against the supposed algal nature of the sacculi, which are so eminently characteristic of certain endocyclic Crinoids, and never occur in the exocylic Actinometra. They occur wherever Antedon is found, from nearly 82° N. to below 52° S., and at all depths down to 2600 fathoms; but they are not to be found in species of Actinometra which live side by side with Antedon in the warmer parts of the world, and I pointed out in 1882[1] that this fact is one of considerable importance in the generic distinction of the two forms. I cannot think that Messrs. Vogt and Yung are aware of it, however, or they would hardly have committed themselves to the following statement:[2]—"Les Comatulides libres (Antedon, Actinometra) offrent fort peu des différences anatomiques, et sauf quelques détails insignifiants, sont construites absolument sur le même plan que notre espèce type." The absence in Actinometra of the sacculi, which are almost always present in Antedon, may be a "détail insignifiant;" but it is, at any rate, one which may possibly turn out to be fatal to Messrs. Vogt and Yung's theory of the vegetable nature of these structures.

I cannot imagine any reason why the "amœboid spores," which have given rise to such abundant "véritables zoospores" along the pinnule ambulacra of Antedon carinata and A. microdiscus at Bahia and at Cape York respectively, should not have had equal opportunities for developing in the

[1] "Preliminary Report on the 'Blake' Comatulæ," 'Bull. Mus. Comp. Zool.,' 1882, vol. ix, No. 4, p. 11.

[2] Op. cit., p. 571.

pinnules of Actinometra meridionalis and Act. paucicirra, which occur at the same localities. The contrast between the pinnules of the two genera is so striking that there must be some reason for it, and I am altogether unable to understand why it should appear at all if the sacculi are only vegetable parasites.

I much doubt, therefore, if we are any nearer to an understanding of their real nature than we were before Vogt and Yung took up the subject.

Since the above lines were written another view of the nature of the sacculi has been published by Walther,[1] who says: "Seine Function ist nach der mir zugänglichen Literatur noch unbekannt, doch vermuthe ich auf Grund folgender Beobachtungen: starke Lichtbrechung, starke Farbstoffaufnahme, Mangel einer zelligen Structur, Aufbau aus polygonalen oder rundlichen Plättchen, Lage an den Punkten stärkeren Wachsthums und eine gewisse fremdartige Einlagerung im Gewebe—dass es Reservematerial (Nahrungsdotter) ist; während ich eine secretorische Function wegen der umgebenden undurchbrochenen Kapsel für ausgeschlossen halte."

I have no remarks to offer respecting this suggestion of Walther's; but I will conclude by a few words upon Professor Perrier's latest utterances upon the subject of the sacculi. He attributes the name to me,[2] being apparently unaware that it originated with my father, as did also the idea that they might be sensory organs.[3] The latter view is also attributed to me by Perrier in the following passage,[4] which refers to my first publication on the subject of the Crinoidea in 1876. "Les

[1] "Untersuchungen über den Bau der Crinoiden, &c.," 'Palæontographica,' 1886, Bd. xxxii, p. 165. I propose at some future time to make some remarks upon this author's application of "Transcendental Morphology" to the Crinoidea.

[2] "Mémoire sur l'organisation et le Développement de la Comatule de la Mediterranée," 'Nouv. Archiv. du Mus. Hist. Nat.,' 1886, 2me Ser., T. ix, p. 154.

[3] 'Proc. Roy. Soc.,' 1876, vol. xxiv, p. 227.

[4] Op. cit., p. 133.

corbeilles vibratiles du canal dorsal et les corps sphériques des bras lui paraissent être des organes des sens." My only positive reference to the sacculi in this paper [1] was in the explanation of fig. 1, which ran as follows. "*s*. Sacculi ('calcareous glands' of Wyville Thomson) of doubtful (? sensory) nature." I put in the "? sensory" in accordance with my father's suggestion made three months previously. But I cannot see that it at all justified Perrier in stating that the sacculi "lui paraissent être des organs des sens."

His assertion that I took the same view of the ciliated cups in the cœliac canal is entirely incorrect. All that I said of them[2] was that Ludwig had well compared them "to the ciliated funnels on the mesentery of the Synaptidæ." Further on in the paper, however, I described how in certain pinnules of Actinometra armata the radiating branches of the axial cords "enter into connection with peculiar cellular organs, of very similar construction to the groups of large epidermic cells described in the tactile papillæ of the integument of the Synaptidæ, by Professor Semper. I am inclined to regard these organs also as sensory in function; their position upon the dorsal or most exposed side of the pinnules certainly favours this hypothesis." Here then Professor Perrier, misled by the double reference to the Synaptidæ, has confused together a variety of statements respecting entirely different structures. 1. The ciliated cups in the cœliac canals of the Comatulæ. 2. The ciliated funnels on the mesentery of the Synaptidæ. 3. The cell-groups in the tactile papillæ of the Synaptidæ. 4. The cellular organs on the dorsal side of the ungrooved pinnules of Actinometra armata. I attributed a sensory nature to the fourth of these, and he describes my views as applying to the first.

But even this is not the full extent of his errors. For on the same page, in summarising my second paper of 1876, he says, "Les corps sphériques (i. e. sacculi) y sont désignés sans

[1] "Remarks on the Anatomy of the Arms of the Crinoids," 'Journ. Anat. and Physiol.,' 1876, vol. x, p. 580.

[2] Ibid., p. 579.

point de doute comme 'des organes des sens' problématiques." This statement is absolutely untrue.

There is no mention whatever of the "corps sphériques" of Antedon in the paper to which Perrier refers. But in describing the two kinds of arms of Actinometra armata, namely, those with and those without an ambulacral groove, I said:[1] "Another difference between these two types of arms is that the curious problematical 'sense organs' which I have mentioned in a former paper are limited to the terminal pinnules of the non-tentaculiferous arms. The three last segments of these pinnules are very small, but the centre of the dorsal surface of each of the next seven or ten segments is occupied by one of these curious bodies, which when viewed from the exterior appears as a rounded brownish mass." Perrier, however, has confused these single, median, and antiambulacral structures of Actinometra with the double row of sacculi on the ambulacral side of the arms and pinnules in Antedon.

It is really lamentable that the historical introduction to his elaborate and magnificently illustrated work, which will become a classic ere long, should be disfigured by errors like these. I could name others of the same kind, all of them alike due to sheer carelessness in consulting the works of his predecessors.

Speaking of the sacculi on another page (154), Perrier says: "Ces corps énigmatiques ont été récemment considérés comme des parasites et designés sous le nom de Zooxanthelles. Herbert Carpenter ne trouve pas qu'il y ait encore de raisons suffisantes pour se ranger a cette opinion." This statement is supported by no reference whatever to any of my published writings, for the very excellent reason that Professor Perrier has none to give.

The section on the sacculi in the "Challenger" Report was written in the summer of 1884. This was the last occasion on which I referred to the nature of these structures in print; and it is impossible therefore that I could have expressed an

[1] "Remarks on the Anatomy of the Arms of the Crinoids," Part II, 'Journ. Anat. and Physiol.,' 1876, vol. xi, p. 92.

opinion adverse to the views of Messrs. Vogt and Yung respecting their vegetable nature, as these were not published till 1885, and I did not become aware of them till March, 1886.

On p. 154, after quoting from the "Challenger" report some facts respecting the distribution of the sacculi, Perrier adds: "Cette inégale distribution montre que ce ne sont pas là des organes de quelque importance, si tant est que ce soient des organes; mais on ne peut rien conclure de ces données relativement à leur véritable nature." I am by no means sure, however, that Perrier has drawn the right conclusion from the facts of their distribution.

I have no means of knowing what is his own view of their nature; but they are lettered *z* in his figures. The explanation of these has not yet appeared, and I am therefore uncertain whether *z* is explained as denoting "zooxanthelles," or whether it is used for some other descriptive term.

DESCRIPTION OF PLATE XXX, fig. 3.

Illustrating Mr. P. Herbert Carpenter's Paper "On the Supposed Presence of Symbiotic Algæ in Antedon rosacea."

FIG. 3.—Portion of a pinnule of Antedon acœla, with a very well-developed ambulacral skeleton. × 20.

p. The pinnule joints. *c.p.* The covering plates of the ambulacra. *s.p.* The side plates. *s.* Notches in the distal edges of the side plates for the reception of the sacculi (Zooxanthelles, Vogt and Yung).

The Function of Nettlecells.

By

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With Plate XXX, fig. 4.

Morphology.

Both in the ectoderm and the entoderm of all Polypomedusæ these elements are met with. They are never absent in the ectoderm in any species, and are generally also found in the entoderm.

Their structure has been investigated by numerous authors; particularly F. E. Schulze (1), O. Hamann (2), Korotneff (3), and the author (4) have studied their structure and action more closely. Also Jickeli (5) has dwelt on this subject.

However different the animals may be on which these Nettlecells are found, the latter nevertheless are always of the same structure, although they may vary very much in size. The large ones are fewer in number and more determinate in their position than the small ones. No Nettlecells of the large kind are found in the entoderm.

The Nettlecells, or, as Schulze calls them, cnidoblasts, consist of a cell the greater portion of which is occupied by the well-known highly refracting vesicle, on the size of which the size of the whole cnidoblast depends. The granular protoplasmatic portion of the Nettlecell is reduced in bulk so much that it only forms a thin coating over the surface of the vesicle. In one locality this plasmatic coat is slightly thickened, and here the flattened nucleus, closely attached to the vesicle, is situated.

From the upper margin of this protoplasmatic sac which surrounds the vesicle, a conical, stout and pointed filament, the cnidocil, projects. This is about as long as the vesicle is broad, and is situated in such manner oblique to the surface as to form with it an angle of 45°. This angle is very constant. The cnidocil always points in a centrifugal direction, that is to say the cnidocils on the tentacles point towards the ends of the tentacles, those on the body point towards the mouth, and so on. The cnidocils invariably point in that direction from which a foreign body is most likely to approach the animal.

The vesicle itself possesses a very distinct, tough, and apparently elastic membrane. It is closed on all sides except the anterior end, where a circular aperture, about a quarter as wide as the vesicle, is situated. This aperture leads into a very long tube, when the cnidoblast has exploded. This tube is about twenty times as long as the cnidoblast and tapers towards the end, which appears pointed. It is surrounded by one or two spiral lines of minute hooks or bristles which are often very large and conspicuous at the base, but which rapidly decrease in size distally and become invisible even with the highest power near the end. The tube is probably closed at the end.

This tube can be ejected with great force from the capsule of the cnidoblast, where it is coiled up very regularly before the explosion. The explosion inverts this tube hanging down from the orifice of the capsule into its interior, so that the external surface of the coiled-up tube becomes the internal surface of the ejected tube and vice versâ. The well-known poisonous effect of these cnidoblasts is due to a poison which is contained in the interior of the coiled-up tube, and which, as the tube is inverted, comes to be situated on the outer surface. The tube penetrates, by the force of its ejection and in consequence of its small size, soft foreign bodies which may come in contact with the animal, and so the poison is transmitted into the body of the victim. Whilst Möbius and others have studied the tube and capsule, the discovery of the cnidocil was made by F. E. Schulze.

After these facts had been made known, the question arose

whether the cnidoblast was in connection with the nervous system described particularly by the brothers Hertwig. Such a connection has been demonstrated in many cases by Jickeli, the author, and Korotneff.

In the lower strata—subepithelium—of the dermis, ganglion cells are met with in those parts generally where cnidoblasts are situated. These ganglia cells are multipolar. Their processes are connected with slender nerve-fibres extending tangentially between the mesodermal jelly (the supporting membrane, Stützlamelle) and the outer epithelium. Some of these processes, however, extend in a radial, centrifugal direction, and these are connected with the large cnidoblasts. No connection has hitherto been observed with sufficient certainty between the ganglion cells and the small cnidoblasts.

The protoplasmatic outer portion of the cnidoblast is prolonged into a process extending centripetally like a peduncle. This is composed of granular protoplasm and forms the connection between the cnidoblast and the ganglion cell below. Hamann found that these peduncles, in some cases at least, were not formed of granular protoplasm, but appeared as transparent and structureless peduncles formed of the same substance as the supporting mesodermal membrane. Subsequently I was able to demonstrate that the large cnidoblasts have two peduncles, one a transparent supporting rod as described by Hamann, and one a granular thread, which connects it with the ganglia cells of the subepithelium. Whilst the former is always quite straight, the latter generally appears more or less curved and irregular.

These cnidoblasts are surrounded by high and slender cylindrical ciliated cells which form the outermost layer, or they penetrate the large cells of the outer epithelium. In those parts where the epithelium is formed by flat and low pavement cells, large cnidoblasts are never observed.

The small cnidoblasts, however, are scattered over the surface more indiscriminately and occur in great abundance also in the pavement cell areas. In these areas a subepithelial layer of ganglion cells seems not to occur.

Physiology.

F. E. Schulze, who discovered the cnidocil, was of opinion that any foreign body touching the cnidocil would cause an explosion of the Nettlecell, much in the same way as touch invariably causes a sting in the case of the stinging-nettle. To this end it appeared that the cnidocil was so placed as to point towards the ends of tentacles, that is, always in that direction whence an enemy would be most likely to approach. The position of the stinging hairs of Urtica is the same.

Others who dwelt on the subject endorsed Schulze's purely mechanical explanation, that direct pressure on the cnidocil is transmitted to the cnidoblast and there causes the explosion of the capsule which is already in high tension.

If this were so there would apparently be no reason for the connection of the cnidoblast with the nervous system of the animal.

Now, it is a well-known fact that touch by no means invariably causes the explosion of the Nettlecells and the ejection of the tube. If a tentacle of an Actinia is viewed under the microscope in seawater under a cover-glass, and if fine grains of sand are placed in the water and a strong current produced by suction on one end, then the sand-grains are carried to and fro with great velocity by the moving water and continually come in contact with the surface of the tentacle. No explosion of a Nettlecell, however, can be observed. But if acetic acid be added to the water then the tubes will be seen shooting forth like rockets all over the surface.

When the animals, as they often do, contract themselves and draw up their tentacles like the Medusæ to one hundredth part of their length, or close them over the mouth like the Actiniæ, there must be a very strong pressure, which according to the mechanical theory would immediately explode all the Nettlecells.

There are some species of Actinia which live in sand, as, for instance, the Cerianthus. Those which live in shallow water,

or between tide-marks, bury the body in the sand and expand their tentacles in the surface of the sand, the waves move the sand and it is evident that masses of sand must be continually falling on the tentacles. I have often observed a species of Actinia exceedingly abundant in the "sands" of Port Phillip, Victoria, and I know that they do not retract their tentacles when the water moves and the sand drops on them.

According to the mechanical theory, each sand-grain which came into contact with the tentacles would cause the explosion of a great number of Nettlecells. It is evident that this cannot be so.

Further, there are Nettlecells embedded in the jelly of the umbrella of some Medusæ (discovered by me, l. c., over the marginal bodies of Crambessa mosaica) which explode if the surface of the body is touched with acetic acid instantaneously and long before the acid could have got to where they are situated.

This will show that touch is by no means sufficient to cause the explosion of the Nettlecells; nor is it the only possible cause.

It would now seem possible that the cnidoblasts were exploded at the will of the animal by a contraction caused consequent on a centrifugally acting nervous irritation in the plasmatic mantle surrounding the capsule. Chun (6) has observed muscular differentiations in this plasmatic mantle in Physalia, which is one of the severest stinging Cœlenterates. Others have been inclined to consider the peduncle as muscular and contractile.

If we were to assume this we should not be able to see the use of the cnidocil.

It seems, therefore, that there can be but one explanation of the mode of action of the cnidoblasts—of the large kind, at least—which is the following:

1. The structureless peduncle is a support and may contract so as to withdraw the cnidoblast with its lid from the surface under certain circumstances, particularly when the parts where the Nettlecell is situated are to be contracted. The animal has

control over these movements by means of the subepithelial nervous layer situated below the cnidoblasts.

2. The granular peduncle is a nerve-fibre connecting the protoplasmatic mantle of the Nettlecell with the nervous system of the animal.

3. By means of this the movements of the protoplasmatic mantle can be controlled.

4. The explosion of the cnidoblast is caused by the contraction of the plasmatic coat which surrounds the capsule, and which in Physalia (Chun, l. c.) has partly been converted into a network of muscular fibres.

5. The plasmatic contractile coat of the cnidoblast is incited to action by the cnidocil. If anything touches the cnidocil then the plasma mantle contracts and the tube is shot forth.

6. The animal can, however, by its volition prevent this reflex action by means of the nerve-fibre connecting the cnidoblast with the ganglia cells below. In this way the explosion may be prevented even if the cnidocil be touched, if this be the wish of the animal.

We find, accordingly, that the complicated machinery of nerve-centres controlling reflex actions of a low order in man and the higher animals is found also in these low forms of animal life, the Cœlentera.

The Ctenophora, which are destitute of cnidoblasts, possess in their stead certain structures, the "Klebezellen" of Chun and the "Stiftzellen"[1] of Hertwig, which appear homologous with these Nettlecells in the Ctenophora. Their action is very different and they do not explode like the cnidoblasts of Polypomedusæ.

It seems, however, probable that they are in a similar way subject to the control of the animal, as is the cnidoblast.

[1] These are, according to my investigations of the histology of Nais cordigera, Les., (7), not a sensitive apparatus, as the brothers Hertwig had assumed, but stinging hairs.

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4. R. v. Lendenfeld.—"Ueber Coelenteraten der Südsee. iii., Ueber Wehrthiere und Nesselzellen," 'Zeitschrift für wissenschaftliche Zoologie,' 1883, Band 38, p. 366.
5. C. Jickeli.—"Ueber den Bau der Hydroidpolypen," 'Morphologisches Jahrbuch,' 1882.
6. C. Chun.—"Die Natur und Wirkungsweise der Nesselzellen bei Coelenteraten," 'Zoologischer Anzeiger,' Band 4, p. 646.
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EXPLANATION OF PLATE XXX, fig. 4,

Illustrating R. von Lendenfeld's Paper on "The Functions of Nettlecells."

Fig. 4.—Schematic representation of cnidoblast, &c.—*a*. Mesodermal supporting lamella. *b*. Peduncle (Hamann's) of cnidoblast. *c*. Ordinary cylindrical epithelium cells. *d*. Their nuclei. *e*. Longitudinal striated muscles. *f*. Subepithelial muscle-cells. *g*. Their nuclei. *h*. Subepithelial ganglion cell. *i*. Tangential nerve-fibre. *k*. Nucleus of the ganglion cell. *l*. Epithelial sensitive cell. *m*. Its nucleus. *n*. Palpocil (Wright). *o*. Cilia of the ordinary epithelium cells. *p*. Nerve connecting ganglion cell with cnidoblast. *q*. Protoplasmatic contractile mantle of cnidoblast. *r*. Nucleus of cnidoblast. *s*. Nematocyst. *t*. Its aperture. *u*. Cnidocil (Schulze). *v*. Thread coiled up inside the cnidoblast.

Some New Methods of Using the Aniline Dyes for Staining Bacteria.

By

E. Hanbury Hankin.

I HAVE lately been staining bacteria in tissues for Dr. Klein by some new methods that seem to offer certain advantages over those already in use.

Dr. Klein has kindly supplied me with tissues containing various bacteria. But most of my results have been obtained with Bacillus anthracis.

In the methods about to be described the hardening of the tissue that generally precedes section cutting must be carefully attended to. It is always necessary to use Müller's fluid. The tissues must be cut into very small pieces, and the liquid frequently changed. If this is not done the nuclei will be found to show more affinity for the dye than the bacteria, and only those organisms that are placed near the margin of each section will be visible.

Nearly equally good results are obtained with perfectly fresh material.

Method A. Materials required:

(1) A strong watery solution of methyl blue or Weigert's aniline oil solution of the same dye.

(2) A saturated alcoholic solution of eosin. This should be made simply by shaking up the dye with the alcohol and filtering. By heating the alcohol or letting the solid eosin remain for some days in contact with the liquid a stronger solution is obtained, which is undesirable for the present purpose.

(3) A pipette to hold the above. A test-tube fitted up as a wash bottle is very convenient for holding the eosin.

(4) Absolute alcohol, which must be used in a capsule provided with a cover, to prevent the access of watery vapour from the air. Every precaution should be taken to keep the alcohol as free from water as possible.

(5) Benzine and clove-oil mixture, made by mixing equal volumes of benzine and oil of cloves, and adding sufficient absolute alcohol to dissolve the turbidity that appears on shaking together the above reagents.

(6) Oil of cloves, which must be fresh and nearly colourless. By exposure for a few hours to light and air oil of cloves loses a great deal of its power of dissolving the aniline colours.

(7) Benzine. This may be replaced by xylol or cedar oil.

Modus Operandi.—The sections are taken from spirit and placed in the methyl blue solution. Immediately the eosin is dripped in from the pipette. About equal parts of the eosin and methyl blue solutions are employed. If too much eosin is used the background of the section will have a dull purplish tinge, contrasting badly with the blue-tinted bacteria; while if too little eosin has been dropped in its red shade is scarcely visible.

By adding eosin to the solution its power of dissolving methyl blue is diminished. Part of the excess is precipitated in the form of a granular deposit; part of it combines with the tissue in the form of stain. The precipitate thus rapidly formed is readily dissolved by the alcohol used in dehydrating, and never spoils the appearance of the specimen. As soon as the eosin has been added the sections are removed one by one to absolute alcohol, shaken about in it for a few seconds, and then placed in the benzine and clove-oil mixture.

The sections are washed in this till the effects of the eosin begin to be apparent, then washed rapidly in another capsule of the same mixture, spread out on a section lifter, placed in benzine, and mounted. If, however, they are not sufficiently decolorised they should be removed to oil of cloves, which will readily dissolve the excess of methyl blue. If the red tint of

the background is not sufficiently pronounced they may be treated for a minute or less with eosin dissolved in clove oil, afterwards washed in benzine and clove-oil mixture, lastly in benzine, and then mounted.

The whole process does not take much more than a minute, of which about twenty seconds are occupied in adding the eosin solution to the methyl blue. The dehydrating in alcohol should be accomplished as rapidly as possible.

Sections stained in this manner show the bacteria stained blue; the nuclei are similarly coloured, but of a lighter shade, the eosin has stained the red blood-corpuscles orange, and the background of the tissue is of a rose-red tint.

Ganglion cells are stained purple if the section has not had too much of the colour removed. Besides the blood-corpuscles eosin can stain several other things of a yellow or orange tint, and if these structures have also an affinity for methyl blue the two dyes combine, giving a bright green shade. Hæmoglobin crystals and sometimes red blood-corpuscles are thus stained.

In sections through the lung of a sheep which had suffered from foot-and-mouth disease this eosin and methyl blue method showed large amorphous caseous deposits of a bright emerald-green colour, while other methods of staining failed to differentiate them.

Sections of the lung of another sheep, which contained encysted thread-worms, showed within their rose-red epidermis the protoplasm of the nematodes stained green, and their nuclei of a purple tint.

Sections through a congested spleen showed orange-red blood-vessels on a background of blue and green nuclei almost as well as an injected specimen.

While oil of cloves is generally used to help the alcohol in taking the excess of colour out of a section, I, on the contrary, use it for keeping the colour in, so far as the bacteria are concerned; for bacteria stained as here described would rapidly become invisible if left for long in the more powerful solvent, namely, the alcohol; and sections may often be kept indefinitely in oil of cloves without the bacteria losing their stain,

although this solvent is quite capable of washing out all the colour from nuclei, connective tissue, &c.

Not only clove oil, but also other clearing reagents, such as benzine, xylol, creosote, aniline, and cedar oil have a greater or less power of fixing the colour in the bacteria, though several of them can dissolve the aniline dyes freely.

Sections stained by any of these methods, quickly dehydrated and cleared, can then be washed for a longer time in alcohol, without the dye leaving the bacteria, than can sections which have been taken direct from the dye into spirit. I even found that clearing the tissue before staining, and then washing in alcohol, would slightly improve the result in some cases.

Concerning this method of staining it may be remarked that, firstly, a mixture of methyl blue and eosin has scarcely any staining power. If the sections are put into the dye after the addition of the eosin no result is obtained, and consequently it is immaterial how long the sections are left in the mixed staining solution. Secondly, the eosin solution is much stronger than that in general use. If a section is stained in methyl blue, and then placed in a saturated alcoholic solution of eosin, as here used, in a very few seconds all the blue colour is turned out, and the section stained of a uniform red tint; but in this method the presence of the methyl blue in solution seems to protect the tissue from the eosin. Thirdly, when eosin and methyl blue solutions are mixed some of the methyl blue is precipitated, and after some time will be found sticking to the sides of the containing vessel. Fourthly, if a greater quantity of the eosin solution than that above mentioned is employed, instead of a preponderance of red in the resulting stain, it will be found that the methyl blue has commenced to stain the background besides the nuclei, and the whole section will have a purplish colour. Fifthly, if the conditions of solution of the dyes are reversed, and alcoholic methyl blue is added to watery solution of eosin, no staining effect is produced, unless the saturated eosin is diluted with about five times its bulk of water. By this method the bacteria are stained slightly better than in the manner above described,

the only objection to its use being that the dilute eosin employed (though stronger than the eosin solutions in general use) is incapable of staining the background of the tissue strongly. I have sections stained in this way showing dark blue bacilli on a nearly colourless background.

Moreover, the eosin may be replaced by certain other reagents, which have the property of precipitating methyl blue; for instance, by dilute alcoholic solution of picric acid and by a dilute alcoholic solution of tetrabromofluorescein. This last reagent is made from a strong watery solution of eosin by adding hydrochloric acid, filtering off, and washing the precipitate, and then dissolving it in alcohol.[1]

In the former case I noticed that if the picric acid solution is too strong all the methyl blue is precipitated in the solution, and no staining effect is produced. By diluting the picric acid the bacteria were stained, and by still further dilution of the picric acid no more effect was produced than in the first experiment. These facts seem to show that the precipitation of the methyl blue plays some part in producing the stain.

Most of the aniline dyes in common use are more soluble in alcohol than in water. It occurred to me that if a saturated alcoholic solution was added to a strong watery solution of the same dye a precipitate would occur, and that possibly bacteria present would be stained. I first tried with Weigert's aniline water solution of Spiller's purple, and obtained very poor

[1] See 'The Chemistry of the Coal Tar Colours,' by Benedict and Knecht, published by Bell and Son. Since writing the above I have been looking over some of the first specimens that I made by the eosin and methyl blue method about six months ago. I find that some of them are faded, but in others the bacteria are still dark blue on a pink background. In the latter case, instead of using the eosin solution above described, I had used a mixture of three parts eosin saturated in alcohol, and one part of tetrabromofluorescein saturated in alcohol. When first made the bacteria were stained nearly black.

The bacteria in question were small bacilli, which Dr. Klein found associated with some as yet undescribed disease of a sheep. They were not very easy to stain by this method. Scarcely any anthrax specimens stained in this way show any sign of fading.

results. On adding the alcoholic Spiller's purple a whitish tinge was produced, as if milk had been added to the solution. This I found to be due to minute globules of aniline. I also found that these globules have a great affinity for the dye, for if aniline or any other oily liquid that can dissolve Spiller's purple is shaken up in a test-tube with a watery solution of this dye nearly all the colouring matter is removed from the solution and dissolved in the oily liquid.[1]

I then tried with watery solutions of Spiller's purple, and obtained successful results, in the following manner:

Method B. The materials required are:

(1) Saturated watery solution of Spiller's purple.

(2) Saturated alcoholic solution of the same dye.

It is very important that both these solutions should be saturated. The best way to effect this result is to boil the dye with the solvent used in each case, allow the mixture to cool, and then filter.

(3) Absolute alcohol, benzine and clove-oil mixture, and benzine as in Method A.

(4) Eosin dissolved in oil of cloves made by mixing about as much eosin as can be lifted on the point of a penknife with a watch-glass full of oil of cloves. The mixture should be used fresh, as after standing a quantity of the eosin is precipitated in crystals, especially if any acid fumes or traces of picric and other acids are about.

Modus operandi.—The sections are removed from spirit and placed in the watery Spiller's purple.

At once an equal bulk of alcoholic Spiller's purple is dropped in from a pipette. The sections are then dehydrated in absolute alcohol as quickly as possible and removed to the benzine and clove-oil mixture.

Sometimes it may be necessary to dehydrate, not in alcohol, but in alcohol saturated with Spiller's purple. By this means

[1] All dyes that stain nuclei also stain the globules of an oil emulsion. Hence when staining—at any rate by these methods—care must be taken that no trace of oil of cloves or any other oil is introduced (on needles, section-lifters, &c.) into the staining solution. Mere traces will often spoil the result.

the exit of the dye from the bacteria can be effectually prevented.

When cleared the sections are removed from the benzine clove-oil mixture to oil of cloves containing eosin. The eosin stains the background red and at the same time turns out the excess of Spiller's purple; sometimes a few seconds, sometimes a few minutes, are required to do this. The sections are then washed in oil of cloves, passed through the benzine and clove-oil mixture to benzine and mounted.

The removal of the excess of colour can be greatly hastened by moving the sections backwards and forwards between the oil of cloves and benzine and clove-oil mixture.[1]

I attempted also to stain in this manner with fuchsin and gentian violet, but found that although the sections were left in the dyeing solution only for a few seconds they were hopelessly overstained, and it was impossible to remove the excess of colour by means of oil of cloves.

After some trouble I succeeded in finding out how to avoid this difficulty. The method is as follows:

The sections are subjected to double treatment with fuchsin or gentian violet in the same way as above described for Spiller's purple, then rapidly dehydrated in alcohol and placed in the benzine and clove-oil mixture. They are then removed to oil of cloves in which picric acid has been dissolved. This quickly turns out the excess of fuchsin or gentian violet and at the same time stains the background. The sections are then washed in pure oil of cloves, benzine and clove-oil mixture, placed in benzine and mounted. Or if a green background is preferred to the yellow tint of picric acid the sections may be contrast stained with a mixture of methyl blue snd picric acid dissolved in oil of cloves or aniline. They must then be washed rapidly in benzine clove-oil mixture and placed in benzine.

The only objection to the use of gentian violet by this method

[1] This is really the reiteration of a "tip" kindly shown me by Mr. Lingard at the Brown Institution. He showed me that in staining by Gram's method it is possible to turn out the excess of colour by moving the sections from alcohol to oil of cloves, and back again repeatedly.

is that the bacteria, if at all easy to stain, are dyed black, which result, for obvious reasons, should generally be avoided.

In all these methods of staining, advantage is taken of the well-known fact that benzine does not dissolve, and therefore fixes the aniline dyes. Sections when stained so feebly that prolonged washing in alcohol would render them quite colourless, are placed in benzine after dehydration, and the loss of colour being thus checked, they are mounted as permanent sections. If, however, they were removed directly from the alcohol to the benzine, a precipitate of dye would probably be formed on the surface of the section, which would spoil the result. Hence the necessity for the benzine and oil of cloves mixture, which, refusing on the one hand to dissolve much more of the dye, and on the other to precipitate any, forms a link between the alcohol and the benzine. Sections should generally be placed successively in two or three watch-glasses or capsules full of this mixture before removal to benzine; and in the last of the series they should only remain while they are being spread out on a lifter. By taking this precaution the last capsule will contain little dissolved dye, and all granular precipitate on the section is prevented.

Another advantage in placing sections in benzine before mounting is that any residue of clove oil is removed. By this means the section is far more likely to be permanent than if the excess of clove oil is merely drained off with a piece of blotting paper, as is usually the case.

In using these methods it is necessary to remember that fuchsin and other dyes which show such an affinity for nuclei when dissolved in water or alcohol, act quite differently when dissolved in oil of cloves. Under these conditions they are almost incapable of staining bacteria or nuclei, but the whole of the tissue becomes dyed of a uniform tint. This influence of the solvent in modifying the action of the dye is not of a chemical but rather of a physical nature, for the oil of cloves may be replaced by aniline, creosote, or any clearing agent, with scarcely any alteration in the result.

If a section is placed in water and some alcoholic fuchsin

dropped in, the nuclei and bacteria will be found to have taken the dye. But if the section is placed in an alcoholic solution of fuchsin, and if the dye is precipitated by the addition of a considerable quantity of benzine, the whole of the tissue will be found to be strongly stained a uniform crimson tint. The dye has acted as a background stain. From these considerations it is evident that firstly, after the nuclei or bacteria in a section have been stained with Spiller's purple, fuchsin, or gentian violet, if the excess of colour is being removed by means of oil of cloves, care must be taken to move the sections into a fresh quantity of the reagent as soon as it has become strongly tinted by the dye. And, secondly, after staining bacteria by double treatment with Spiller's purple or methyl blue, they may be contrast stained with fuchsin dissolved in an oily medium. Aniline is the best for the purpose. After leaving in this reagent for about a minute they should be removed to picric acid and clove oil for a few seconds, then washed and mounted. If methyl blue was the dye previously used, the bacteria will be found to be stained green on a brick-red background.

Another method of staining, which, though a little more complicated seems to offer certain advantages, is as follows:

Sections are subjected to double treatment with fuchsin as above described, then washed for about five minutes in picric acid dissolved in oil of cloves; this last reagent is removed by washing in benzine and clove-oil mixture. They are then treated with methyl blue dissolved in aniline oil for about ten minutes. The effect of this is that every structure that was previously stained red with fuchsin is turned black, blue black, or dark purple. This, then, is the colour of the bacteria, and the background tint is green or blue. This can be changed to red by placing the section in oil of cloves and eosin for a minute. The sections are then washed and mounted as in the other methods. Not only do sections stained in this way promise to be remarkably permanent, but the contrast between the dark blue of the micro-organism and the pale pink of the background is as strong as can be desired.

The question arises whether sections stained by these methods

are likely to be permanent. It is well known that, cæteris paribus, bacteria that have been stained for six hours are more likely to retain their colour than those that have been in the staining solution for only a few minutes. Is it then likely that these methods will be permanent, where the whole process takes only a few minutes, and the bacteria are only exposed to the action of the dye for a few seconds? Though the only way to settle this question is to observe how long sections thus stained will last, I thought that some indication of their permanency or otherwise might be obtained by exposing them to the action of sunlight. To do this I fixed up several sections in a window with a southern aspect on sunshiny days and observed the effect of the light at intervals. The results obtained were very different in different cases, but seemed to show that the permanency under these conditions depended rather on the amount of colour that had been washed out of the section after staining, than on the time that the section had been left in the staining solution.

A section that had been stained in gentian violet for twenty-four hours in the ordinary manner was completely bleached by sunlight in three hours. A section stained by the eosin and methyl blue method, and in which all blue colour had been washed out of everything except the bacteria, faded in the same time, while other sections stained by the same method, but in which a trace of colour had been left in the nuclei, stood a whole day's sunlight without much change, so far as the micro-organisms were concerned. Another section which had been stained in this method for histological purposes when exposed to six hours' sunlight lost the colour previously adhering to the nuclei, but left the bacteria present still strongly dyed. It is thus clear that the fastness to light is due to the quantity of dye present in any stained part of the section; and I employed this fact in differentiating out certain bacteria by means of light as follows. I had some sections of the liver of a mouse that had died of some form of septicæmia investigated by Dr. Klein. The sections when stained with Spiller's purple and eosin showed here and there dark blue apparently homo-

geneous masses plugging the smaller blood-vessels and expanding the capillaries. There was absolutely nothing to show whether or not these were masses of bacteria. But after I had exposed the mounted sections to sunlight for a day, the Spiller's purple present in the interspaces between the bacilli was completely destroyed, and the masses that before seemed to be amorphous were now seen to consist of clusters of minute bacilli marked out with perfect distinctness.

Some sections stained by the method last described were exposed to ten days' sunlight during the hottest part of last July, without appreciable change, and they still show everywhere the bacilli of a dark blue black tint, though the background of the tissue has become so bleached as to be nearly invisible. This permanency of the stain produced by methyl blue and fuchsin in combination is remarkable, when it is remembered that manufacturers regard a dye as "fast to light" when a tissue stained by it is unchanged by three hours' exposure.

These methods of staining are generally inapplicable to coverslip specimens. But preparations of anthrax blood or pneumonia sputum, very excellent for demonstration purposes, can be made as follows:

The films are stained with methyl blue or Spiller's purple, washed and dried as usual, and then a drop of eosin dissolved in oil of cloves is placed on them for a few minutes; this is then washed off with clove oil and benzine mixture. This again is removed with benzine and the coverslips are then mounted. Bacteria are seen to be stained blue, red blood-corpuscles are red, pus-cells or leucocytes generally have purple nuclei, and the background has a pinkish tint.

The method of staining by means of eosin and methyl blue gives very good results from a histological point of view, but will only succeed with bacteria that are easy to stain.

The methods of double treatment with fuchsin and Spiller's purple are successful with nearly all bacteria.

Tubercle bacilli, however, cannot be satisfactorily stained by any of these methods.

Illustrations of the Structure and Life-History of Phytophthora infestans, the Fungus causing the Potato Disease.

By

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With Plates XXXI and XXXII.

Some time ago I was commissioned to prepare for the Science and Art Department, South Kensington, a series of drawings illustrating the structure and life-histories of certain parasitic fungi; the intention being that these drawings should eventually be enlarged to form wall-diagrams suitable for museum and teaching purposes. I soon found that if the drawings were to be what I should wish them to be—i. e. to form a properly connected series, and to be taken from original preparations—the task promised to develope into one of greater magnitude than had been anticipated. In fact it became necessary to go over the entire field.

On the completion of the drawings from which the diagrams illustrating the phenomena in the life of the potato disease fungus (Phytophthora infestans) were to be selected, it was suggested to me that they form in themselves a series which would probably be welcomed as bringing together facts about this fungus in a connected form, whereas what other original figures exist are scattered through various foreign periodicals, &c., or are not easily accessible to students.

The text, it will be noticed, applies to the drawings through-

out, any theoretical matters touched upon being referred to incidentally and in connection with points of structure, &c., in question. It has not been my intention to discuss hypothetical matters at any length, and hence the form of the descriptive letterpress.

Fig. 1 was drawn from a preparation made by cutting off the epidermis of the leaf at the margin of a disease-spot, where the tissues are as yet green, and the white aërial hyphæ of the fungus form the well-known velvety "bloom." Two stomata are shown, and a number of intervening cells of the epidermis. The stomata are very wide open, and each guard-cell contains chlorophyll-corpuscles and a nucleus (see the stoma on the right). The vertical boundary walls of the epidermis-cells are sinuous. From each stoma emerge branched aërial hyphæ of Phytophthora infestans, and their continuation into the leaf can be traced for a short distance. The aërial hyphæ branch considerably and produce the ovoid "conidia" at the tips of the branches, the tip often growing forwards and developing another "conidium" before the first one has fallen, and so on repeatedly, as indicated by the peculiar joint-like swellings (see fig. 3 for further explanation of this process). Septa are found here and there below the joint-like swellings; otherwise the hyphæ are not segmented. Conidia in various stages are still attached to the hyphæ, others have fallen or been knocked off during manipulation; such a fallen conidium is seen lying on the epidermis. The preparation was made from a perfectly fresh leaf, and examined in water.

Fig. 2.—This transverse section of a potato leaf was made across a similar part of the disease-spot, but passed somewhat closer to the dark brown part of the spot, where the cells are already dead; this is evident at the right hand of the drawing. The epidermis is very similar on both sides, the stomata being less numerous on the proper upper surface of the leaf. The mesophyll to the left of the preparation is still quite healthy and free from the mycelium of the fungus; the cells (seen in

optical section and in plan) are turgid, and the chlorophyll-corpuscles bright green and sharply defined. A fibro-vascular bundle consisting of a few vessels, wood cells, and soft bast, and surrounded by a sheath, has been cut across transversely; the palisade cells on its dorsal surface are arranged in a peculiar manner, and a depression in the epidermis of the upper side of the leaf corresponds with its course. To the right hand of the section the parasitic mycelium of Phytophthora infestans is rampant, though not as yet abundant in this younger portion of the disease-spot. The tubular, non-septate hyphæ, are seen in the intercellular spaces, but not in the cells themselves. Many of the cells are already losing their turgescence and the chlorophyll-corpuscles are becoming discoloured and misshapen, the protoplasm and cell walls eventually turning brown and becoming disorganised. The hyphæ in the intercellular spaces send branches through the stomata, especially on the lower side of the leaf, partly because that is more sheltered, and there is more moisture, and these branches become aërial hyphæ which again ramify and produce the conidia, as seen in the centre of the figure. The stoma to the extreme right is cut through transversely, but not in the median plane, and the tip of a hypha is already protruding through it; one hypha in the lacuna below has been cut across obliquely, and the extreme tip of another is just making its way outwards towards the orifice of the stoma. The stoma to the left of this is cut longitudinally, and the tips of two hyphæ are about to protrude through to the exterior; the stoma to the left, again, is also in longitudinal section, and two branched conidiophores have passed through and developed their conidia outside. The details of these are similar to those in the preceding figure. To the left of this stoma is one of the numerous capitate hairs, with yellow, oily-looking contents; and still further to the left one of the longer pointed hairs, the outer cell walls of which are dotted with minute papillæ. Similar minute papillæ exist on the outer walls of the epidermis-cells; the enlarged epidermis-cells surrounding the base of the hair are often much larger than in the specimen drawn. A stray

hypha is to be seen between the cells of the spongy mesophyll close to the epidermis at this part: it is cut through. The dark grey cell below the smaller hair is filled with fine granular particles of calcium oxalate. Below and to the right of this cell a hypha is making its way in the spaces between the palisade cells to the stoma (in oblique longitudinal section) on the morphologically upper surface of the leaf. It will be noticed that the cell walls of the epidermis turn brown over the badly infected portions, but, as elsewhere, the discolouration and disorganisation do not result immediately on the access of the mycelium; the process is accelerated or retarded by wet or dry weather—possibly apart from the mere quantity of mycelium, though certainly not independent of its presence.

Fig. 3.—Portion of the tip of a branch of one of the conidiophores which had grown out from a piece of potato-tuber, lying in a drop of water beneath the microscope. The order of development is from *a* to *e*; the conidium marked * is the same throughout. In *a* the tip of the hypha is commencing to swell up, and soon becomes an ovoid body full of fine-grained protoplasm (*b*), still continuous with that of the conidiophore. Soon afterwards the now larger conidium presents a more granular clouded appearance, and its proximal or basal end is cut off from the hypha below by a septum, At its distal end a colourless papilla has appeared, due to the deliquescence and swelling of the cellulose wall at this point. In *d* this conidium is nearly ripe, and in *e* it has just fallen away by the rupture of the short, slender pedicle. Meanwhile, as the conidium referred to was approaching maturity, the hypha continued to grow by bulging out below the septum in *c*, and (in this case) to the right. This pushed the point of union between the above conidium and its hypha over to the left, and thus caused the displacement of the conidium in *d*. In the latter figure the new growing point of the hypha has already swollen up to form a new terminal conidium, which was in its turn displaced (to the left also), as shown in *e*, by a renewed growth, and so on. In each case a flask-like dilation of the hypha

marks the previous place of origin of a conidium, which had been thus developed and displaced.

Fig. 4.—In this drawing the germination of the conidium and of the zoospores to which it gives origin are represented. The conidium (*g*) was one of five which had been isolated in a drop of water, suspended in a damp chamber beneath the microscope. The sowing of conidia was taken from fresh, damp, diseased leaves, and made at 11.35 a.m., and the conidium (*g*) was then drawn. The contents were densely granular, and looked dark grey. The cellulose wall was sharply marked, and the pale papilla at the anterior end distinct, but not prominent. The short, broken-off pedicel was also evident. The first changes of note occurred at about 2 p.m., when the contents appeared much more translucent and watery, the dark, coarse granules being no longer obvious. This seemed to be owing to their having altered in character and become more translucent. The cell wall was also paler, perhaps because the contrast between it and the closely fitting contents was less marked. The apical papilla was thicker, and slightly more prominent. During the next two hours or so (2 p.m. to 4 p.m.) the peculiar hyaline granular contents were obviously undergoing changes, and very slow movements of the translucent granules occurred, producing variations in the faint cloudy markings shown in the drawings (*h* and *i*). Towards 4 p.m. certain vacuoles appeared, and at length became sufficiently distinct to draw (*i*). There were about nine of these altogether. The papilla also became more prominent, as if it was swelling up. At 4.50 it was obvious that each of the vacuoles formed the centre of an angular block of protoplasm, as seen in *k*. These angular blocks were separated by thin, sharply marked plates, and the whole mass was no longer close to the outer wall of the conidium, but a pale, watery looking line lay between them. The apical papilla was still more gelatinous in appearance. That these angular blocks were incipient zoospores was proved by what followed. Their arrangement appeared to be as follows—one at the apex, three in the next tier (two visible in the drawing), four in the next tier (three visible

in the drawing), and one at the base. The drawing was made at the higher focus.

Four minutes after the drawing of *k* was completed the papilla at the apex gave way, and the zoospores glided out as shown at *l*. They appeared to be quite passive, as if being pushed out from behind. Special attention was paid to the fact that they were squeezed through the narrow aperture. On reaching the exterior the zoospores did not immediately move away, but remained some seconds, as if hesitating, as it were; possibly the shock of meeting the water outside affected them. Two of the zoospores remained united for several seconds, presenting a superficial resemblance to conjugating amœbæ; after slight amœboid writhings they separated, but did not move out of the field. The zoospore (*n*) moved away briskly, but came to rest in less than a minute, and close to the now empty conidium, or zoosporangium, as it may be called.

The movements and changes of the other zoospores could not be followed, as attention was devoted to the specimen *n*, the further fate of which was followed, and noted as accurately as possible (see *o* to *z*). Owing to its movements being nearly circular, and its coming to rest close to the empty zoosporangium, it was easy to see the two cilia and two vacuoles (*o*) as the movements ceased. The zoospore then became rounded off and seemed to throw off its cilia—at least I saw one detached (*r*, *s*), and lost sight of the other one. The two vacuoles became smaller and soon disappeared, as if the zoospore in diminishing its volume squeezed out water. As far as it was possible to judge, these processes occupied one minute, and the zoospore had then come to rest as a spherical mass (*s*), which soon clothed itself with a recognisable but thin membrane.

It should be pointed out that the zoospore here followed moved for a very short time compared with others. I have frequently seen the zoospores still active twenty minutes or more after emergence, and they are said to move even longer. At the same time they often come to rest in from one to five minutes, and sometimes only give one little flirt and then come to rest.

The spherical resting zoospore remained unaltered, to all appearance, from 4.56 till 6 o'clock, but soon after that was seen to be putting forth a protuberance (*t*) (6.14 p.m.), which soon elongated into a hypha (*v*) (6.34) as long as the diameter of the zoospore. At 7.10 this germinal hypha was twice the diameter of the zoospore in length, and a large vacuole had formed in the rapidly emptying zoospore (*w*). This vacuole occupied nearly the whole of the cavity at 7.50—in other words, the protoplasm had nearly all passed into the developing germinal hypha (*x*). A tiny protuberance on the hypha also indicated an incipient branch, which, however, did not attain any considerable length, and soon became emptied. At 11.20 p.m. the state of affairs was as shown in the drawing (*z*). The whole of the protoplasm was in the apical one fourth or one fifth of the germinal tube, the rest being empty like the zoospore, and having three very thin septa across at pretty equal distances. Whether these septa are really cellulose walls it was impossible to determine. Next morning there was no appreciable change, and the protoplasm seemed to be dying towards evening.

The other four of the five conidia sown did not develope zoospores; two of them germinated directly in the manner shown in fig. 7. The development of the zoospores is delayed or even arrested by direct daylight, even if not very strong, and it is not improbable that in the present case the formation of the zoospores was arrested in the other conidia by the repeated and continued exposure of the preparation during the observations.

Fig. 5.—In this drawing two zoospores are represented germinating on the epidermis of a potato leaf, and one has become rounded off, but has not yet put out a germinal tube. The preparation was obtained by painting the lower surface of a fresh leaf with a camel-hair pencil dipped in rainwater and then passed over freshly-developed conidia at the margins of disease-spots on other leaves. At the end of twenty hours the leaf was examined, and numerous zoospores were found on the epidermis, many of which had put forth germinal tubes which

were entering the leaf. The drawing is combined from two preparations to save space, the zoospore which is putting forth a tube through the stoma having been observed on a different part from the other—in other words, instead of only one cell intervening between the stoma and the lower of the two germinating zoospores, there were very many. The zoospore close to the stoma simply protrudes its tube into the orifice. The one lower down has germinated on an epidermis-cell, and the tip of the tube at once commenced to bore through the outer wall; once inside, the germinal tube swells up and has in this case branched. The empty remains of the zoospore and part of the tube are left outside. The chlorophyll-corpuscles are shown in the guard-cells, but the nuclei are omitted.

The germination of the conidia on the living leaf is often very rapid, and may certainly take place in two hours after the sowing was made. I could not satisfy myself that it is affected by light, as is that on glass slides. It is accomplished readily during the night, on leaves kept wet under bell-jars. The results seem to be more satisfactory if rainwater is employed, in preference to well-water; and the same is true of experiments on glass slips. It is improbable that temperature was the important factor in these differences, possibly the oxygen present in the rainwater was of more significance.

It occasionally happens that a zoospore germinates in an angle of the venation of the leaf, and sends its germinal hypha through the epidermal cells of the rib or "vein" lying at its side; in such cases an optical section of the tube and zoospore can be obtained, but the best proof of the entry of the germinal tube through the wall of the epidermis-cell is obtained as follows.

Fig. 6.—The preparation is part of a vertical longitudinal section of a young internode of the potato plant, and shows a stoma in longitudinal but not quite median section, and to the right a germinating zoospore, the germinal hypha of which has pierced the cuticle and cell-wall and is growing on inside the epidermis-cell. The method adopted was to sow large quantities of the conidia on one of the flat sides of the tetra-

gonal, winged internodes of the potato plant; several such preparations were then laid with the sowings upwards, on damp blotting paper, in soup-plates covered with bell-jars, and the air kept damp. After twelve hours or longer, sections were cut longitudinally vertical to the flat places on which the sowings were made, and the section examined. It was not difficult to obtain evidence of the germination of the zoospores, and entry of the germinal hyphæ, but it was only after many weeks that I succeeded in preparing the really satisfactory case here drawn. The razor had passed close to but not through the germinal tube; the empty zoospore and first part of its germinal hypha are seen lying close on the exterior of the cell wall—the zoospore had come to rest in a slight depression at the junction of two cells—the very fine hole through which the germinal tube passed was clearly visible on focussing. On reaching the anterior of the cell the hypha thickened considerably, and passed along the roof of the cell, and was just about to turn and run down the vertical wall (or possibly to bore through it) where the section was made. The sub-epidermal cells frequently contain a crimson-coloured sap, but none of the cells in the preparation were so coloured.

Figs. 7 and 8.—When the conidia of Phytophthora infestans are sown in water on glass slips, they frequently assume the appearances figured at figs. 4, *g*, *h*, and *i*, after a few hours, and then cease to develope further in the direction of producing zoospores. Instead of doing that, the cloudy, vacuolated condition of the protoplasm which usually heralds the development of the zoospores (fig. 4, *i*) is again replaced by the more uniformly hyaline appearance of the earlier stage (fig. 4, *h*), and the papilla either commences to grow out as a hypha, or a protuberance from it (fig. 7) does so; occasionally, but rarely, this hypha branches, as in fig. 8. This germinal hypha, the development of which stamps the conidium as an ordinary spore (in contrast to its behaviour in other cases as a zoosporangium) elongates considerably, and in some specimens attains a length equal to ten times that of the conidium; its apex then dilates into an ovoid body much like the conidium from which it originated (fig. 7).

In one instance (fig. 8) this took place at the end of each of the two branches. The protoplasm of the original conidium passed entirely into the hypha, and along it (fig. 7) wholly into the new or secondary conidium, which is usually somewhat smaller and sometimes much smaller (fig. 8) than the primary conidium. One or two fine transverse septa may be formed in the germinal hypha (fig. 7). As a very general rule the secondary conidium is oblique or misshapen; fig. 7 was an exceptionally symmetrical one.

The conditions which determine this mode of germination, in preference to the formation of zoospores directly, are not quite clear; but they seem to be connected with the nutrition of the germinating conidium. When the sowings of conidia are exposed to light these secondary conidia are often formed; and I found it more difficult to obtain the zoospores in well-water than in rainwater. I here speak more especially of water from a particular well, which has proved to contain considerable quantities of organic matter. In large sowings, i. e. where eighty to hundred or more conidia existed in the drop of water, by far the majority of the conidia germinated in this manner; and wherever the germination was delayed from obscure causes beyond twelve hours, this was the prevalent form it assumed. Sowings in very dilute infusions of organic matter (jam and horsedung were tried) never yielded zoospores, whereas several conidia would germinate like this. When large quantities of the conidia were sown on a small area of the potato leaf or stem, a larger portion of them germinated thus (see fig. 9, below). No connection was established between differences of temperature and of mode of germination. Putting all these facts together, it seems not improbable that the difference is due to nutrition, and possibly three factors were concerned in affecting this, (1) the amount of free oxygen available, (2) the comparative maturity of the conidia themselves, and (3) the intensity of the light. It is not inconceivable that direct sunlight increases the oxidizing processes during the early stages of the germination; and I feel convinced that the presence of numerous competing conidia, or of organic matter generally,

affects the germination by influencing the amount of oxygen available for any one conidium. I am satisfied that it is easier to obtain zoospores in dewdrops on the living leaf than in water on glass. Nevertheless, it must not be overlooked that the zoospores will develope on a wet leaf during the night, including the early hours of the morning. There can be little doubt that an interesting field of investigation in comparative physiology is here open.

As to the significance of the secondary conidia and their formation, I have found them empty, as if they had developed zoospores which had escaped; but have never seen zoospores come from them. I have also seen a secondary conidium with what looked like a germinal hypha developed from it; but this died before developing very far, so that it was impossible to say whether a tertiary conidium could be developed. I have not seen these tubes enter a stoma. The secondary conidium would seem to be a second attempt on the part of the zoospore-producing protoplasm to prepare for the development of zoospores again; there is a loss of substance from respiration, and the cellulose of the hypha has to be formed, and the energy expended is no doubt evolved at the expense of materials in the protoplasm, and not replaced by nutrition.

Fig. 9.—This drawing was from a preparation of the epidermis of an internode on which several of the crowded conidia had germinated as above. The drawing is to scale. As usually happens in such cases as this, the germinal hypha is shorter than those developed in hanging drops, and the secondary conidium oblique.

Fig. 10.—The preparation shows hyphæ of the parasite in the cortical tissues of the internode three days after infection. The chief point of interest is the course of the hyphæ, they run between the thin-walled, closely-fitting cells, in the middle lamellæ, and even push aside the other layers of the cellulose walls; the diameter of the hypha is considerably greater than that of the walls they traverse. The same thing occurs in the tuber (see fig. 15, below). The hyphæ branch often; they are devoid of septa and have very thin walls and abundant finely

granular protoplasm. In the preparation drawn the hyphæ are particularly luxuriant and thick, the diameter varying, however, in different parts of their course. Several interesting questions here suggest themselves. How are these hyphæ nourished in the "middle lamella"? Is any protoplasmic substance present? Processes are very rarely sent into the cells of the cortex; the branches in the upper part of the drawing are running over the cells and in the middle lamella, between the cell which is drawn and one that would have lain nearer the observer. The longitudinal hyphæ often run for some distance in the line of junction between three cells. The cells contain pale chlorophyll-corpuscles; a nucleus is present in the one to the left, and a few crystals lie in the large cell to the right of it.

Fig. 11.—A potato-tuber was sliced across, and the mycelium of Phytophthora, from a diseased potato planted on the clean cut. The preparation was then put aside, and was not kept particularly damp. On examining the sections cut vertically to the cut surface, some time later, the mycelium was seen attacking the tuber, as in the drawing. The cut surface of the wound (at the top in the drawing) had undergone partial healing by means of tangential divisions of the exposed cells (the rotten remnants of the cut cells were destroyed), and the two or three tiers of cells thus cut off had become cork-like. The contents of the cells were removed to a large extent in making the preparation. The protoplasm or its remains turns coarsely granular, and eventually rusty brown; the starch-grains are dissolving, remains of the nuclei are found as opaque, coarsely granular masses, and here and there a crystal is noticed. The mycelium is strictly intercellular, the branches running between the cells in the narrow lacunæ, or in the substance of the walls. Eventually the cell walls soften and swell, and split apart, and finally turn rusty red and decay.

Figs. 12 and 13.—Cells of a diseased potato in a somewhat more advanced condition. In addition to the changes above referred to the cell walls are now swelling, and the cells separating from one another at the middle lamella. In the pre-

paration from which fig. 13 was drawn it was easy to see the remains of the protoplasm as a sort of matrix in which the starch-grains (some partially eroded) were embedded. The nucleus and proteid seem to be destroyed long before the starch, and it is even doubtful whether the starch is directly attacked at all until bacteria gain access, and hasten the decomposition. In fig. 13 starch grains are seen to be displaced from the matrix in which they were lying.

Fig. 14.—Portion of mycelium from such a preparation as the last. The two branches running across from the vertical ones were passing along the surface of a cell, and a brownish tinge was given to the cell wall in the immediate neighbourhood. The same is seen in fig. 15, *a* and *b*. The hypha corrodes the walls, as it were, in its immediate neighbourhood: the rest of these cell walls were as yet not coloured.

Fig. 15.—Portions of hyphæ in the middle lamella between the cells of the potato tuber. The corrosive action of the hyphæ is indicated by the rusty hue which they cause the cell walls to assume.

On the Formation and Liberation of the Zoospores in the Saprolegnieæ.

By

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I. The Formation of the Zoospores.

The zoosporange of the Saprolegnieæ has long been a favourite object for the study of cell development; but only within the last few years has an insight been obtained into the great complexity of the phenomena attending the formation and the liberation of the zoospores. These were first described by Büsgen ("Die Entwicklung der Phycomyceten-sporangien" in 'Pringsheim's Jahrbücher,' vol. xiii, 1882);[1] and, a little later, independently by Marshall Ward ("Observations on Saprolegnieæ" in 'Quarterly Journal of Microscopical Science,' N.S., vol. xxiii, 1883).

The following is an abstract of the nearly concurrent results obtained by these two observers. As is well known, the zoosporange is formed by the enlargement of the end of a hypha. The protoplasm streams into this enlargement and becomes dense, its transverse septum then isolates the zoosporange, which contains one large centre vacuole, or, in Achlya, often several small ones. A blunt beak-like enlargement forms at an undetermined place (usually the apex), and through this the spores are finally emitted.

In Achlya the following processes occur. There appears in

[1] This paper contains an excellent summary of the literature of the subject.

the protoplasm a network of lines, formed of fine granules, and marking out the protoplasm into polygonal areas. These lines broaden out, and become converted into clear bands, which slowly swell up "like transitory cell plates." This we may term the first stage of preliminary division.

The second or homogeneous stage consists essentially in the almost instantaneous disappearance of the clear bands; the protoplasm becomes lighter and homogeneous; the central vacuole or vacuoles disappear at the same time that the basal septum, hitherto concave towards the sporange, now bulges in and becomes convex, showing that the turgescence of the sporange has diminished. This stage hardly lasts more than half a minute, and passes on to the

Third stage, that of the shifting vacuoles. The protoplasm loses its homogeneity owing to the appearance of a number of minute vacuoles, some of which would seem to occupy the centre of the meshes bounded by the network of the first stage, others to lie along the lines bounding them. These vacuoles come and go, fuse or disappear, and reappear. This stage gradually passes into the next.

Fourth stage of final division. Now, as the protoplasm shrinks from the wall, leaving a clear space interpreted by Büsgen and De Bary as a substance, new lines appear, clearly marked and more numerous than in the preliminary division. They are the optical expression of the planes separating the zoospores, which now contract, round off, and escape a little later.

These are the facts as described by Büsgen and Ward, and as accepted by De Bary, their master, in his great work on Fungi. In the above summary I have alluded to the fact that Büsgen regards the lines of the preliminary division as rudimentary cell plates. Ward explains them as nuclear plates. Büsgen and De Bary seem to consider them to be the ultimate source of the expulsive substance lining the sporangial wall in the last stage, which, swelling in water, would determine the expulsion of the zoospores: they deny the existence of the flagella on the sporangial zoospore of Achlya as described by

Cornu in his classical memoir, so that, for this genus, the existence of an expulsive matter would be essential to liberate the zoospores. In Dictyuchus (genus), Saprolegnia, and Leptomitus the processes and stages differ only in minor details from the above.

In July, 1884, I had the opportunity of going over this matter in Strasburg, under the direction of the same illustrious botanist, Prof. A. De Bary, and with the kind help of Dr. Büsgen. I had in hand a species of Saprolegnia which we were unable to determine, as during the intense heat it formed no sexual fruit. I anticipated nothing more than the confirmation of my predecessors' results, but to my surprise I found an undescribed phenomenon at the homogeneous stage. As soon as it came on, a crowd of bacteria swarmed from all parts to the neighbourhood of the sporange, and executed a vigorous dance there till the conclusion of the stage, when they dispersed. Inferring that some excretion must take place thus to attract the bacteria, I put in the eye-piece micrometer, and found indeed that the sporange narrowed greatly, by as much as one seventh of its previous calibre. This unexpected result set me thinking, and on my return to Cork I took up the study afresh. Here I have only obtained two species: Achlya polyandra, and a form with the constricted hyphæ of a Leptomitus, but which seemed to be identical with Saprolegnia Thuretii,[1] sent to me recently by Prof. De Bary's kindness. Though these forms were convenient for study and easily cultivated, they just failed to show the marked contraction so interesting in the undetermined Saprolegnia. I now proceed to describe the chief new points I have made out.

In the Leptomitus form, and in starved sporanges of Achlya, where a narrow layer of parietal protoplasm surrounds an immense vacuole, it is easy to study the real signi-

[1] I now think I must have contaminated my culture of S. Thuretii with my Leptomitus form, which I hence regard as distinct, and define thus: Saprolegnia corcagiensis (n. sp.), diplanetica, habitu, constrictionibus, zoosporangiis que omnino Leptomiti lactei, polygama, oogoniis fenestratis.

ficance of the lines of preliminary division. They are the optical expression of thinnings of this parietal layer between prominences rounded towards the vacuole. These prominences enlarge, and the protoplasm aggregates more and more in them as they become nearly hemispherical; and the intermediate protoplasm becomes thinner and thinner, so as to give the impression of clear spaces in surface view; but in optical section it is easy to assure oneself that the protoplasm lining the sporange wall is everywhere continuous and closely applied thereto. Careful focussing everywhere shows the continuity of the clear bands and the vacuole. The granules which first marked out the lines of demarcation in Achlya do not disappear; they form a layer at the edge and over the free surface of each hemispherical prominence, and are seen as lines bounding it in plan and in optical section.

When the sporange of Achlya is normal the central vacuole is replaced by several, owing to the abundance of protoplasm; and these in the first stage become converted into a continuous system of lacunæ. The inner masses of protoplasm are all connected by thinner bands.

In the narrower sporanges of Leptomitus there is no room for a double row of prominences; hence in section they project alternately, and the central vacuole becomes zigzag. Here it is easy to see that the lines or bands of the preliminary division are merely thinnings of the protoplasm.[1]

In the undetermined Saprolegnia the central vacuole persists, communicating, I think, with a lacunar system of spaces in the thick parietal layer of protoplasm, which includes several layers of prominences (or rather aggregations of protoplasm).

The homogeneous stage consists essentially in the swelling up of the protoplasm and the loss of its resistance to osmosis. On examining a normal sporange of Achlya, and carefully focussing a lacuna with a high power (Zeiss E $\frac{1}{9}''$ for instance), we see at the onset of this stage that the margins of the lacuna advance and meet from the angles inwards until the space disappears completely, much in the same way as the con-

[1] As indeed figured by Büsgen (op. cit., T. xii, figs. 11, 12).

tractile vacuole of an Amœba is seen to disappear. In a starved sporange the protoplasm contracts into a bossed gut-like mass towards the centre of the sporange; for the cell wall is rather thickened and rigid, so that it cannot present the contraction so marked in the undetermined Saprolegnia and in a less degree in the Leptomitus form (which, however, is usually too narrow for easy measurements). I have tried to account for the causes of this curious phenomenon, of which I have just given the first complete description. It occurred to me that the following was a possible explanation. The protoplasm is acted upon in two ways: 1. The tendency of protoplasm to stick together into a single mass. 2. The tendency to aggregate around numerous centres (to form the prominences), aided by the turgescence of the sporange. If then the thinning at the intermediate bands went on to complete rupture at any one point, the turgescence would be lost; so the first force would overcome the second, now left unaided by the turgor lost for the moment, and would thus lead to homogeneity; though the second force ultimately gain the upper hand in the next and last stage. In this case loss of turgescence should always bring on homogeneity. I tried to induce loss of turgescence by De Vries's method of plasmolysis with solutions of cane sugar and of saltpetre. I found, however, that plasmolysis to a very considerable extent was not sufficient to induce homogeneity. Hence the loss of turgescence must be a concomitant or follower of homogeneity, and not its cause. The explanation had seemed so simple that I was much disappointed at having to give it up; but the facts were too strong.

I then experimented with the aqueous solution of eosin, which De Vries has shown does not diffuse readily through the "Hautschicht" or external layer of protoplasm, nor its internal layer, the "vacuolar wall." The protoplasm only stains readily at the stage of homogeneity; whence we may conclude that at this stage the resistant layers do not exist, at least as continuous layers. I think it probable that the Hautschicht and vacuolar walls break up at this stage, and become re-

constituted later on, and that herein is the true essence of the homogeneous stage.[1] Probably, also, the stage of shifting vacuoles is due to the reconstitution of these resistant layers.

As to the nuclei which exist in the first stage (of preliminary division),[2] Büsgen adduces facts which make it probable that nuclear multiplication takes place during the homogeneous stage. The observation of the nuclei is extremely difficult, and I am still seeking a satisfactory technique for the pursuit and elucidation of this division of the subject.

II. The Liberation of the Zoospores.

As already stated, the emission of the zoospores has been ascribed by previous observers to the secretion of an expulsive matter in the sporange, which swells up in the water to expel them. Now, if such a substance existed it should be visible by some difference of refrangibility or staining in the sporange or outside. But there is no matter lining the sporangial wall that will stain in any reagent, or refract differently to the water of the preparation. I have tried aniline dyes, hæmatoxylin, picrocarmine, before and after fixation by osmic acid, picric acid, absolute alcohol, and obtained no sign of its existence. There is no streakiness in the water (even on staining) at the emptying of the sporange. In this case we may fairly say, "De non apparentibus et de non exsistentibus eadem est ratio." But if we follow the process of expulsion fully and minutely, we shall be led to another explanation, admissible as involving a vera causa: acceptable, as covering all the facts. To understand it we must review in detail the processes of the definitive separation and emission of the zoospores.

The protoplasm which hitherto filled the beak usually forms

[1] I must here note that in Leptomitus the first lines of demarcation never wholly disappear; the homogeneity is never absolute.

[2] Whence Marshall Ward's identification of the lines as nuclear plates is inadmissible. His words are rather ambiguous, "A phenomenon of nuclear division in which the cell plate first formed becomes used up again" (l. c., p. 286).

at least two zoospores, which, as they round off, become too large for the calibre of the beak and retire from it. The end wall of the beak, convex outwardly, is now seen in optic section to be menisciform, thickened in the middle and thinning off at the edges; and it presents that peculiar brilliant lustre which is so characteristic of diffluent or collenchymatised cell walls. And, indeed, it does shortly disappear, a phenomenon which De Bary ascribes with great plausibility to the secretion of some ferment. In some cases, especially in the undetermined Saprolegnia, we may actually perceive the disappearance, followed by the immediate outrush of the zoospores; but usually in Achlya and Leptomitus the foremost zoospore enters the beak, and closes up against its end wall, which, possessing the same refractive index, ceases to be distinguishable. In this case the outrush of the zoospores is the sole indication of the dissolution of the end wall of the beak. As soon as the way is clear, the zoospores crowd to the opening, closely serried, leaving a clear space along the side wall of the sporange, and giving at first sight the impression that they are indeed pushed by such a vis à latere as the expulsive matter of De Bary would exert. They force their way through the opening, often becoming constricted as they do so, and emerge obtusely pyriform or of a stumpy "biscuit shape," with the anterior end the narrower, and possessing two flagella (tractella). The hinder ones in the sporange, as room is made for them, also lose their rounded or polygonal form, and assume this. In Achlya, as the zoospores emerge, they remain near the entrance, grouped in a hollow sphere, their narrower rounded ends turned in towards the centre. Each new comer presses in between the others, so that the sphere grows in size till the zoospores have all settled there.

The outrush of the spores, so rapid at first, is seen to slacken after some time, and then we can note more readily the real mode of procedure. A man up in a balloon, observing a crowd at the doors of a theatre, might well regard the inrush of sightseers when the doors open as the expression of a vis à tergo; but he can correct his judgment by observing the behaviour of

the isolated later arrivals. We may often see in the half-emptied sporange a file of say eight or ten equidistant zoospores going towards the opening; the hinder ones move leisurely enough, keeping their distance; the front ones quicken up their motion and lengthen their distance as they get to the mouth, and leave it with a run, like a late arrival when he is stimulated by coming in sight of the theatre. At length, when there are but two or three left in the sporange, they may be seen to move to and fro leisurely, as if careless of any goal, till when they happen as it were to get towards the apex; then they too quicken speed and go out, but less fast than in the earlier stages of emission, and so finally leave the sporange empty. Only when the water is not well aerated a number of zoospores may remain inside.

We now turn to those that have left the sporange in Achlya, grouped in a sphere outside. Each revolves on its long axis for a short time, then goes to rest, rounds off and becomes encysted in a cellulose wall, closely united with its neighbours. Sometimes, however, a few zoospores of Achlya may escape from the sphere and swim off a short distance to turn on themselves for a short time (sometimes becoming amœboid), round off, and encyst quite isolated. As these motions clearly indicated a motor organ, I used the usual reagent for cilia and flagella, iodine solution, which at once demonstrated the flagella in the moving zoospores, inside or outside the sporange, as seen by Cornu and denied by Büsgen and De Bary.[1]

[1] Cornu's words are most explicit. "Le trait d'union entre les Saprolegnia et les Achlya a cependant échappé jusqu'ici à tous les botanistes.

"Les zoospores sont de deux sortes, comme chez les Saprolegnia [italicised in the original]. Les premières, au lieu de se mouvoir pendant plusieurs minutes ont juste assez d'agilite pour gagner l'ouverture du sporange; elles sont munies de deux cils antérieures, visibles dans les conditions favorables. Elles adhèrent les unes aux autres en général par le moyen de ces cils. . . . Au bout de ce temps [three or four hours] elles présentent, soit le premier mode de germination, qui consiste à s'allonger en filaments, soit le deuxième, et émettent alors des zoospores de deuxième nature" ('Monographie des Saprolegniées,' p. 11).

In the face of this clear and detailed statement by so trustworthy an inves-

In Leptomitus and Saprolegnia the flagella are easily seen even in the sporange. In these forms the zoospores, instead of assembling in a hollow sphere at the mouth of the sporange, swim away freely in all directions for a few minutes, and then encyst after the fashion described for Achlya. In all these genera the cyst opens after a few hours and the zoospore leaves in a different form, kidney shaped, with two flagella diverging from the notch, one anterior (tractellum) and one posterior (pulsellum). This phenomenon of two distinct mobile conditions to the zoospore separated by an interval of rest, has received the name of Diplanetism. It is obvious that Achlya is also diplanetic.

We have now to consider the full explanation of the outrush, which study has already led us to regard as really due to a vis à fronte, an attraction outside the sporange. No expulsive matter could produce the exit of the last few zoospores nor effect the acceleration of their movement near their mouth. Now, Engelmann and Pfeffer have by their brilliant researches familiarised us with the action of chemical stimuli. The swarming of the bacteria at one stage, evidently due to such a stimulus, led me to undertake this research, and I must invoke the theory again at this point. Saprolegnieæ are among the most aerobic of plants; their culture only succeeds when the water in which they grow is kept constantly oxygenated. When the oxygen is used up, the hyphæ and young sporanges become deformed; the mature sporanges open by the disappearance of the end wall of the beak; but the zoospores remain inside; they encyst there and form the so-called "Dictyuchus state," which never occurs in well aerated cultures of the above three genera.

It is obvious then that oxygen dissolved in the ambiant water exercises the stimulus which is the true source of the liberation of the zoospores. That such a stimulus is sufficient to account for the squeezing out is obvious from the observation

tigator, it is astonishing that such excellent observers should have denied the existence of the flagella, without exhausting every means of ascertaining if they were there.

of Juranyi, cited elsewhere by Marshall Ward, that in Œdogonium the "relatively large antherozooid forces its way through an aperture too small for it to reach the attracting oosphere."

The exit is so rapid at first because of the contrast between the external medium and the small amount of liquid within the sporange, vitiated by the close-packed thousands of zoospores, and with its gases slowly changed through the sporangial wall, and because of the immense number of zoospores, all influenced at once by the stimulus. Later on the contrast is lessened, partly by the exit of so many zoospores, partly by the influx of aerated water from without to occupy the room left by their exit. Only near the very mouth of the sporange is the contrast marked enough to accelerate the pace of the foremost zoospores.

But when the water is left unaerated there is no difference as regards oxygenation between the inside of the sporange and the surroundings; the beak may open, but the zoospores, feeling no attraction to without, stay where they are, and the Dictyuchus state is produced; or, if the aeration be imperfect, only some of the zoospores leave the sporange till those within are no longer attracted and remain inside.

Finally, we may note that this is only one instance of the extraordinary susceptibility of this group to chemical stimuli. Others are well known, such as the growth of the hyphæ of germinating zoospores (especially in the Dictyuchus condition) towards food material, the germination of the oospores only in presence of food material, the growth of the antheridial branches towards the oogonium, &c:

The following is an abstract of the chief points I claim to have established:

1. The clear bands of the first stage of the zoosporange are neither cell plates nor nuclear plates, but thinner parts of the protoplasm due to the aggregation of the greater part thereof around distinct centres.

2. At the homogeneous stage the protoplasm acquires an extreme perviousness to liquid; this is probably due to the temporary loss of the resistant layers (Hautschicht, vacuolar walls) as continuous layers.

3. The homogeneous stage is accompanied by a loss of turgescence, and in many cases by a marked contraction of the sporange.

4. The clear spaces seen in the final separation are merely the watery liquid of the sporange between the contracting zoospores, and do not represent expulsive matter. No such expulsive matter exists.

5. The sporangial zoospores of Achlya possess at their exit the two tractella described by Cornu, just like those of Saprolegnia and Leptomitus. Achlya is therefore diplanetic.

6. The escape of the zoospores is not due to any such expulsive matter as has been assumed, but to the chemical stimulus of the oxygen in the medium acting on the auto-motile zoospores.

It would seem probable that the escape of the protoplasm from gonidia of so many Peronosporeæ (Phytophthora and the plasmatoparous Peronosporæ, for instance) is due to the same chemical stimulus of well-aerated water. There is no evidence for the existence of an expulsive matter in the sporange or spore of any aquatic fungus. Sporangial walls being diffusible to water and gases it is obvious that the conditions of the constantly immersed sporange are totally different from those of the aerial ascus of the higher fungi, where such a material does certainly exist.

The above observations were chiefly made on plants grown on mealworms in tumblers, and floated out on large glass slides for observation, seldom covered, and replaced in the tumblers afterwards. In some cases I have used small cultures in the hanging drop with the cardboard or blotting-paper moist chamber.

P.S.—A suggestion as to the physiological value and the filiation of diplanetism, &c., may not be out of place. The zoospore on leaving the sporange has enough reserve material to carry it a certain distance, and to enable it to germinate. In the first swarming the zoospores get scattered; and then during the long stage of encystment the further work of dis-

semination is effected by the movements of the water at no cost to the organism. And we must bear in mind that a considerable amount of dissemination is needed, lest the zoospores should be uselessly attracted to the host on which their parent thallus is living.

In Achlya the limited first swarming forms a sort of globular colony of resting spores, easily broken away as a whole by a slight impact, as of a dead animal floating down stream. The swarmers when liberated form a host of invaders effecting rapid and complete infection, which isolated ones might fail to do.

In Dictyuchus (gen.) the resting state of Achlya is reached without the expenditure of energy required to swim to the outlet of the sporange. A further economy is thus effected.

The Termination of Nerves in the Liver.

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With Plate XXXIII, figs. 1 to 6.

After the completion of my studies on the termination of nerves in the cutaneous epithelium of the tadpole, I began investigations on the distribution and arrangement of nerves in other organs, and have now arrived at what I consider important results, more especially in the case of liver. That of man was first employed at the outset of the investigation, but I soon perceived that on account of the small size of the cells here I would have to resort to some other Vertebrate for control purposes; not that the liver of man does not yield definite results, but that these might always be open to doubt if taken alone. Fortunately at that time there were a number of Necturi in the Laboratory Aquarium, and to these I resorted, on the advice of Professor Wright, obtaining from them my most valuable preparations of the liver. The hepatic cells in these are from two to four times in diameter those in man. It is obvious, therefore, that for ascertaining the relations of nerves to the hepatic cells the liver of Necturus (=Menobranchus) is the most favorable that can be at the disposal of any histologist.

I made preparations also from the livers of the dog, rabbit, and frog, which turned out to be of but indifferent value, and recognising that the narrower the field of investigation is the more could attention be bestowed on the necessary details of technical manipulation and of observation, I devoted nearly the whole of my time to winning successful results from the livers

of man and Necturus. There is besides another justification for narrowing the range of the work as I have done, namely, that one of the highest and one of the lowest Vertebrate types are embraced in the investigation. I do not wish to be understood as believing that the results which I here advance are typical of every Vertebrate liver. Indeed, the following pages show a not very close agreement of results from the two types, and it would be hazardous to say which presents the form of nerve termination which has the most general occurrence in other Vertebrate livers.

I may be allowed to insist on one point about which the vaguest opinions are allowed to pass currently as correct: the hepatic cell and nerve-tissue are in close connection, not merely by contact, but by actual union.

The literature on this subject, what little there is, is full of contradictions or negative statements. Pflüger, the first observer in this line, came to definite conclusions, it is true, but although experimental physiology has partially confirmed his view, taken as a whole and not in detail, yet the workers since that time who have published descriptions of their researches on the nerves of the liver have found no such connection between these and the hepatic cells as he describes, or, in fact, none at all. The reason for these contradictory results partly is that in nearly every case the researches were based on the Mammalian liver, the cellular constituents of which are too small to admit of definitely deciding so difficult a question.

I proceed now to give a résumé of the literature on the subject, coupled with a description of the methods employed in each case. A reference to these methods is necessary in order that I may briefly outline their advantages and disadvantages.

Pflüger[1] used osmic acid to determine the course of the nerves. He found them rarely single, often in bundles, each single fibre dividing frequently and anastomosing, and finally penetrating the membrane of the liver-cells in order to terminate in the latter. The fibres retain their myeline investment up to the point of penetrating the cell. The fibres in the

[1] 'Archiv für die ges. Physiologie,' ii, 1869, also 1871.

interior of the cell terminate in a series of fine fibrils with regularly placed granules or swellings along the course of each.

Hering[1] found a rich supply of nerve-fibres entering the portal canal and branching with the vessels running in Glisson's capsule. Only a few were medullated, the finest bundles containing only non-medullated fibrils. Hering was unable to trace any nerves into the hepatic lobules.

Nesterowsky[2] injected the vessels of the cat and dog with coloured glue, and left sections of the organ so treated in a $\frac{1}{4}$ per cent. solution of gold chloride for twenty to twenty-five minutes, after which he put them in a weak solution of glycerine acidified with acetic acid, till they took a violet colour, which usually happened in five to fifteen days. In some cases he added a little of a solution of ammonium sulphide in order to bring out the nerves more prominently. He found branches of the portal vein surrounded by a plexus of coarse and fine nerve-fibres. Out of the coarser plexus arise fine anastomosing fibres, forming loops; they enter the lobules and closely twine about the blood-capillaries. Nesterowsky never observed even a connection between these nerve-fibres and the hepatic cells. He could not determine whether the nerves were medullated or not, although he thought he saw in one case examples of the former.

Kupffer[3] followed Nesterowsky's methods, and came to the conclusion that the fibres considered by the latter as nerves are simply those of connective tissue. He treated sections of the liver obtained by means of a Valentine knife with weak chromic acid solution (0·05 per cent.) and then left them for several days in a 0·01 per cent. solution of gold chloride, when they attained a red or violet colour. By means of this method he demonstrated the so-called "stellate cells," and at the same time found that the tissues immediately about the central vein of the lobule acquired a violet tint, a fact which indicated, he first thought, the presence of nerve-fibres, but he afterwards

[1] 'Stricker's Handbuch,' p. 452, Leipzig, 1871.

[2] "Ueber die Nerven der Leber," 'Virchow's Archiv,' Bd. 63, p. 412, 1875.

[3] "Ueber Sternzellen der Leber," 'Arch. für Mikr. Anat.,' Bd. xii, p. 353.

considered the structures in question to belong to connective tissue, since they acted towards a solution of nickel oxide in ammonia like the latter, and as he found the same sort of fibrils directly entering the lobules from the hepatic serosa.

Kolatschewsky[1] used two methods. In one, fine sections of the liver were pencilled out and treated for ten to twenty minutes with $\frac{1}{4}$—$\frac{1}{8}$ per cent. solution of gold chloride; these, put in water acidified with acetic acid, were left there for one or two weeks exposed to the light until they became coloured rose violet. According to the other method, sections of liver hardened with $\frac{1}{10}$—$\frac{1}{15}$ per cent. solution of ammonium bichromate were pencilled out and placed in a solution of the double chloride of gold and sodium of the strength recommended by Gerlach. The reduction is accomplished as in the first method. By these methods he found deeply coloured fibres running in the interlobular spaces and entwining ultimately about the capillaries of the lobules. Some of the fibrils end in the nuclei on the capillary walls. The fibres branch, enter into the depth of the lobules, and form there plexuses of fibrils running parallel to and around the vascular channels. The smaller the capillaries the narrower are the meshes of the plexus. Kolatschewsky was not certain that these fibres are nerves, and he never saw their connection with the hepatic cells, if such occurred. His results agree in the main with those of Nesterowsky.

Holbrook[2] made sections of the fresh liver when it was frozen, which he left in a $\frac{1}{2}$ per cent. solution of gold chloride for thirty to forty minutes. The reduction of the gold was accomplished with formic acid. In some cases he hardened the tissue first of all with chromic acid, and then used the foregoing method. He found the nerves in the portal canal provided with a large number of nuclei and occurring usually in bundles of from three to five fibres, which enter the lobules and branch at acute angles along the capillary channels. The finest nerve-fibrillæ

[1] "Beiträge zur Histologie der Leber," 'Arch. für Mikr. Anat.,' Bd. xiii, p. 415.

[2] "The Termination of Nerves in the Liver," 'Proceedings American Society of Microscopists,' p. 95, 1882.

are found running around the capillaries between these and the hepatic cells. They touch, pass between, but do not enter the latter as Pflüger maintains. Holbrook asserts that the fibrils are connected with the cement substance or protoplasmic bridges between the cells, and thereby with the outer portion of the cell reticulum. He also corroborates the results of Nesterowsky's researches.

Methods.

To demonstrate nerve-structures in the liver of Necturus the method employed was as follows: Pieces of the liver were hardened for a week or more in Erlicki's fluid, or for several days in a $\frac{1}{6}$—$\frac{1}{5}$ per cent. solution of chromic acid. After the hardening was sufficiently completed in alcohol, sections of the frozen tissue were made with a Cathcart microtome. These, when the gum was carefully removed, were put in a weak solution of formic acid (5 per cent.) for an hour, transferred to a 1 per cent. solution of gold chloride for about twenty minutes, then washed in distilled water, and the gold afterwards reduced in the dark with a 10 per cent. solution of formic acid. About thirty hours sufficed for this reduction when the temperature of the room was 20° C. The sections then had a deep red colour, but sometimes the tinge was violet. The chromatine of the nuclei of the hepatic cells took a deep blue violet tint, the caryoplasma light violet, while the cytoplasma came out very distinct as a meshwork with a pink or light carmine colour. The nerve-fibres appeared deep violet, but the connective tissue of the interlobular spaces attained a light red, sometimes a deep red colour.

When chromic acid was used as a hardening reagent the addition of any organic acid at the same time, such as acetic acid more especially, seemed to me to have the effect of robbing the nerve-fibres of their selective capacity for gold, while it increased the effect of the latter on the remaining constituents of the liver.

I do not know whether chromic acid or Erlicki's fluid offers in the method described more advantages. If there is any advantage at all it is to be obtained from the former reagent,

as with it one is apt to get beautiful preparations of the liver in which the gall-capillaries, gall-ducts, blood-capillaries, the nerves, and the elements of the hepatic cells and their nuclei are demonstrated in a way that I have found equalled by no other method of manipulation. The value of chromic acid and gold chloride in this respect I shall refer to again in a subsequent paper.

Sections of the liver of Necturus are not of any value when they are of less than 0·020 m. in thickness, that being less than half the average diameter of the hepatic cell.

In the case of the human liver chromic acid was the only reagent used in hardening. The sections were made with the paraffin method, and were subsequently treated in the manner already outlined. I found that uniformly thick or uniformly thin sections did not answer well, for in these either but short pieces of nerve-fibres or fibrils could be seen, or else they were obscured by the thickness of the section. I managed to obtain sections about half an inch square, which had a thickness at one edge two to three times greater than at the opposite one, so that the thickness decreased gradually from one edge to the other. With these sections I was able to see and follow a fibre in its full extent, together with its divisions or branchlets, and thereby gained all the advantages of a thick and a thin section, with the faults of neither so far as tracing the nerves is concerned.

The success of the preparations of the human liver was the exception and not the rule. About 10 per cent. or at most 20 per cent. of them only were valuable for all the purposes for which I made them. Sections from the same piece of liver, when treated under exactly like conditions but in different dishes, proved to be not equally successful, some being indifferent or worthless. Why this is I do not know. In the case of a very strong colouring with the gold so much as to obscure the structure, I used a ½ per cent. solution of potassic cyanide as recommended by Cybulsky.[1] By putting the over-stained tion in this solution the proper depth of colour is obtained

[1] 'Zeit. für wiss. Zool.,' Bd. 39, S. 657.

by the solution of the excess of the fixed gold, this process of course being carefully watched. In this reagent one finds an additional advantage; the nerve-fibres are the last to part with the violet colour, thus being distinguished from connective-tissue fibres. It, however, does not always operate in the latter way satisfactorily.

The sections of the human liver received from the gold a dull violet or a dull red tint, while in other preparations a blue violet tint was found. In two cases I obtained preparations which to the eye appeared almost colourless, but which on examination demonstrated the nerve-fibres very distinctly.

All the sections were cleared in oil of cloves, and mounted in balsam.

In the study of the ultimate terminations of the nerves I have used the Leitz $\frac{1}{12}$ inch homogeneous immersion with special illumination. In the human liver, more especially, it was impossible to do anything with a less efficient objective. In the Necturus liver it was quite easy, however, to see the required structures with a system 7 of Leitz, but I have endeavoured in every case to verify my observations with the higher power objective.

The value of gold chloride as a reagent for differentiating nerves is not admitted by all histologists. It has been urged also that the elements it selects in a fresh tissue and those it differentiates in a tissue hardened by a reagent such as chromic acid are not necessarily the same structures. This objection has a great deal of force, especially in view of the fact that gold chloride gives a violet tint to connective tissue which has been first hardened with chromic acid; the corium of Necturus and the connective tissue around arteries are cases in point. Moreover, the tendency of a hardened tissue is to reduce equally the gold so as to give to all the tissue elements a violet colour. Yet with all its faults the method of hardening with chromic acid and the subsequent treatment with gold chloride has many advantages over other micro-chemical and staining reagents, and so far as the demonstration by it of nerve-structures are concerned no greater suspicion should be attached to results

obtained with it than to those of other histo-chemical reagents. Gold chloride employed in any way is not an infallible test for nerve-structures, for these have in the end to be determined by their intrinsic form and arrangement, by their origin and termination, or either separately. The violet colour given by gold chloride to fibres otherwise undemonstrable is therefore of accessory value only.

It is not known definitely to what organic compound is due the capacity of nerve-fibres for fixing in themselves gold chloride. R. Gscheidlen,[1] after a series of experiments, came to the conclusion that the reduction is caused by a fatty substance. He treated pieces of the ischiadic nerve of a frog with ether, alcohol, and water respectively, and found that the extract obtained with ether reduces gold in a few hours, while that obtained with alcohol took longer to do the same, the aqueous extract, on the other hand, a very long time. As 90 per cent. of the solid extract obtained with ether is fatty in its nature Gscheidlen drew the inference that a constituent of this fat reduces the gold. I do not think that this explanation will suffice, for nearly all the fat of such an extract must come from the myeline investment of the fibres, and we find that no reduction usually occurs in the medulla. Fol[2] points out that the violet colour may have another explanation than a mere reduction of the gold, and calls attention to the fact demonstrated by Lindet that this reagent forms double salts with phosphorus compounds, especially the chlorides, which give aqueous solutions of a violet colour. Whether gold chloride undergoes reduction or enters into a more complicated condition it is outside the province of the histologist to determine. It is possible, however, without transgressing limits, to consider some aspects of this question and to suggest some points which may help in the solution It seems to me that the substance which favours the production of a violet colour with gold chloride is diffused through all forms of tissue, and that it is found in a concentrated condition in nerve-tissue only. If a section of

[1] 'Arch. für Mikr. Anat.,' Bd. xiv, p. 225.

[2] 'Lehrbuch der Vergleichenden Microscopischen Anatomie,' p. 175.

liver be treated with gold chloride, and the process of colouration be watched, it will be found that the first tinge which the nerve-fibres take is red, and afterwards they show all stages transitional between that colour and violet, while the other systems of tissue slowly pass through the same order of colours to the violet tint. The nuclear chromatine is an exception, being, like nerve-tissue, quick to attain a violet tint. Occasionally other structures act like nerve-fibres towards gold, and among these may be mentioned certain paranuclear bodies in the cutaneous epithelium of Necturus which are first coloured red, then rose violet, and finally deep violet. This appears to show that the substance which fixes the gold in a violet form is not confined to nerves, but is diffused to a small degree in other tissue elements.

The finest nerve-fibrils being hardly thicker or less delicate than the trabeculæ of the cytoplasma, it is wrong to suppose that a reagent which does not specially preserve and fix the latter will do this for the former. It is in this respect that I find the reason for the failure of Nesterowsky, Kolatschewsky, and others to resolve the finer nerve terminations, seeing that the reagents they used for hardening the tissue do not render the cytoplasma distinct and firm, and with it the finer nerve-fibrils. Ammonium bichromate is not a suitable reagent for this purpose, neither is the weak solution of chromic acid such as Kupffer used. The same objection can be urged against the method of freezing the fresh liver in order to obtain sections. The method of gold colouration must not be allowed to injure the cytoplasma. The test which I always exacted of the method employed was the distinct demonstration of the cell reticulum; that being in a good state of preservation, it was only a question of the number of trials with gold chloride in order to get the desired demonstration of the termination of the finest fibrils. I think also that the clearing up of fresh tissue with formic or acetic acid previous to steeping in gold chloride is apt to destroy both the cytoplasma and the finest nerve-fibrils. It is on this ground that I advocate the use of chromic acid to fix these before subjecting them to the action

of gold chloride, and to the subsequent treatment with formic or acetic acids. Osmic acid, although useful in the case of medullated nerve-fibres, is of no value for demonstrating the finest non-medullated fibrils.

Here a few words are necessary concerning the structure of the cytoplasma. In figs. 3, 4, 5, 6 it is represented as a network with thickened nodal points. It must be admitted that it does not always appear in such a regular arrangement. The meshes are often much larger and round as if occupied by fat droplets. Often also the trabeculæ thin out toward the periphery of the cell, so as to be nearly indistinguishable. The specimens of Necturus from which these preparations were made were caught early in March, 1885, and consequently there was but a small amount of fat in the hepatic cells. The appearance presented in the figures is a normal one, for chromic acid material with hematoxylin or aniline dyes show a similar arrangement. Flemming[1] believes in the arrangement of the cytoplasma in threads throughout the cell, but doubts if these form a network such as Klein[2] describes. Structures, however, like those drawn in figs. 5 and 6, leave hardly any doubt as to the occurrence of a reticulum.

The Nerves of the Human Liver.

In sections of the liver treated successfully with gold chloride the tissues immediately about the interlobular and central veins take a rose-violet or blue-violet colour. These strongly coloured fields, observed with a low-power objective, seem to consist wholly of violet-coloured fibres, but when more highly magnified the latter, which are commonly arranged in bundles, are seen to constitute but a small part of the interlobular tissue, or of that about the central vein, there being between the bundles a quantity of connective tissue coloured light violet or red. The thickest fibres are of about 0·0035 mm. in diameter. Each bundle is composed of a varying number of fibres, and is

[1] 'Zellsubstanz, Kern, und Zelltheilung,' Leipzig, 1882, p. 28.

[2] "Observations on the Structure of Cells and Nuclei," this Journal, vol. xviii.

usually separated from its neighbour by a narrow interspace less in diameter than that of the bundle. The fibres when seen in transverse section are round, and possess nuclei which are closely applied, sometimes at definite intervals. The fibres are wavy in their course, and are clear and homogeneous. They branch frequently, the branches being of diminished size, round, and lacking the nuclei of the larger trunks. They appear in no way to be related to or derived from connective-tissue corpuscles, they do not anastomose with one another, and they nearly always have a parallel direction, decreasing in size as they pass into the smaller divisions of the interlobular canal, where their arrangement in bundles is not so common.

The violet colour of the fibres render them remarkably distinct in contrast with the rose-violet connective tissue in which they lie scattered. Sometimes, however, the connective tissue is not coloured at all, but comes out as a granulo-fibrillar appearance which is apt to be overlooked in the presence of the deeply coloured fibres. In these cases the bundles are separated by the granulo-fibrillar substances which penetrates much less prominently between the individual fibres.

Where connective tissue and nerve-fibres are coloured alike, it is useful to differentiate between the two with the aid of a weak solution of potassic cyanide. The section being placed on the slide a drop or two of this reagent is added to it and the decolouration watched with a moderately high power. When the interlobular tissue is deprived of its colour to the degree required the section is mounted in the usual way. Under the high power one now finds only a portion of the interlobular tissue retains its violet tint, and this portion is composed of the fibres above referred to. This does not necessarily show that the fibres so revealed are nerve-fibres, or definitely distinguish them from those of connective tissue. It, however, seems to agree with the experience of Cybulsky, that in tissue stained with gold chloride, and subsequently treated with potassic cyanide, the nerve-fibres retain their colour longest.

I have never seen the connection of these fibres with medullated nerves, having never found the latter in the liver, but the normal or abnormal occurrence of which in the interlobular canals I do not doubt. Medullated nerve-fibres are sometimes found in unusual places. For example, Cybulsky found a medullated nerve-fibre penetrating the cutaneous epithelium, and I also have seen the same thing in a preparation of epithelioma. One may be inclined to believe, therefore, that medullated nerve-fibres can and do occur in the liver. It is to be remembered too that gold chloride is not a good reagent for demonstrating the myeline investment of nerves, the occurrence of which may escape the eye in preparations obtained with the one method.

It is quite true, as Kupffer asserts, that in gold preparations violet-coloured tissue passes at places in from the serous covering of the liver between the hepatic cylinders. I gather from his statements that he supposes that no nerves can reach the hepatic tissue in this way. Such a supposition is groundless, seeing that the serosa and the interlobular tissue are of one and the same origin, and one is as likely as the other to contain nerve-fibres. Where in my preparations the serosa was coloured violet throughout I added a drop of the solution of potassic cyanide, and found in consequence the same to be true here which I have described for the interlobular canals, namely, the presence of the two types of tissue—nerve and connective, the latter, however, very largely predominating.

There are at times interspersed between the bundles of large, violet-coloured fibres, fibrils in which the violet colour is not so distinct, and is more readily removable with potassic cyanide than that of the large fibres, but less so than that of connective tissue. I am doubtful of the significance of these, but they apparently answer to the smaller nerve-fibres of Nesterowsky. I have had no means of determining their connection with the larger fibres.

Around the central vein of a lobule both the connective and the nerve-tissue are in small quantity. The nerve-tissue is

found absent frequently in otherwise successful preparations, and the fibres usually are not more than half a dozen, each separated from the other by a considerable interval of space.

For tracing the nerve-fibres further to their termination it is necessary to resort to the special sections which I have referred to, namely, those which decrease in thickness from one edge to the opposite one. In a section of this sort, if the thick edge includes a longitudinal view of one of the interlobular canals, every facility is thereby afforded for following these fibres. A uniformly thin section is not of much value for this purpose, as in it the fibres can be followed but a short distance, on the average equal to the combined diameter of four or five cells, and the connection of the smaller with the larger fibres is difficult to make out. Part of a section prepared in the manner described is drawn in fig. 1, which represents the border of a hepatic lobule. At such a point are found medium-sized fibres coloured deep violet, always with a clearly defined border, quite different in this respect from connective-tissue fibres. One sees them arise from the large deep violet fibres of the interlobular canals, often as a direct continuation, without branching until after they enter the lobule. They are not numerous, there being usually two of them to each capillary channel, and they run between the capillary wall and the hepatic cells. They are easily distinguished with a low power objective. At first view they appear to form a network of anastomosing fibres, but a further examination shows that the branches of these fibres cross rather than join each other. Fibres of such a diameter are never found outside the capillary channels, that is, they do not penetrate between the liver-cells. These fibres belong to what I have denominated the coarse intralobular plexus. They possess no nuclei and branch at acute angles, the resulting branches being either quite as large as the original trunks or much finer. The large ones may be considered as belonging to the plexus just mentioned. The finer may be resolved into two classes : a perivascular plexus or network and an intercellular one. The perivascular network can be best seen when one looks from above into a capillary channel cut

longitudinally. The meshes of this network are irregular and greater or less in area than that of a hepatic cell, and the fibrils are very fine, without varicose swellings, and with a violet tint appearing quite distinct against the duller tint of the background formed by the hepatic cells. This perivascular plexus is in direct continuation with the fibres of the coarse interlobular plexus, and is therefore of a nervous nature. Whether it belongs to the walls of the capillary channels or to the hepatic cells bordering on these, or to both, I do not know. Sometimes it appears to belong to one, sometimes to the other. I cannot even say whether it is distinct from the intercellular network. The latter, also, is formed of fine fibrils, which are commonly seen unconnected with each other, but which in good preparations show anastomoses enclosing a varying area and extending between the hepatic cells. The finest of these fibrils possess varicosities regularly arranged and observable only with homogeneous immersion lenses such as a $\frac{1}{12}$ inch.

All my efforts to find a further resolution of the fibrils of the perivascular plexus availed nothing in the result. They might, as Kolatschewsky found in one or two cases, terminate in the nuclei of the capillary wall, but as to this I can bring no observations either for a negative or for an affirmative view, since in my preparations the capillary walls and their nuclei, unstained by gold chloride, appeared under high magnifying power as a hyaline refracting membrane. The perivascular plexus may, as I have already pointed out, serve as origin to the intercellular plexus.

From the fibrils of the intercellular network excessively minute twigs are given off which terminate each in a delicate bead in the interior of the hepatic cells near the nucleus. In a section such as that given in fig. 1 one often suspects such intracellular terminations, but the use of homogeneous immersion objectives does not demonstrate them satisfactorily, and only a careful search of very thin sections gave in five or six cases results not at all doubtful. I found, indeed, in some specimens excessively fine fibrils of a violet colour passing from the capillary side of the hepatic cell to the

neighbourhood of the nucleus and there end with the characteristic bead-like swelling; but I could not prove to my own satisfaction that they were other than prominently coloured trabeculæ of the cell reticulum. A view of a specimen such as I have represented in fig. 2 lends itself easily to interpretation. Here a fine nerve-fibril running along the side of the hepatic cylinder gives a fine twig to each cell which reaches the vicinity of the nucleus. Sometimes a twig divides after it enters the cell, the divisions running to opposite sides of the nucleus. The terminal points of all the intracellular twigs are delicate beads. I was always compelled to believe that such twigs are really within the cell when I found their terminal beads to be on the same level as the nucleus in an optical view of the latter. It is, of course, impossible in the greater number of cases to say whether the fibrils which give rise to these intracellular twigs belong to the perivascular or to the intercellular network. In fig. 2 one finds great difficulty in determining to which network the nerve-fibril belongs, but in several cases the demonstration of the intercellular origin was quite distinct, and this has led me to conclude that only the intercellular fibrils give off intracellular twigs.

The Nerves of the Liver in Necturus.

The hepatic cells in Necturus measure 0·042—0·05 mm., and consequently in a given area of a thin section the number of cells is less correspondingly than in the human liver. From this one would expect to find a less rich supply of nerve-fibres, and results bear out this opinion. The nerve-fibres in the interlobular canals are few in number, and each has a diameter much narrower than that of the larger ones of the human liver; their course is straighter till they enter the lobules, where they pass along the capillary channels to their termination. The small quantity of connective tissue in the interlobular canals usually takes a deep violet stain and then appears homogeneous and structureless. In such a case I have not found it necessary to remove the excess of the stain, for the nerve-fibres are clearly outlined against the connective tissue. Apart from the larger

interlobular canals the connective tissue also is not demonstrated by the gold, so that one finds no difficulty in tracing a nerve-fibre for a long distance, providing it lies in the plane of the section.

Nuclei were rarely observed on the largest fibres, and whether these belonged to the sheath of the fibre or to nerve-corpuscles it was impossible to determine. The division of the fibres in the interlobular canals is not common, but branching occurs more frequently in the capillaries of the lobules. Here they give rise to fibrils of two sorts; those which form the perivascular plexus surrounding the capillary walls, and those which, few in number, apparently course between the hepatic cells. I am unable to say whether there is any morphological or physiological difference between these kinds of fibres. I am inclined, however, to think they are one and the same, for they terminate in the majority of observed cases in a like way. The intercellular fibrils arise from fibres which serve as origins to the perivascular plexus; this also supports the conclusion already stated as to their physiological value. In a thin section the intercellular fibrils are the most commonly visible. I have applied the term intercellular to them because, although they do not always run between the cells, they unquestionably lie outside the capillary channels for the greater part of their course. I have followed them in some cases for a distance equal to the combined diameters of over twenty of the cells and have found them to accommodate themselves but very little to the windings and tortuous course of the capillaries. In thin sections, where the fragment of one cell is seen to lie over that of another, one of the fibrils in question has been again and again observed to pass between their contiguous walls. Part of their course is in the capillary channel, i.e. between the wall of the blood-capillary and the adjacent liver-cells.

The meshes of the perivascular plexus vary much in size and form, being usually less than the area covered by a hepatic cell and of an irregular quadrangular or triangular shape. The fibrils which form them are wavy in their course and apparently anastomose completely with one another. Now and then only

a longitudinal section of a capillary contains a view of this plexus in all its relations, on the one hand with the hepatic cells, and on the other with the capillary wall. The latter appears closely embraced by the network which also, in well-preserved specimens, borders the hepatic cells. In this respect one has great difficulty, as in the human liver, in deciding to which the network belongs physiologically, if to the capillary wall or to the cells; the terminations of this network show that it belongs to the latter. Is it to be supposed, however, that nerves are not distributed to the capillaries themselves?

It was of course much easier to determine how the long intercellular fibrils terminate than to do the same thing for the fibrils of the perivascular plexus. My first conclusion, after some observation, was that both terminate in a like manner, but I soon perceived that it was rarely possible in thin sections to decide whether a fibril which runs in the capillary channel and gives off intracellular twigs belongs to the intercellular class or to perivascular network. Both class of fibrils are equally delicate. The intercellular ones I found again and again to terminate within the hepatic cells. I had therefore to guard against confusing the two sets of fibrils as to their terminations. One of the several cases where an absolute decision was possible is drawn in fig. 3. Here fibrils of the network are seen to give off twigs which penetrate the adjoining hepatic cells, while on the opposite edge of the capillary channel a fibril, apparently belonging to the intercellular order, terminates in a like way.

The intercellular fibrils branch at certain intervals, each branch running at sharp angles with the main trunk. I believe several times to have detected a network of these branchlets. In such a case these intercellular fibrils would correspond to those of the intercellular network in the human liver. I must, however, leave this point in abeyance. If this network is usually present obstacles to its demonstration are certainly to be found in the deep tinting which the cell reticulum acquires from the gold method. The perivascular network, on the other hand, is not obscured, for the capillary walls over against which

it is usually seen are uncoloured, or nearly so. The fibrils of the intercellular order are generally so delicate that it is difficult to arrest the removal of the colour of the overlying or underlying cells with potassic cyanide at a point where the distinctness of the fibrils is retained. I had therefore no success in trying to determine definitely the common occurrence of an intercellular network.

The simple intracellular nerve-twigs always terminate in the neighbourhood of the nucleus, either singly or after branching, each terminal point being a delicate bead. The unbranched twig may end on the side of the nucleus facing the point where the twig penetrates the cell, or after curving around the nucleus on the opposite or on one of the lateral sides. When a twig branches two or more of the branchlets may terminate in the positions mentioned. In many cases it is possible to trace a twig for a certain distance after it enters the cell, but not to determine how it ends, this being usually due to the deep colour which the nucleus and the dense cytoplasma immediately about it take from the gold. On the other hand, the cytoplasma may acquire such a deep stain relatively that the determination of the presence of intracellular nerve-twigs is almost impossible. Frequently also these have but a pink or red tint while the fibrils from which they arise are deep violet.

All the forms of intracellular nerve terminations to be found in the liver of Necturus are not so simple as that just described. A form which I have several times met with having the intracellular twig branching dendritically is represented in fig. 4. A complicated mode of ending, a very common one in good preparations, appears to belong to the perivascular network. I am inclined, from reasons which I shall state further on, to regard it as a general one for the hepatic cells of Necturus. Fig. 5 shows how widely it differs from the other modes. Here a branch from the perivascular network penetrates a cell and becomes continuous with the cell reticulum in such a way as to leave the impression at first that this reticulum belongs to the nerve-twig rather than to the cell itself, this

opinion being at the same time strengthened by the fact that the cell network is coloured deep violet. The trabeculæ of the reticulum in such cases as this are very much more slender than they are as ordinarily demonstrated, but an exception is to be made of cases like that in the figure, where the trabeculæ become thickened along two or more lines so as to give the intracellular nerve-twig the appearance of a branching which extends toward the nucleus. In fig. 6, however, the reticulum is formed of trabeculæ nearly as coarse as that usually observed in the cell.

The demonstration of the simple intracellular termination occurring in the same cell with the more complicated form is apparently not possible. The former, if such is present when the other mode of termination is demonstrated, must necessarily be obscured by deep violet colour of the cell reticulum; if this depth of tint be lacking it is possible to see the simpler terminations. Both forms are often demonstrated in the same section, and therefore one cannot consider that the method of hardening previous to treatment with gold chloride may account for the presence of the one or the other on the ground of their being artificially produced.

It is probable that every hepatic cell in Necturus presents both forms of termination; otherwise we ought to conclude that there are two kinds of specifically different glandular cells in the liver according to the doctrine of the physiological homodynamy of nerves. The simpler form of termination may be regarded as the same as that found in the cutaneous epithelium of the tadpole,[1] while the more complicated mode is apparently the glandular one. At present it is useless to discuss further the specific function of these, but I hope in a future paper on nerve terminations to treat more fully this aspect of the question.

General.

In the liver of Necturus there is a mode of nerve termination which I have been unable to demonstrate in the human

[1] This Journal, November, 1885, p. 53.

liver. In this mode the nerve fibril fuses or is continuous with the reticulum, but the fibril first penetrates the cell in order to accomplish this fusion, and in this way differs from the method of termination described by Holbrook by which the fibrils are connected with the intercellular bridges or cement substance. I thought at times to see with oil immersion objectives such a fusion of the nerve-fibrils with the cell reticulum in the human liver, but it was in every case impossible to obtain demonstrations as definite as those yielded by the liver of Necturus.

This method of termination resembles to a certain extent that described by Pflüger ; the resemblance would be a more complete one were the cell reticulum regarded as of nervous origin, and then the words of that observer would be applicable here also : " Man könnte demgemäss sagen, dass die Leberzelle eine Kernhaltige Anschwellung eines Nerven sei." The points of difference between the method here described and that of Pflüger's are, however, too many to permit the supposition that they are one and the same but viewed according to different modes of preparation. When one compares the observations of Nesterowsky, Kolatschewsky, and Holbrook on the extreme fineness of the nerve-fibrils so far as they could trace them, the termination of medullated nerves in the hepatic cells appears exceedingly improbable except in pathological cases.

With regard to the methods of technique employed by the other observers several objections may be urged ; these I have already put forward. The methods are no doubt useful in demonstrating the course but not the termination of the nerves. It must be thoroughly understood that the nerve terminations in the interior of liver-cells are as delicate, as easily injured, and as difficult of demonstration as the finer cell structure. If this point is admitted I do not apprehend that a confirmation of the description of the nerve terminations here given will be a tardy one. Of course there are reagents which preserve well the cell structure but which do not fix it in such a way as to permit the selective capacity of gold chloride full play, and one must then endeavour to find such reagents as will give with gold chloride the best results. I have found these in Erlicki's

fluid and chromic acid, but it is possible that by varying the methods of the fixation of the gold some other reagent for hardening will give better results than I have obtained.

In conclusion, I may state that I have demonstrated these terminations to several competent observers, and among these I may mention Professor Ramsay Wright and Professor Osler. To the former I owe my thanks for his having gone over and verified every point here advanced and for the advice of which I availed myself at various times during the research.

EXPLANATION OF PLATE XXXIII, figs. 1 to 6,

Illustrating Mr. A. B. Macallum's Paper on "The Termination of Nerves in the Liver."

All the figures are representations, as exact as possible, of the structures drawn.

Fig. 1.—A section of the edge of a lobule of the human liver to show the course of the nerve-fibres. The clear spaces represent transverse and longitudinal sections of the radial capillaries where they are continuous with the interlobular capillaries. *a*. The coarse intralobular plexus. *b*. The perivascular plexus. *c*. The intercellular fibrils. *h*. The hepatic cells. The capillary walls are indistinguishable. Some of the larger nerve-fibres appear at places to run over the liver-cells, but at these points they actually follow the capillary pathways. Leitz, Oc. 3 and Obj. 7. The outlines of the cells, nuclei, and fibres were drawn with camera.

Fig. 2.—Two cells of the human liver, showing the termination of nerve-fibrils in their interior. Leitz, Oc. 3, and oil immersion $\frac{1}{12}$ inch.

Fig. 3.—Shows the simple intracellular termination of nerves in the liver-cells of Necturus. *a*. A larger nerve-fibre. *b*. An intercellular fibril, with *c*, the perivascular plexus arising from a common fibre. Erlicki's fluid, gold chloride, formic acid. Leitz, Oc. 3 and System 7.

Fig. 4.—Shows the dendritic branching of a nerve-fibril in the interior of a single hepatic cell of Necturus. Erlicki's fluid, gold chloride, formic acid. Leitz, Oc. 3 and System 7. The branching of the fibril in the interior of the cell was drawn with hom. imm. $\frac{1}{12}$ inch.

Fig. 5.—Two hepatic cells of Necturus with nerve-fibrils of the perivascular plexus; a fibril from the latter enters each of the cells and becomes continuous with the cell reticulum. Erlicki's fluid, gold chloride, formic acid. Drawn to the scale of Leitz, Oc. 3. and System 7; but the course of the fibrils outlined as seen under hom. imm. $\frac{1}{12}$ inch.

Fig. 6.—A single hepatic cell of Necturus, showing the relations of nerve-fibrils and the cell reticulum. The optical plane is above the nucleus and near the upper surface of the cell. The nerve-fibril passes over the upper surface of the cell, and gives off a branch which divides into twigs to penetrate the cell and fuse with its reticulum. Erlecki's fluid, gold chloride, formic acid. Leitz, Oc. 3, and oil imm. $\frac{1}{12}$ inch.

On the Nuclei of the Striated Muscle-Fibre in Necturus (Menobranchus) lateralis.

By

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With Plate XXXIII, figs. A and B.

While examining, several months ago, some preparations made for the purpose of demonstrating the mode of nerve termination in the striated muscle-fibre of Necturus, I found many of the isolated nuclei to possess on their surface furrows and striations hitherto undescribed, and which, I now believe, are of importance in determining the structure of the contractile element of muscle-substance. A careful study of these nuclei has further revealed other peculiarities which point to a conclusion as to the origin of the contractile element, the muscle reticulum. In view of this, I give a detailed description of the appearances presented by the nuclei, and refer them to their supposed causes.

The best preparations were obtained with gold chloride and formic acid. This method is valuable for two reasons, the nuclei are completely isolated after a stay of thirty to forty hours in the acid, and the furrows on their surface take a deep violet tint, while the remaining portion of the nuclear membrane is coloured light, or rose violet. The isolation of the nuclei from the muscle-substance prevents any confusion which might be caused by the striation of the muscle-fibre were one to examine them in situ. It is to be noted that the muscle-fibre in

Necturus macerates and disintegrates very easily in solutions of formic acid.

The long diameter of the nuclei, the largest and the smallest, measures 0·037—0·053 mm., and the transverse diameter 0·010—0·025 mm. The form of the nucleus varies somewhat, the oval being the most common; oblong and quadrate ones were occasionally observed. The membranes of old nuclei have apparently the capacity for attracting the reduced gold. In what might be considered as young nuclei, the membrane is not furrowed or but slightly so, and the chromatine is usually quite distinct, arranged in short, variously looped pieces along the long axis of the nucleus, or in the form of minute nodules (nucleoli) in different positions in the nuclear cavity (fig. A, 1 and 24). The reticulum of achromatine, or, better, caryoplasma, with the meaning attributed to that term by Carnoy, is very delicate with very narrow meshes in the nuclei just referred to. A very large number of the nuclei observed lacked chromatine while their caryoplasma was more prominent and its meshes larger. In a few, a further modification of the reticulum, to be described below, was observed.

In view of Nicolaides'[1] work on the division of nuclei in the frog's muscle, I made a careful search for like cases of division in Necturus, but the result was disappointing. Out of many hundred nuclei which I examined I found but one case of division, and that from the heart-muscle (fig. A, 11). Flemming[2] observed indirect division of the muscle nuclei in larval but not in adult Amphibia. My failure to find in Necturus what was observed by Nicolaides in the frog could not have been due to the reagent, gold chloride, for I found negative results when a mixture of chromic and acetic acids, or of these and osmic acid, was employed.

Nine out of ten of the old nuclei, i. e. those in which there remains no trace of chromatine, have markings and furrowings on their surface. Sometimes, as in fig. A, 2, a series of parallel

[1] "Ueber die Karyokinetische Erscheinungen der Muskelkörper," 'Arch. für Anat. und Phys., Phys. Abth., 1883, p. 441.

[2] 'Zellsubstanz Kern- und Zelltheilung,' Leipzig, 1882, p. 337.

furrows occupy one part of the surface of the membrane, the remainder of which is possessed of a series running in a contrary direction. They may be arranged parallel to the length of the fibre, or, as in 8, perpendicular to the long axis of the nucleus, or, as in 7 and 9, oblique to the same. Often one finds a combination of the two last mentioned modes of arrangement as represented in 10. Cases were observed in which a furrow, followed a part of the way around the nucleus, was found to divide at an acute angle, the branches completing the rest of the nuclear circumference. If the nucleus is flattened it is usual for the furrows to be confined to one surface. In 7 and 9 the number of these is respectively three and two, and in 8 they are very numerous. The depths of the furrows vary with their breadth, the latter being estimated by the breadth of the violet line of reduced gold. The depth in some nuclei is such that when one takes an optical section of them the nuclear cavity is almost divided into a series of separate compartments (25). The distance of the furrows from each other is subject also to considerable variation (10, 12, 15).

The cause of these furrows is in all probability the pressure exercised by the trabeculæ of the muscular reticulum described by Melland,[1] the transverse trabeculæ giving rise to the transverse furrows, the longitudinal ones to the furrows running parallel to the long axis of the nucleus.

Here in passing I may refer to my own observations on this muscular reticulum. These were made on specimens obtained with gold chloride from the rabbit, frog, Necturus, crayfish, and grasshopper, and as a result I accept in full the views of Melland. In my preparations of the muscle-fibre obtained from the rabbit and Necturus this reticulum is in all its aspects splendidly demonstrated. Carnoy[2] apparently holds the same view as to the cause of the striation of the muscle-fibre in Hydrophilus piceus, for he describes it as due to a rearrangement of the reticulum of the cell (cytoplasma of that

[1] "A Simplified View of the Histology of the Striped Muscle-fibre," this Journal, July, 1885, p. 371.

[2] 'La Biologie Cellulaire,' Paris, 1884, pp. 192 and 193, fig. 38.

author) in longitudinal and transverse trabeculæ, the myosine arising from that part of the cellular contents enclosed in the meshes of the primitive reticulum of the cell.

It is not too great an inference to draw that this reticulum is the true contractile element, while the myosine shifts and accommodates itself to the conditions of the latter. In support of this I refer to fig. A, 6 and 13, in which one sees that the furrowing takes the shape and arrangement of the meshes of the reticulum.

Why the furrowing on the surface of some nuclei is longitudinal only, on others transverse, I cannot with certainty say. Of one thing I am sure: where one series of parallel furrows alone is visible, these are in by far the greater number of cases transverse to the long axis of the nucleus. The greater part of the muscle-fibre used in my preparations was in the uncontracted condition, and it is in this condition that the muscles are for the greater part of life. Following out the theory as to muscular contraction, given in the last paragraph, it is to be supposed that during rest the transverse trabeculæ of the muscle reticulum are contracted, but lengthened during contraction of the fibre, the conditions with regard to the longitudinal trabeculæ being in the reversed order. As now the contraction or shortening of the transverse trabeculæ must occur during the greater part of the life of the muscle, or during its resting periods, it is obvious that transverse furrows should be seen on the nucleus oftener and more prominent than longitudinal ones or than both combined. In this way we may explain away part of the difficulty of accounting for the occurrence of one or other series only of furrows.

E. Weber[1] found that the nuclei of the frog's muscle are provided with longitudinal striæ, which he thought due to the pressure exercised by the muscular fibrillæ. I endeavoured for the sake of comparing them with the nuclei in Necturus to isolate, after the manner already described, the nuclei of the frog's muscle, but I met with a very indifferent success. In

[1] "Note sur les noyaux des muscles striés chez la grenouille adulte," 'Archives de Physiologie,' 1874, p. 489.

the case of the few isolated, however, I could make out a certain amount of transverse striation of the membrane.

Further, as to the transverse furrows being often at acute angles with one another, an explanation for this may be found in the facts now to be described.

A few nuclei presented other appearances than those mentioned (fig. A, 9, 15, 16, 18, 19). Here the form of a oblong-meshed reticulum like that of the muscle-fibre was observed and considered by me at first to be caused by an impression produced by the latter on the surface of the nuclear membrane. I found, however, in cases where it could admit of no doubt, that the reticulum was not on, but within the nucleus. In other words, there is in the interior of some nuclei a reticulum like in every respect to that found in the muscle-substance. In these cases no chromatine or caryoplasma could be observed.

On the other hand, I observed a few nuclei in which the caryoplasma was approximately square meshed (fig. A, 23). The quadrangular form of the meshes is exhibited oftener and better in the nuclei from the heart muscle (fig. A, 20, 21). This form of the reticulum appears to be one of the stages transitional between the reticulum of the young nucleus and that referred to in the last paragraph.

In view of these facts it is safe to infer, as Carnoy and Melland have done, that the muscle reticulum is simply the modified cytoplasma, the caryoplasma being derived from the latter.[1]

Nuclei with such a modified caryoplasma as represented in fig. A, 20 and 21, must be capable of movement, or contraction and extension. This movement has been observed in the nuclei of vegetable cells by Hanstein,[2] and in the nuclei of white blood-corpuscles of Necturus by Gage,[3] and is amœboid in character. But the possession of a square-meshed reticulum implies extension and contraction in definite directions—the nucleus con-

[1] See Carnoy, op. cit., p. 250.
[2] 'Bot. Zeit.,' 1872, p. 22.
[3] 'Science,' Jan. 8th, 1886.

tracts with the muscle-fibre and extends with it again, yet not passively.

The possession of an irregularly meshed reticulum by the nucleus would imply on the part of the latter movements, if any at all, of an amœboid character. It is possible that this is just what occurred in the nuclei represented in fig. A, 10, 12, and 17, an unequal extension or contraction of all parts of the nucleus, resulting in a misplacement of the furrows and in their irregularity.

Nicolaides suggests that all muscle nuclei take a more than passive share in muscular contraction.

Some nuclei have part of their surface completely free from furrows (fig. A, 5). I think this is due to the fact that the whole of the nuclear body is not surrounded by the muscle-substance, a part of it lying between the latter and the sarcolemma.

EXPLANATION OF PLATE XXXIII, figs. A and B.

Illustrating Mr. Macallum's Paper on the "Nuclei of the Muscle-fibre in Necturus lateralis."

FIG. A, 1—25.—Nuclei of striated muscle-fibre of Necturus lateralis, 11, 20, 21, 22, representing some from the heart muscle. Mode of preparation: gold chloride, formic acid.

FIG. B.—A part of a striated muscle-fibre with its nuclei, from Necturus. Gold chloride, formic acid.

(Note.—In drawing these figures, Leitz obj. 7, and oc. 1 or 3 were employed.)

The Development of the Cape Species of Peripatus.

PART III.

ON THE CHANGES FROM STAGE A TO STAGE F.

By

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With Plates XXXIV, XXXV, XXXVI, and XXXVII.

The early development of Peripatus capensis, as described in Parts I and II of this series, is apparently so different from that of the West Indian and New Zealand species, and at the same time our knowledge of the early stages of these species is still so incomplete,[1] that it is difficult at present, and would indeed under the circumstances be waste of time, to attempt to institute any detailed comparison between them. With regard to the later stages, however, the matter stands otherwise; for not only do they seem to be alike in the three species, but in the case of the West Indian species at any rate we have in Dr. Kennel's memoir, if not a complete still a detailed and coherent account of them.

While able to speak, to this extent,[2] favorably of Kennel's

[1] In spite of Dr. Kennel's objection, I must adhere to the view expressed in Part I of this series, that his account of the early stages of the Trinidad species presents internal evidence of the incompleteness of his observations. To particularise; his account of the relation of the embryo to the uterine wall, and of the origin of the so-called amnion and placenta, seems to me unsatisfactory.

[2] I am obliged to confess that Dr. Kennel has made some rather important

scientific work, I regret to be obliged to take up an altogether different position with regard to the personal remarks directed against myself, with which he closes his memoir.

He there devotes a considerable amount of space to a criticism of my first papers on the development of the Cape species. I do not desire to enter into a controversy with Dr. Kennel, nor do I intend to deal with his remarks in detail, but there are one or two points which require notice from me.

His criticism has two main objects. In the first place he is anxious to prove that there is nothing new in my results: either they were known before, or he has already obtained them, or they are not true. With regard to this, I have only to say, if it please Dr. Kennel to take this view he is quite welcome to do so. His opinion is a matter of no interest to me. The second point is, however, more serious. Dr. Kennel's object is apparently to impugn my personal honesty. He accuses me of trying to take credit for discoveries really made by another man. Not only is this charge false, but an examination of Dr. Kennel's works on the development of Peripatus proves that he knew it to be false when he made it.

He accuses me of unjustly laying claim to having proved that the mouth and anus of the Peripatus capensis are derived from the blastopore of the embryo. I may, perhaps, be allowed to state the facts concerning this matter.

Balfour made the discovery that the blastopore of Peripatus capensis became closed in the middle so as to give rise to two openings. The question then arose—a question which was stated in Balfour's memoir on Peripatus (No. 4, p. 255), and again in my paper on the "Origin of Metameric Segmentation" (No. 32, p. 55), both of which papers Dr. Kennel was

blunders, which, however, are pardonable, inasmuch as they concern the points which are the most difficult—in fact the only points of any difficulty in the whole of the later stages, and at the same time are the points of the greatest interest—to follow. The blunders relate, as will be seen, to the fate of the cœlom and the origin of the various parts of the body cavity.

familiar with, as is shown by his constant reference to them—do these openings give rise to the mouth and anus of the adult, or do they become closed up, the mouth and anus being new formations?

Balfour was unable to settle this, because he had no embryos between Stages B and F. The presumption was that they did not, both of them, persist, because Moseley (No. 23 *a*) had stated that he could find neither mouth nor anus in his youngest embryos (about my Stages D and E). Dr. Kennel was acquainted with this statement by Moseley, as is shown by his remarks on p. 195 of Part I of his work, where he actually uses it to cast doubt upon my view that the mouth and anus of the adult are derived from the blastopore of the embryo. There can be no doubt then that Kennel knew that Balfour had not proved the thesis that the mouth and anus of the adult were derived from the two parts of the blastopore. The intermediate stages which were necessary for its proof were obtained by me a year later, and I first announced the fact that the two parts of the blastopore persist into the adult, in my paper on the "Origin of Metameric Segmentation," in a passage which reads as follows (p. 55): "In the paper referred to (Balfour's memoir, edited by Moseley and myself) 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 Moseley in his original paper, so that I think there can be no doubt that they do become the mouth and anus of the adult."

Dr. Kennel brings another charge against me of the same nature, equally baseless and wanton.

I believe Dr. Kennel to be an honest worker, and I do not wish to speak of him in an unnecessarily harsh manner, but I am bound to confess that I do not understand his attitude of mind in bringing charges of this kind against me. I need only say with regard to them that the accusation of plagiarism in the matter of the mouth and anus may be taken as a fair sample of the kind of criticism, if criticism is the word

to apply to such clumsy attacks, which he has throughout directed at my work on Peripatus.

The Ectoderm.

During Stage A, the ectodermal or outer part of the embryo consists of a closely reticulated protoplasm, which contains a single layer of oval nuclei of fairly uniform size. At the lips of the blastopore this layer, with its contained nuclei, is prolonged inwards for a short distance to the more vacuolated endodermal part of the embryo. These nuclei, which are arranged in a layer immediately within the lips of the blastopore, resemble in their shape the ectodermal nuclei (Pl. XXXIV, figs. 2, 3), and they may be regarded as ectodermal or endodermal according to the inclination of the observer. I am inclined to call them intermediate or undifferentiated nuclei, that is to say, nuclei which cannot be definitely assigned to the endoderm or ectoderm, and in this capacity I shall have occasion to return to them when I come to speak of the later history of the mesoderm.

The protoplasm surrounding these nuclei projects into the cavity of the blastopore and into the archenteron, in the form of delicate processes, which anastomose with other similar processes (Pl. XXXIV, fig. 3; Part II, fig. 26 *b*).

When the mesodermal bands are formed, the nuclei of the ectoderm overlying them become slightly longer than elsewhere, and the ectoderm has the appearance of being composed of columnar cells. The nuclei are, however, still in a single layer.

At the beginning of Stage c, a number of smaller, more rounded nuclei appear in the ectoderm within the outer layer of oval nuclei (Pl. XXXIV, fig. 6 *c*). These nuclei are, in the next stage (Pl. XXXIV, fig. 9, *v. n.*), found along the inner i. e. ventral portion of the ectodermic thickening just mentioned as overlying the somites. They constitute the rudiments of the central nervous system.

The nuclei of the outer, i. e. latero-dorsal portions of the original ectodermal thickening (Pl. XXXIV, fig. 9), remain in

a single layer, and the ectoderm containing them becomes pushed out by processes of the mesoblastic somites. These processes constitute the rudiments of the appendages. The latter are, as I have already mentioned in Part I, formed from before backwards, the first to appear being the appendages of the pre-oral somites—the later antennæ—which exactly resemble in their development the other paired appendages of the body.

After this general account I will now describe, in greater detail, the development of the ectoderm up to Stage E, under the following heads:

1. The dorsal and ventral ectoderm which intervenes between the lateral thickenings.
2. The lateral thickenings.
3. The ectoderm of the proctodæum and stomodæum.

It will be convenient to defer a description of the ectoderm of the primitive streak to the section of this paper on the history of the mesoderm.

4. The slime-glands.

1. **Dorsal and Ventral Ectoderm.**—During Stages A and B the ectoderm on the dorsal and ventral surfaces is composed of what may be called cubical cells with oval nuclei, but it must be remembered that these cells are not isolated from one another or from the endoderm. During Stage C, the nuclei of the dorsal ectoderm become spherical (Pl. XXXIV, fig. 6 *c*), as do also the nuclei of the ventral ectoderm on each side of the mouth (fig. 6 *b*). But the greater part of the ventral ectoderm, namely, that which intervenes between the mouth and anus (Pl. XXXIV, fig. 6 *c*), and that which is placed on each side of the anus, becomes reduced to an extremely thin layer (Pl. XXXIV, fig. 6 *c*, *d*), the character of which will be obvious from an inspection of the figures. In Stages D and E, the ventral ectoderm retains the characters already described, but its width becomes less, the lateral thickenings having somewhat approached one another on the ventral surface (figs. 9, 25). The dorsal ectoderm, on the other hand, becomes in Stage D somewhat reduced in thickness (figs. 9—13), though it never becomes as thin as the ventral portion. Subsequently, in

Stage E, it undergoes a striking change. It becomes much thickened. The thickening first appears in Stage D in the region of somites 7—10 (fig. 25), where indeed it lasts longer and is more marked than elsewhere, and gives rise to the prominence marked *d* in the figures of Pl. XXXII, Part I. The change begins at the sides and gradually extends dorsalwards. The ectodermal hump (*d.*) seems to retain until its disappearance indications of this bilateral origin. The thickness of the dorsal ectoderm varies in different specimens, and no doubt depends to a certain extent on the amount of contraction which the protoplasm has undergone at death.

This increase in thickness is mainly due to the appearance, outside the nuclei, of a layer of vacuolated protoplasm. The vacuolation is not shown in my figures, but it is a very striking feature. The surface of the dorsal ectoderm, particularly of the hump, is very rough in these stages, and in the best-preserved embryos without a definite external boundary. It presents very much the appearance which a bath sponge would present in section, fraying out, as it were, into the surrounding fluid; and one may fairly conclude that during life it possesses the power of sending out processes into the fluid surrounding the embryo, and that the superficial vacuoles open to the exterior. In short, I am inclined to think that this surface ectoderm during Stages E to F has a nutritive function, absorbing the fluid in which the embryo lies, and it seems to me conceivable that the placenta described by Kennel in the Trinidad species may be a more specialised organ of the same nature. During the progress of Stage F the nuclei which have hitherto been placed in the deep parts of the layer (Pl. XXXV, figs. 23 *a*—*e*, 25) acquire a superficial position, excepting in the hump, where they retain their deep position until after Stage G. Contemporaneously with this change the deeper parts of the ectoderm become filled with very large vacuoles, so that the protoplasm is reduced to fine cords, passing inwards from the superficial nucleated layer. This vacuolated deeper part of the dorsal ectoderm now becomes much reduced, so that in Stage F the dorsal ectoderm consists mainly, if not entirely, of a thin layer of

nucleated protoplasm derived from the superficial layer of the preceding stage. The hump, however, still persists, retaining the characters it had in Stage E.

With regard to the internal boundary of the ectoderm, in the gastrula stage there was no line of demarcation between it and the endoderm. In Stage B the mesoderm appears, but causes no break in the continuity (Pl. XXXIV, fig. 5 *a*—*f*). In Stages C and D, however, a definite separation occurs, firstly by the appearance of the cavity in the somites, and secondly—and this happens later, in Stage D—by the dorsal and ventral separation of the endoderm from the ectoderm. The endoderm is, however, still continuous with the splanchnic layer of mesoderm, and the ectoderm with the somatic. In the subsequent development this continuity seems to be retained and to be extended in consequence of the growth of the mesoderm over the internal surfaces of the at first uncovered parts of the two primary layers. At any rate I have never been able to see any well-defined boundary between the layers in question, even in the best-preserved embryos, if a careful examination was made with a high power. The defined line drawn in my figures has only an existence with a low power; it is therefore extremely difficult to say whether or no nuclei pass in from the ectoderm to the mesoderm, and often not possible to settle for certain whether a given mass of nuclei belong to the ectoderm or somatic mesoderm. In the later stages (F) this absence of a defined line continues, so that it becomes difficult to decide whether the external transverse muscles of the body wall are ectodermal or mesodermal. To this point I shall return in Part IV of this series.

2. The lateral thickenings, which give rise, amongst other things, to the nervous system, contain during Stages A and B a single layer of long oval nuclei (Pl. XXXIV, fig. 5 *b*). They are at first confined to the ectoderm immediately overlying the mesoblastic somites, and constitute on each side a continuous band extending from the pre-oral region, where they are more ventrally placed and continuous with each other

across the middle line, to the primitive streak behind. They diverge from one another as they pass backwards, and end in the ectoderm of the primitive streak. During Stages A and B they contain a single layer of oval nuclei, which rapidly increase in number, and become arranged in Stage C in parts in more than one layer. The latter fact is especially conspicuous in the pre-oral portions, i. e. in the portions which will give rise to the cerebral ganglia (Pl. XXXIV, fig. 6 *a*).

In Stage D the lateral thickenings have increased considerably in extent, occupying the whole of the sides of the body, and encroaching on the middle region of the body, where in the previous stages they were widely apart, somewhat on the ventral surface (Pl. XXXIV, figs. 9, 11), so that the relation to the somites mentioned above (p. 473) is lost. The increase in the number of nuclei is now more marked, and is found to concern chiefly two regions: (*a*) nearly the whole of the pre-oral parts of the lateral thickenings (Pl. XXXIV, fig. 14); (*b*) the ventral (inner) portions only in the circumoral and postoral regions (Pl. XXXIV, figs. 9, 13). The increase has for its results, as may be seen by reference to the figures, the production of some roundish nuclei lying internally to the oval nuclei. These round internally placed nuclei are much more numerous in Stage E, and eventually give rise to the whole of the cerebral ganglia and ventral nerve-cords. They remain up to the close of Stage E in close connection with the external layer of oval elements, though in some sections there are, in the post-oral region, indications of a commencing separation between them (Pl. XXXV, fig. 23 *a*—*d*). The manner of separation of the central nervous system from the superficial ectoderm will be described in Part IV.

It is only necessary for me to point out here that in the region of the brain this separation does not occur, as the ectoderm is invaginated, and remains in connection with the nervous tissue throughout life (Pl. XXXV, fig. 22 *a*, and Pl. XXXVI, fig. 33).

In Stage D a small amount of the so-called punctated tissue appears on the dorsal surface of this rudimentary central

nervous system; this tissue, which is earlier and more largely developed in the cerebral rudiments than in the ventral cords, consists of a fine protoplasmic network, which stains but slightly and is almost entirely without nuclei. A similar network exists in the ventral nucleated parts of the nervous system, but is there obscured by the crowded nuclei. At first the whole central nervous system was similarly crowded with nuclei (Pl. XXXIV, figs. 15, 16 *a*, &c.), but in Stage D the latter began to withdraw from the dorsal part, thus allowing the non-staining protoplamsic network to stand out with great distinctness (Pl. XXXV, figs. 22, 23 *a*, &c.). At the boundary between this white matter and the nuclear mass there are, in Stages E, F, some nuclei which are somewhat larger than the rest, and more loosely arranged, so that they may be said to lie in the ventral part of the white matter (Pl. XXXVI, figs. 38, 39, &c.). Four such may generally be seen in each section of the ventral cords.

I must now pass to describe the changes of the more dorsal parts of the lateral thickenings in the postoral region. In Stage D they contain a single layer of oval nuclei, and become at the same time pushed outwards by the outgrowths of the hinder part of the mesoblastic somites (Pl. XXXIV, fig. 9). These outgrowths are arranged in pairs, one pair from each pair of somites, and they constitute the rudiments of the postoral appendages. The first pair to be formed is the pair which will become the jaws; the next pair will form the oral papillæ, and so on in order from before backwards. When the appendages are well established—in Stage E—it may be seen from an inspection of transverse sections that they are special developments of the posterior portion of lateral ridges of ectoderm which extend on each side for the whole length of a somite (Pl. XXXV, figs. 18 *a*, *b*, 21 *a*, *l. r.*). Immediately within these ridges there is a thickening of the somatic mesoderm (*m. t.*) which I shall speak of again later, and which is continuous with the thickened mesoderm of the appendage itself. The postoral appendages, therefore, may be described as special developments of longitudinal ridges, which,

however, are not continuous up the whole length of the body, but are interrupted at the lines of segmental division. These limb-ridges in Stage E become separated from the appendages and continuous with one another dorsal to the appendages (Pl. XXXVI, fig. 36). They now, therefore, form one continuous ridge on each side of the body, placed just dorsal to the insertion of the appendages. They eventually disappear.

In Stage E the portions of the lateral thickenings dorsal to the line of insertion of the appendages, and the dorsal ectoderm itself undergo a peculiar modification. The ectoderm here becomes thicker, and this increase in thickness is due, mainly, to an increase in the protoplasmic layer, elsewhere of extreme tenuity, on the outer side of the nuclei (Pl. XXXV, fig. 23 *a*—*d*). This modification, which has already been referred to (p. 472), is seen first, and is always most conspicuous in the region of the seventh (Pl. XXXV, fig. 25) to about the tenth somite, where the nuclei become numerous and arranged in several layers.

There is only one other portion of the postoral ectoderm which needs consideration, viz. the inner portion of the original lateral thickening—the parts which give rise to the nerve-cords.

These are at first perfectly continuous from end to end of the body. They consist, in Stage E, when they first become well marked, of a number of oval nuclei with intermixed round nuclei in the deeper parts (Pl. XXXV, fig. 23 *a*—c). In Stage D they were wide apart, being separated by an area of extremely thin ectoderm (Pl. XXXIV, figs. 9—13). The latter, however, soon becomes of less extent (*vide* sections of Stage E), so that they approach one another until, in Stage F, they meet and coalesce in the middle line (Pl. XXXVI, figs. 38—41). They retain, however, always a trace of their paired origin. In late embryos of Stage F, the nerve-cords separate from them, and they become segmented in such a manner that they persist only between the appendages, the intervening portions having disappeared. In this condition they have been called by Kennel (No. 13) the ventral organs. The ventral organs, as Kennel has already described, persist into the adult, in

which they are much more conspicuous in some species than in others. The ventral organs of the jaws and oral papillæ undergo special changes which have already been quite correctly described by Kennel, and which I shall have occasion only to refer to hereafter.

It now only remains for me to describe the changes which take place in the pre-oral portions of the lateral thickenings. I have already stated that these are from the first continuous with one another in front and with the postoral portions behind the mouth, that the internal rounded nuclei appear sooner in them than elsewhere, and in greater numbers. In fact, nearly the whole of the pre-oral lateral thickenings give rise to the internally placed rounded nuclei (Pl. XXXIV, fig. 16 *a*) which will form ultimately the cerebral ganglia. These rounded nuclei extend, though only in a thin layer, even across the middle line in front of the mouth, in which position they first appear in Stage c (Pl. XXXVI, fig. 28, *com.*), and rapidly increasing in number, remain in connection with the ectoderm until Stage E, when they become detached (Pl. XXXV, fig. 22 *a*, Pl. XXXVI, fig. 31, *com.*) though the cerebral rudiments themselves are still in connection with the ectoderm. It thus appears that the two cerebral ganglia and their connecting commissure are developed as a single structure from the ectoderm.

The ventral cords, beginning at the level of the jaws, are, as we have seen, continuous developments from the lateral thickenings; and the question arises: Does this continuity extend in front of the jaws to the preoral region, or is there a sharp separation between the cerebral rudiments and the ventral cords?

There can be no doubt whatever that in Peripatus capensis there is no such break at any stage of development. A study of transverse sections of many different embryos from Stages c—E conclusively demonstrates that the ventral nerve-cords, which are developments of the inner portions only of the lateral thickenings, are continuous at all stages of their existence, both before and after their separation from the ectoderm, across the boundary between the

first and second somites with the inner portion of the cerebral rudiment, which is a development of the whole surface of the pre-oral part of the lateral thickening. It is true that at first this part of the central nervous system is weaker than the parts in front and behind it, but long before the separation from the ectoderm occurs (Pl. XXXV, fig. 19 *b*; Pl. XXXVI, fig. 35, *c.o.n.*), it forms a well-marked cord with a layer of white matter. The whole central nervous system of Peripatus capensis develops, therefore, as a continuous structure from the ectoderm, and the independent origin and secondary connection of the cerebral ganglia and ventral chain, which has been asserted for some Arthropoda, e. g. for Spiders by Balfour (No. 3), for Annelida by Salensky (No. 27), Lumbricus by Kleinenberg (No. 15), and for Mollusca by various observers, does not hold for Peripatus.[1] Balfour held the same view with regard to this point in Peripatus (No. 2 p. 337).

I entirely agree with the remarks of Hattchek (No. 12, p. 8) on this subject. He holds, in opposition to Salensky, that the circumoral part of the nervous system in the annelid larvæ which he has investigated, develops fron the ectoderm in continuity with the apical ganglion and ventral nerve-cords; and I am strongly inclined to think that a further and closer investigation will show the same fact to hold for other Annelids and Molluscs.

The Cerebral Grooves.—The cerebral ganglia gradually increase in size (Pls. XXXIV, XXXV, figs. 14, 16 *a*, 19 *a*) and their ventral surface becomes in Stage E markedly flattened (Pl. XXXV, fig. 22 *a*) and then invaginated (Part I, fig. 35), so as to form two grooves, which rapidly become narrower and deeper, until in Stage F they form two slits, longitudinally arranged, ending blindly in front, but opening behind into the buccal cavity (Part I, fig. 36, and Pl. XXXVI, fig. 33). Eventually, in old embryos of Stage F, they lose their external openings, become reduced in size, and form two vesicles in the ventral

[1] Kennel apparently holds the opposite view on this point. If he is right I can only suppose that P. Edwardsii differs in this respect from capensis.

portions of the cerebral ganglia. Owing to the relatively greater growth of the latter they appear to form, in the adult, small hollow appendages of the brain (No. 2, Pl. XVII, fig. D, *d*).

The most important features which remain to be described in the development of the preoral part of the central nervous system, is the part which it takes in the formation of two organs—the eyes and the tentacular nerves.

The eyes arise in Stage D as a pair of invaginations of the postero-lateral parts of the cerebral rudiments (Part I, fig. 29). The two pits so formed are placed immediately in front of the point of origin of the lip-folds from the pre-oral lobes (Part I, fig. 30). They are at first shallow, but soon become deeper, and eventually (by the end of Stage E) constricted off from the surface, so as to form closed vesicles (Pls. XXXIV, XXXV, figs. 16 *a*, 15, 19 *a*, 22 *a*). The eyes, therefore, are nothing more nor less than invaginations of the lateral portions of the rudiments of the cerebral ganglia, and of the surface ectoderm, which gave rise to and covers the latter. Two elements enter into their composition, (*a*) the surface ectoderm with its oval nuclei, and (*b*) the rounded nuclei of the cerebral rudiment. The columnar surface nuclei form the lining of the optic chamber, while the rounded elements which lie behind the inner wall give rise to part of the retina and optic nerve. Figs. 14, 15, 19 *a*, 22, show very clearly the method of development of the optic rudiment. In the later stages the connection between the posterior wall of the optic vesicle and the cerebral ganglion becomes somewhat constricted, but persists throughout life as the optic nerve. The eyes of Peripatus are, therefore, as I stated in my original paper (No. 33), cerebral eyes, and are from the very first in connection with the cerebral ganglia.[1]

[1] Kennel states that in P. Edwardsii the eye arises independently of the brain, and secondarily enters into connection with it. Not having examined the development of the eye in P. Edwardsii I am not in a position to affirm or deny the correctness of this statement. But there are certain other points in Kennel's account, particularly those relative to the early stages and to the fate of the cœlom, which incline me to think that his material was not so good either in quality as might have been wished.

The tentacular nerves are to be regarded, from their method of development, as forward prolongations of the cerebral ganglia.

In the outgrowths from the pre-oral lobes to form the tentacles the cerebral thickenings, which extend dorsalwards on to the anterior face of the former, participate in such a manner as to form the ventral surface of the developing tentacles (Pl. XXXIV, fig. 7). The deep rounded elements of these forward tentacular continuations of the cerebral rudiments eventually separate from the surface ectoderm, and become the tentacular nerves.

The later stage of the cerebral ganglia will be considered in the next part of this series. By the stage reached (Stage F) they are in connection with the walls of the cerebral grooves.

The latter are almost if not entirely constricted off from the surface, and are lined by the ectoderm which gave origin to the cerebral ganglia. Kennel regards this invaginated layer as the homologue of the ventral organs of the postoral region. It differs, however, from the latter in being invaginated. Notwithstanding this, the view seems to me a plausible one.

The cerebral ganglia of Peripatus resemble, therefore, in their development and method of removal from the surface, the central nervous system of the Vertebrata, and the cavity of the ventral appendages of the adult brain corresponds[1] with the central canal of the latter.

These cerebral grooves seem a fairly constant feature in Tracheate embryos. They do not, so far as I know, persist into the adult of any other Arthropod, but disappear in the course of development. In these cases their walls are said to become transformed into parts of the supraœsophageal ganglia. Structures of a similar nature are found in other animals. They have been described by Kowalevsky in the embryo of Dentalium (No. 16), in which animal the cerebral ganglia are formed from the walls of two invaginations of ectoderm at the apical pole of the body. They persist for some time, and then vanish. In Sipunculus Spengel (No. 35) has de-

[1] By this, of course, I do not mean that the two structures are homologous.

scribed a single canal leading from the cerebral ganglion to open at the base of a tentacle. In Nemertines, as is well known, canals opening on the surface penetrate into the cerebral ganglia, and in Balanoglossus a single canal traverses part of the central nervous system. Whether these canals leading to this important part of the central nervous system are homologous it is difficult to say. Probably they are not, but are simply analogous, their function being, or having been, in some aquatic ancestor, respiratory. I have already (No. 31) suggested this as the probable genetic explanation of the central canal of the Vertebrate nervous system.

Summary of the Early Development of Nervous System.—The lateral thickenings are from their origin continuous from somite to somite. They begin in front of the mouth, where they are connected with one another across the middle line, and they end behind in the ectoderm of the primitive streak.

The rounded elements which give rise to the nervous system are derived from the ventral parts of these thickenings. They are formed first in the pre-oral region, and then in the lateral cords; that is to say, the nervous system at its very first appearance is a continuous structure beginning in front of the mouth, where it is continuous across the middle line, and extending backwards on each side of the mouth.

The portions of these two cords in front of the mouth become the cerebral ganglia, which give rise directly to the eyes and tentacular nerves; the portions around the mouth become the circumoral commissures, while the portions behind the mouth are the rudiments of the ventral nerve-cords of the adult. The ectoderm from which the rounded elements arise remains thickened, and give rise to structures which have been called by Kennel the ventral organs. The ventral organs are at first placed at a little distance from the middle line. Eventually, however, they approach one another and meet, excepting in the region of the mouth and pre-oral lobes.

On the pre-oral lobes the structures corresponding to the ventral organs of the posterior part of the body become invaginated and separated from the surface in such a manner as to

form the linings of the vesicles, which are attached throughout life to the ventral surface of the cerebral ganglia.

3. **The Stomodæal and Proctodæal Ingrowths.**—At the time when the blastopore is a continuous slit and traversed by strands of anastomosing protoplasm, i. e. during Stage A, the part of the endoderm which is continued into the ectoderm at the lips of the blastopore, resembles, in the small size and number of the vacuoles and the regular shape and arrangement of the nuclei, the ectoderm. In fact it is impossible to say whether this layer is really ectodermal or endodermal. By its position and development it resembles endoderm, by its characters ectoderm.

When the blastopore closes in the middle these cells are left inside and form the median ventral wall of the mesenteron (Pl. XXXIV, figs. 5 *b*, 6 *c*, *v. en.*), and eventually assume the characters of endodermal nuclei. At the primitive mouth and anus, however, they still persist as rows of nuclei intervening between undoubted ectoderm and endoderm (Pl. XXXIV, fig. 6 *b*, *d*), and they extend forwards for a short distance, forming the median ventral wall of a portion of the pre-oral enteron (Pl. XXXIV, fig. 4, and Pl. XXXVI, fig. 28). It is by the growth of this tissue that the lining of the stomodæum and proctodæum is formed.

The details of the development of these structures will be best treated in the next section dealing with the alimentary canal.

4. The slime-glands arise in Stage E as hollow invaginations of the ectoderm of the oral papillæ (Pl. XXXV, fig. 23 *d*, *s. gl.*). They gradually increase in length and project into the central compartment of the body cavity. Their further development will be described more conveniently in Part IV of this series. Kennel's description of the origin of these organs agrees with my observations.

The Endoderm.

The endoderm during Stage A and earlier stages consists simply of the inner portion of the vacuolated protoplasmic wall of the embryo. Its vacuoles are somewhat larger and its nuclei fewer and more irregular in shape than those in the outer or ectodermal portion. But the two are perfectly continuous (*vide* figures on Pl. XIV, Part II). The vacuoles of the layer immediately within the ectodermal nuclei are larger than those in the innermost layer, i. e. in the layer next the gut cavity.

Processes from the endoderm cells project into the enteron and anastomose with each other. This is a well-marked feature in embryos rather younger than Stage A, and indicates the origin of the gut as a vacuole or a concresence of vacuoles (Pl. XIV, fig. 23, Part II).

These processes persist until Stage B at the blastopore, as I have already mentioned in Part II of this series (*vide* figs. 24 *b*, 26 *a*, on Pl. XIV, Part II). At the hind end of the blastopore they become particularly well developed (Pl. XIV, fig. 25 *a*, Part II), so much so that it is impossible to say in which section the blastopore ends and the primitive streak begins. In other words, the blastopore passes quite gradually into the primitive streak. Or again, to put it another way, the primitive streak only differs from the hind end of the blastopore in the fact that the anastomosing protoplasmic strands, which everywhere traverse the blastopore, contain nuclei in the former case, but not in the latter. On this view the primitive streak, in favour of which I may refer to Pl. XXXIV, fig. 1, which represents a section through the primitive streak of Stage A, and to Pl. XIV, fig. 25 *a*, Part II, which is a section through the hind end of the blastopore, is the hindermost portion of the blastopore.

The nuclei of the endoderm are large, and particularly remarkable for the irregularity of their shape. They do not, excepting those near the lips of the blastopore, ever present the karyokinetic figures characteristic of dividing nuclei; they appear to divide directly. Some of them are much branched

like connective-tissue cells; a feature which is not at all exaggerated in the figures.

The nuclei of the lips of the blastopore, which have already been described as intermediate between ectodermal and endodermal nuclei, constantly present the karyokinetic figures characteristic of dividing nuclei (Pl. XXXIV, fig. 3, *d. n.*, and Pl. XIV, fig. 26 *b*, Part II).

During Stages B and C, the endoderm, though diminishing somewhat in thickness, retains all the characteristics just described.

During Stage B the blastopore, which has grown considerably in length, and markedly dumbbell-shaped in surface views closes in its middle portion (Pl. XXXII, figs. 23—25, Part I). This is effected simply by the approximation and fusion of its lips (Pl. XXXIV, fig. 5 *b*). The connection, which has hitherto existed between the latter by the anastomosing strands already mentioned, now becomes closer and they completely unite with one another. The result of their union is that the intermediate nuclei come to lie inside and form a definite part of the endoderm, viz. the ventral endoderm in the median line between the mouth and the anus (Pl. XXXIV, fig. 6 *c*). By their fate then these intermediate nuclei are endodermal, and so indeed I think we must regard them, unless indeed we are willing to take the view that the median ventral endoderm of the alimentary canal of Peripatus is ectodermal in origin. Up to the close of Stage C the endoderm has been in close contact and continuous with the ectoderm, excepting where the mesoblastic somites, which appear in Stage B, intervene. In Stage D, however, a remarkable change takes place, the endoderm separates ventrally and dorso-laterally from the ectoderm, and there is now a direct connection between the two only along the dorsal middle line (Pl. XXXIV, fig. 9). This is soon lost in Stage E, and henceforth the endoderm layer is only connected with the ectoderm through the walls and cells of the mesoblastic somites, and at the mouth and anus.

This completes all I have to say about the endoderm till the close of Stage E, when it consists of a layer of vacuolated

protoplasm which contains nuclei of irregular shape. But I still have to describe the development of the stomodæum and proctodæum, and the change produced by it in the adjoining parts of the alimentary canal.

The enteron at first reaches the front end of the body. Until the end of Stage c it has a considerable pre-oral extension (Pl. XXXVI, fig. 28). The anterior end of the body now becomes retracted, so that in Stage d the mouth lies at the very front end of the middle ventral line (Pl. XXXVI, fig. 29), though laterally the two pre-oral lobes project for a considerable distance (Pl. XXXVI, fig. 32). The result of this is that the intermediate nuclei, which in Stage c extended forwards from the mouth (Pl. XXXVI, fig. 28), now extend backwards and form the dorsal wall of the developing stomodæum. At the same time the lateral walls have appeared as special developments of the same intermediate nuclei (Pl. XXXVI, fig. 32).

The median ectoderm of the front end of the body so far has been in contact with the front end of the stomodæum (Pl. XXXVI, figs. 29 and 32). It now separates from it (cf. Part I, figs. 33, 34) and grows forward, so that a space becomes established between the dorsal wall of the stomodæum and the front end of the body (Pl. XXXVI, fig. 31). At the same time the dorsal wall of the stomodæum grows rapidly backwards, while the front end of the enteron maintains its position, or is, perhaps, thrown slightly forward. In this way a blind pocket of the enteron is established, lying on the dorsal side of the stomodæum (Pl. XXXVI, fig. 31, *p.p.*). This anterior blind diverticulum persists for some time (late in Stage f) and then disappears without leaving a trace. It has been observed and described by Kennel.

The stomodæum, the sides and roof of which are first developed (Pl. XXXVI, fig. 32, and Pl. XXXIV, fig. 16 *a*, *b*), soon increases in extent, and by Stage f has acquired a well-developed floor (Pl. XXXVII, fig. 49). It is now definitely established, and has a thick lining of oval nuclei and a narrow lumen (Pl. XXXVI, fig. 36). It has also acquired a mesodermal covering from the splanchnic walls of the first and second so-

mites (Pl. XXXVI, fig. 32, and Pl. XXXIV, figs. 14, 16 *a*, &c.). Its further development will be followed in Part IV, but I may now state that it becomes the pharynx and œsophagus of the adult.

Behind, the walls of the enteron extend to the hind end of the body below the primitive streak, and the anus not being terminal (Part I, fig. 25), there is at first a postanal gut. This state of things continues (Pl. XXXVI, figs. 28, 29) until the formation of the proctodæum, which happens when the anus has shifted to the hind end of the body, and the embryo has acquired its full complement of somites (Pl. XXXVI, fig. 30). The proctodæum is due to the growth of the intermediate nuclei. It eventually becomes of considerable extent, acquires a mesodermal investment from the splanchnic walls of the adjoining somites, and finally constitutes the rectum of the adult (Pl. XXXVII, fig. 42).

The nuclei of the embryonic endoderm of Peripatus are remarkable for being branched and angular. Nuclei of a similar character are found in other animals. Leydig (No. 19) has described branched nuclei in the Malpighian tubules and epithelium of the alimentary canal of Arthropoda, and Balfour (No. 3) speaks of large angular nuclei as occurring in the yolk-segment of Aranea. The angular shape is not retained in the adult Peripatus.

The Mesoderm.

The early stages in the formation of the mesoderm, up to the end of Stage A, have been fully described in Part II of this series.

The nuclei of the mesoderm, which arise from the nuclei of the primitive streak, extend laterally and grow forward on each side of the blastopore, at a little distance from it, as the lateral mesoblastic bands. The mesoblastic bands are therefore, primarily at any rate, outgrowths of the lateral portions of the primitive streak nuclei. This is shown clearly by fig. 26 *a*, *b*, *c* of Pl. XIV, Part II. In this embryo (Stage A) the mesoblastic bands (*mb.*) had only a very small extension forwards (five sections in front of the blastopore). They consist of

bands of nuclei placed in the vacuolated protoplasm intervening between the ectoderm and endoderm.

The mesoblastic bands gradually acquire a greater extension forwards as the embryo and the blastopore increase in length. They very soon become segmented—before they have reached the level of the front end of the blastopore. This fact is clearly shown by Pl. XXXII, figs. 23 and 24, Part I. The segments so formed do not at first (Pl. XXXII, fig. 23, Part I) possess any distinct central cavity around which the nuclei are arranged. By the stage figured in fig. 24, Part I, in which five mesoblastic somites are present, the cavities have appeared. The appearances presented by surface views are confirmed by an inspection of sections. I have sections of embryos, in which three solid somites could be made out on each side. Pl. XXXIV, fig. 2, represents a transverse section through a stage with one solid somite. The section is slightly oblique and passes through the somite of one side only; on the other side the section passes in front of the mesoderm. Fig. 5 *a*—*f* represents sections through an embryo of the stage of fig. 25, Part I, in which the somites had reached the level of the anterior end of the blastopore. A distinct cavity, around which the nuclei of the somites are arranged, is present in the somites. By the close of Stage B (fig. 25, Part I) the somites of the anterior pair have reached their permanent position in front of the blastopore. They are separated from one another by the alimentary canal, which at this stage extends in front of the mouth (Pl. XXXIV, figs. 4, 6 *a*).

With regard to this development the following questions, which can only be settled, if they can be settled at all, by a study of transverse sections, present themselves:—(1) How do the mesoblastic bands grow forward before they have augmented into the somites? and (2) How do the somites, which are established before the bands have reached the front end of the blastopore, reach their final position?

1. Do the bands grow forward independently of surrounding structures? or do they receive nuclei from the adjacent ecto-

derm and endoderm? It must be remembered that the first somite is separated from the anterior end of the mesoblastic bands before the latter have reached the level of the middle of the blastopore, so that we are now only concerned with the old embryos of Stage A. After prolonged and careful study of a large number of embryos, I have come to the conclusion that this is a point which it is impossible to settle with certainty by a study of preserved specimens. Still there is a certain amount of evidence, which, on the whole, tends to show that the intermediate nuclei at the lips of the blastopore do contribute to the bands. The evidence depends upon the appearances presented by these nuclei at the lips of the blastopore. These are constantly met with in a state of division, i. e. presenting the figures which are characteristic of binding nuclei. This is shown clearly in many of my figures, e. g. (Part II, fig. 26 *b*; and Pl. XXXIV, fig. 3, of this paper). Further, immediately within these intermediate nuclei, and lying between the ectoderm and endoderm, there are often to be seen nuclei, which may fairly be supposed to have been derived by division from the intermediate nuclei. These are present in sections through the young mesoblastic bands (Part II, fig. 26 *b*), and also in sections in front of the latter (Pl. XXXIV, fig. 3, close to *d. n.*); in the former case indicating that the nuclei in question are reinforcing the nuclei of the mesoblastic bands; in the latter that they are laying down the same structures.

2. With regard to the second question, I can only state that the completed somites attain their final position by an alteration in the relative position of the structures in the anterior region of the embryo.

Pl. XXXIV, figs. 4, 5 *a—f*, are illustrations of the structure of the embryo at the close of Stage B (Part I, fig. 25). Fig. 4 is in front of the mouth, and is through the first pair of somites, now pre-oral. Fig. 5 *a* is through the mouth and second pair of somites; fig. 5 *b* between the mouth and anus, through the region in which the blastopore lips have come together and fused; fig. 5 *c* is through the anus; fig. 5 *d, e, f*, behind the

anus through the primitive streak. The somites of this embryo differ from those of the earlier stages only in the fact that they contain a well-marked cavity. Their walls are still in continuous contact with the ectoderm and endoderm respectively. The primitive streak of this stage needs, however, more consideration. In the first place the primitive groove has become much more marked and extends over a greater distance than in the earlier stages (Part I, fig. 25, and Pl. XXXIV, fig. 5 *d—f*). Immediately behind the blastopore the primitive streak consists of but few nuclei, which form a layer placed between the ectoderm and endoderm, and extending laterally as far as the mesoblastic bands. The latter structures here bend in towards the middle line, so that a few sections behind (fig. 5 *e*) they have reached and become indistinguishable from the primitive streak. The primitive streak in this region is much more bulky and consists of a large mass of nuclei. It extends back for about thirty sections behind the blastopore. There can be no doubt that the mesoderm of the developing hind end of the embryo, which now begins to grow forward so as to lie with its ventral side on the ventral side of the anterior part (*vide* Part I, figs. 26—31), is derived from the continually proliferating cells of the primitive streak. The latter is indeed simply the growing point of the embryo, and in it the three layers of the embryo are united and indistinguishable from one another. It persists until the full complement of somites is obtained and then disappears. Some time before its disappearance it becomes relatively of less extent, and the anus consequently comes to lie nearer the hind end of the body until, at its complete disappearance, the anus has gained its permanent position at the hind end of the body. Pl. XXXIV, fig. 6 *d*, illustrates the structure and appearance of the streak during Stage c. The section was taken at the hind end and cuts the embryo, the ventral flexure of which is beginning, at two points, viz. in the region of the primitive streak and in the region of the anus. The later stages of the primitive streak are shown best in longitudinal vertical sections (Pl. XXXV, figs. 28—30).

The large pole-cell of the primitive streak, visible during

Stage A, and in the earlier embryos of Stage B, vanishes later in Stage B. I do not know what becomes of it.

Of the mesoblastic somites during Stage C there is but little to say. They maintain the same relations and structure (Pl. XXXIV, fig. 6 *a*—*d*) as in the preceding stage, that is to say their walls are everywhere in contact with the adjacent ectoderm or endoderm. The cells of the somatic layer are thicker than those of the splanchnic layer. This difference was observable in the previous stages (Pl. XXXIV, fig. 5 *a*), and becomes, as we shall see, much more marked in the subsequent stages. During Stage D, somites are still being formed at the hind end from the actively growing tissue of the primitive streak. This stage may be said to mark the close of the formation of new somites, i. e. by Stage E the embryo has acquired its full complement, though the posterior are so small and rudimentary that they are not visible from the exterior.

Summary of the Development of the Body Cavity, Nephridia, and Generative Organs.

The elucidation of the further changes in the somites, and the development of the body cavity and heart, has presented some difficulties,—difficulties which have very much delayed the publication of my work, but which I am glad to say I have at length completely overcome. It will, I think, be convenient to explain the terms I shall use, and briefly to summarise the results I have obtained, before proceeding to a detailed description.

The somites are obviously comparable to the somites of other animals. It is no less clear that the cavity in them is homologous with the cavities of the somites of other types, e. g. other Arthropoda and Annelida. I propose, therefore, to call the system of somite cavities and its derivates the cœlom or enterocœle.

The system of cavities, on the other hand, which arise partly in consequence of the withdrawal of the ectoderm from the endoderm, and partly secondarily in masses of mesoderm, is

what in other animals would be called a pseudocœle. For this second system of cavities and its derivates I shall therefore use the words **pseudocœle** or **vascular space**.

My results can be summed up as follows:

1. The adult body cavity comes entirely from pseudocœle. The enterocœle has no part in its formation.

2. This statement applies also to the heart and pericardium. These are both pseudocœlic, and have nothing to do with enterocœle.

3. The only products of the enterocœle cavity are:—(*a*) The nephridia. (*b*) The generative glands and their ducts.

4. The nephridia do not open either in the embryo or in the adult into the body cavity proper (i.e. in Peripatus the pseudocœle), but into a vesicle in each appendage which has hitherto been unnoticed.

These results, when taken in conjunction with the following peculiarities of Arthropod organisation, viz. the feeble development of the somites, the apparent absence of nephridia, the vascular character of the pericardial cavity, and the possession by the heart of lateral ostia opening into the pericardium,[1] will not be without interest to morphologists.

Kennel (No. 13) was the first to point out that the median chamber of the body cavity and the pericardial chamber were not products of the enterocœle; but Kennel erred in supposing that the cavities in the legs and the so-called lateral sinus (No. 13, p. 202) were derived from the somites. In his later memoir he apparently gives up this view so far as the lateral sinus is concerned, but still maintains that a portion of the cœlom becomes broken up by muscles, &c., and persists as the body cavity of the legs. In this, I think, he is mistaken, but it must be borne in mind that he has worked at a different species in which it is possible, though not likely, that the development of the structure in question may be different.

Kennel's observations of the fate of the so-called median

[1] This Arthropodan character was first pointed out to me by Professor Lankester. I have never seen attention called to it in any works or memoirs on this subject.

divisions of the somites and of the origin of the generative tubes, pericardial cavity, and heart, differ, as will be seen, considerably from mine. His account, however, seems to me to lack precision, and I cannot help thinking, especially when I consider that he has altogether confounded the nephridial vesicles of the adult with the leg body cavity (see below), that he has erred here also.

In my preliminary paper to the Royal Society (No. 33) I also made the mistake of supposing the leg cavities were cœlomic, but my account of the generative organs and their ducts was correct. I expressly stated in that paper that I had not succeeded in following the later changes in the somites. My words were as follows:

"So far the development of the somites is quite clear and easy to follow. But the changes by which the dorsal divisions of the somites are converted into their permanent form take place at a late period of development—during November—and are, in consequence of the thinness of the walls, extremely difficult to follow. I have not succeeded entirely in following them. I will content myself, therefore, with making the following statement, of the truth of which I am by no means confident. The dorsal divisions unite with each other transversely and longitudinally, and give rise to a continuous cavity—the pericardial cavity. The portion of this cavity containing the generative cells become separated from the rest as two tubes which form the generative glands and part of their ducts, and come to lie ventral to the pericardium in the central compartment of the body cavity. The external parts of the generative ducts appear to be derived from the modified leg cavity of the anal papillæ."

With regard to previous observers of the adult anatomy, Balfour—the discoverer of the nephridia—overlooked the small vesicle in the leg cavity into which the funnel of the nephridium opens. This oversight is not to be wondered at in the case of the species (capensis) which he worked at, for the vesicle is extremely difficult to see in the adults of Peripatus capensis. Gaffron—the discoverer of the pericardial cavity and cardiac ostia—similarly overlooked the vesicle, though it is quite distinct and easy to see in the species—Peripatus Edwardsii—which he examined.

Description of the Development of the Mesoderm with its Cavities from Stages d—f.

It will facilitate matters if I begin by describing the development of a particular pair of somites and of the mesodermal structures around them, and I will take for this purpose the third somite of the body (somite of the oral papillæ) as one which is typical of the rest and in which the changes are most easily followed.

During Stage d, at the time when the ectoderm separates from the endoderm, the walls of the dorsal and ventral portions of each somite come together; the cavity is thus obliterated and a single layer of cells[1] lying in close contact with the ectoderm results (cf. Pl. XXXIV, figs. 9 and 12, *d. s.*, *v. s.*).

At the same time some cells appear between the somatic wall of the somite and the ectoderm (Pl. XXXIV, fig. 10, *me.*). These cells, which are undoubtedly derived from the somatic mesoderm, extend the whole length of the somite. In the anterior part, i. e. in front of the limb outgrowth (Pl. XXXIV, fig. 10), they are less numerous than in the region of the latter, i. e. in the posterior part of the somite (Pl. XXXIV, fig. 9).

The same arrangement is present in a more advanced state in the later embryos of Stage d (fig. 29, Part I), cf. Pl. XXXV, fig. 17, *a*—*d*. Fig. 17 *a*, *me.*, shows the mass of cells in the front part of the somite, anterior to its connection with the somatic mesoderm. The same section also shows the anterior part of the limb-ridge overlying this cell-mass. Fig 17 *b* shows the connection with the mesoderm, while fig. 17 *c* and *d* shows this mass in the posterior part of the somite in the region of the limb outgrowth. In the posterior part of the somite some of the cells of this mass project into the cavity of the somite in such a manner as to tend to separate the limb portion from the portion in the body, and at the same time a

[1] It is difficult to say whether these layers of cells arise in this manner or as outgrowths from the dorsal and ventral corners of the somites.

cavity appears amongst the cells at the base of this rudimentary septum (fig. 17 *d*, *b. lat.*). Both these latter features are more clearly shown at *sep.* and *b. lat.* in Pl. XXXIV, fig. 13.

In a slightly more advanced stage (intermediate between D and E) the cells of this parietal mass, as I may call it, are more numerous, and the contained cavity—*b. lat.*—extends forwards to the anterior part of the somite (fig. 21 *a*, *me.*, *b. lat.*); while in the posterior part of the somite, in the region of the limb outgrowth, the rudimentary septum has become more marked (fig. 21 *c*, *sep.*), but the median portion of the cœlom still communicates with the lateral or appendicular portion (Pl. XXXV, fig. 21 *c*). The latter (*l. s.* 3) has developed a ventral outgrowth, which lies along the outer side of the nerve-cord, and reaches the ectoderm. The ectoderm becomes slightly indented at the point of contact, where a perforation is soon formed.

In the next stage (E) four changes are noticeable (Pl. XXXV, fig. 23 *a*—*c*):

(1) The dorsal or median part of the somite has extended itself dorsalwards (fig. 23 *a*). At the same time it does not extend so far backwards as the lateral part (i. e. the part in the appendage), so that the latter is overlapped by the median portion of the somite behind (4) (Pl. XXXV, fig. 23 *d*, *l.s.*3, *s.* 4). (It must be remembered in this connection that the outgrowths into the developing appendage takes place at the hinder part of the somite.)

(2) The space (*b. lat.*) in the parietal mass of mesoderm (*m. t.*) has much increased (fig. 23 *a*), and has, at the same time, become partly divided by a tongue of cells, which eventually give rise to the muscles of the posterior internal projection of the jaw.

(3) There exists a short, anteriorly directed, blind diverticulum (or, may be, constricted-off groove), in the form of a tube, from the neck connecting the median portion with the lateral portion of the somite (fig. 23 *b*, *c*, *a.v.*, and *l. s.* 3).

(4) The lateral portion of the somite has acquired an opening

to the exterior (fig. 23 *e*). This happened in a slightly younger embryo. The opening is already covered over by the lip (*L.*), which is rapidly growing backwards (cf. Part I, fig. 35; embryo with commencing cerebral grooves).

Briefly to recapitulate, the structure of the third somite at this stage is as follows:—In front it is dorsally placed and overlaps the second somite; its middle portion slopes ventralwards and communicates with the posterior part, which is contained in the limb, and is peculiarly bent (fig. 23 *c*—*e*), and opens to the exterior. The middle portion further sends a narrow diverticulum forwards for a short distance (fig. 23 *b*, *a. v.*). The parietal mass of cells is larger than in the last stage, and contains several cavities, which are not derived from the cœlom, but arise independently in it.

As in the earlier stages, there is a sheet of cells closely applied to the ectoderm, and extending from the dorsal end of the somite to the middle dorsal line (*d. s.*), and from the ventral corner (*v. s.*) along the parietal mass of mesoderm and the inner surface of the nerve-cord to the ventral ectoderm. Further, the endoderm has entirely separated from the ectoderm, so that two large spaces are left, the one a dorsal (*b. h.*), and the other ventral to the gut (*b. bc.*). These spaces contain a few scattered, more or less branched cells, which appear to be derived from the splanchnic walls of the somites, and are therefore probably mesodermal in nature.

There are therefore four distinct systems of spaces present in the embryo at this stage (E):

1. The cavity of the gut derived from a vacuolation of the endoderm mass of the gastrula stage.

2. The cavities of the somites, derived from a vacuolation of the protoplasm of the mesodermal bands.

3. The spaces which appear independently of the other spaces in the parietal masses derived from the somatic walls of the somites.

4. The spaces formed by the separation of the endoderm from the dorsal and ventral ectoderm, and derived in all probability from the vacuoles found in a corresponding position in the

earlier stages (Part I, fig. 24 *a*—*d*). These latter spaces are comparable with blastocœle spaces of other embryos, so called because they present the relations of the segmentation cavity of the earlier stages. Such a name is, however, obviously out of place here, inasmuch as the segmentation cavity is never present.

In the next stage (F) the dorsal division of the somite has entirely separated from the ventral, so that the two parts may be considered separately.

The ventral division of the third somite presents the same parts as in the previous stage. These were, it will be remembered, (1) what I may call a vesicular internal part, extending to the hind end of the appendage and forwards as an anterior diverticulum, and opening into (2) a tubular part, projecting ventrally and opening to the exterior.

In Stage F the vesicular internal part (Pl. XXXVI, figs. 36, 37, *l. s. v.* 3) has not only lost its connection with the dorsal (median) part of the somite, but its peripheral part—viz. that in the appendage—has become largely obliterated by the increase in the thickness of its mesodermal walls and by the growth of the slime-gland rudiment. At the same time the tubular part has become longer and more twisted (figs. 37, 38, *l. st.* 3), and its external opening covered up by the lips, which have met on the ventral surface (Part I, fig. 36). The tubular part, therefore, no longer opens freely but into the posterior part of the buccal chamber.

I will now describe more in detail the structure of the two parts.

The inner walls of the internal vesicle retain (figs. 36, 37) the character presented by the walls of the anterior diverticulum of the previous stage (*a. v.* Pl. XXXIV, fig. 23 *b*); i. e. the nuclei are relatively far apart, and separated by a slightly staining protoplasm. The outer wall, on the other hand, is reduced to a thin layer.

The tubular portion I shall now call the nephridium. Its opening into the vesicle, shown in fig. 38, is a well-defined structure, which I shall call the funnel of the nephridium.

The external opening of the nephridium (fig. 37) is anterior to the funnel. The course of the tube at this stage will be understood by an inspection of figs. 37, 38, 38 *a*. It will be observed from these that the nephridium—the part marked *l. s. t.* 3 in fig. 38—projects back as a tube which ends blindly (fig. 38 *a*, *l. s. t.* 3 = *sal. gl.*). This backwardly projecting part gains an enormous extension in the later stages, and is known in the adult as the salivary gland.

Kennel, who has correctly recognised the nephridial nature of the salivary gland, has made what I cannot but regard as one or two blunders in his description of its devevelopment.

He says (No. 14, p. 38), "Von allen Theilen der früheren Segmentblase behält nur der 3. Abschnitt, die blindsackartige Ausstülpung, ihren Charakter, indem dort die Zellen als Epithel angeordnet sich erhalten; er wird zum Trichter des Segmentalorgans." Again, on p. 45, "Man findet ihn (segmental trichter of salivary gland) an seiner alten Stelle, allenfalls ein wenig weiter nach hinten verschoben, als kurzen Blindsac, welcher von seiner Ansatzstelle aus schräg nach vorn dicht am eigentlichen Kanal der Speicheldrüse hin verlauft, *nun aber nicht mehr mit Raumen des Lateralsinus communicirt*, sondern blind geschlossen ist" (my italics).

Combining these passages with a statement on p. 38 as to the breaking up of the Segmentblase of the oral papilla, it seems clear that Kennel imagines (1) that the third somite does not divide into a median and lateral part (though it does so in other somites). (2) That the somite itself breaks up entirely into a system of spaces, of which the lateral sinus is part. This is implied by the first of the above quotations, and the italicised parts of the second. It is also definitely stated on p. 176 of Th. 1 (No. 13) in the following words: "Letzere (i.e. Segmentalhohle) werden später gänzlich in den Lateralsinus und die Höhlung der Füsschen umgewandelt und geben den Hohlorganen daselbst, besonders den Segmentalorganen z. Th. der Ursprung." (3) That the funnel of the salivary gland becomes closed and persists in life as a vesicle. (4) (from other statements on p. 39) that the funnel of the

other nephridia are open throughout life into the broken-up space of the foot (or lateral sinus?).

I admit it is rather difficult to make out his exact meaning, his account being somewhat confused and diffuse. But I think I am right in supposing that he maintains in the paper referred to the above four positions. Well, accepting his idea that the later stages resemble one another in the two species, I have no hesitation in saying that he has erred in each particular. The third somite does divide into two parts. The ventral part does not break up into spaces nor does it become traversed by muscles and connective tissue, but persists through life as a vesicle with an epithelial lining. The lateral ramus has nothing to do with the cœlom, but comes from the space marked *b. lat.* in my sections. The funnel of the nephridium opens always into the lateral part of the somite, of which, indeed, it is a part, and does not become blindly closed. The funnels of the other nephridia do not open into the space of the feet, but into the lateral division of their proper somites exactly as do the salivary glands.

Kennel further maintains that the funnel only of the adult nephridium is mesodermal. I cannot accept this; it is an altogether fanciful view. It may be true, but it is a point quite impossible to settle by sections. With regard to it, I have only to say that the ectodermal ingrowth at the opening of the nephridum is so extremely inconspicuous that at the early stage, immediately before and after the establishment of the external opening, no such ectodermal part as Kennel describes is present.

The dorsal divison of the third somite separates from the ventral in an embryo slightly older than that from which series fig. 23 was taken, viz. one in which the cerebral gooves were slightly more advanced than in fig. 22, but not so much developed as in fig. 33, Pl. XXXVI. After its separation, it becomes much reduced in size (fig. 36, *d. s.* 3), then still smaller (Pl. XXXVII, fig. 45, *d. s.*), and finally vanishes (fig. 46, *d. s.*).

This completes what I have to say about the third somite up till Stage F, by which time the adult condition of the parts

is practically attained. The remaining somites conform, on the whole, to the type described. They do, however, present certain differences, of which, perhaps, the most important are found in the posterior part of the body. In the posterior somites the dorsal divisions do not become obliterated, but persist and give rise to the generative glands (Pls. XXXVI, XXXVII, figs. 41, 43, *d. s.=gen. o.*). It will be more convenient, however, to defer the detailed consideration of this and other peculiar features of the remaining somites until after the description of the changes by which the adult body cavity, pericardial cavity, and heart are formed.

The Body Cavity and Vascular System.

I have already described the first appearance of the body cavity. It arises in Stage D as a space between the dorsal ectoderm and the endoderm (Pl. XXXIV, fig. 13 *b. h.*), and between the ventral ectoderm and the endoderm (*b. bc.*), There also appears at the same stage a space in the parietal thickening of the walls of the somites (Pl. XXXIV, fig. 13, *b. lat.*). In later embryos of Stage D (Pl. XXXV, figs. 17*a—d*), these spaces are all more marked, and cells—apparently amœboid wanderers from the walls of the somites—have made their appearance in the two former (Pl. XXXV, fig. 17 *a—d.*). These cells apply themselves to the ectodermal and endodermal walls of the chambers in which they are contained, and so form the foundation of the mesodermic investment by which the body cavity of the adult is lined. In the next stage the cavities *b. h., b. bc,* remain unchanged; but the cavities in the parietal thickenings become definitely established (Pl. XXXV, fig. 21 *a—c, b. lat.* The latter at this stage appear to be segmentally arranged; each one beginning at the anterior end of a somite, and extending backwards to the level of the appendage, in the mesoderm thickening of which it is lost. They are bounded internally by the septum which runs from the ventral border of the somites along the inner side of the nerve-cord to the ventral body wall, and externally by a mass of mesoderm cells which project into what I have called the limb-ridge (Pl. XXXV, fig. 21 *a, l. r.*).

In the next stage (Stage E, Pl. XXXV, figs. 23 *a*—*c*), the two median cavities, *b. h.* and *b. bc.*, present but little alteration, excepting that the dorsal one *b. h.* has been encroached upon by the dorsal extension of the median division of the somite.

The successive lateral spaces have now become continuous, extending through the region of the appendage immediately within the septum (*v. s.*). This is shown in Pl. XXXV, fig. 23 *c*—*e*, and more clearly by Pl. XXXV, fig. 25. (The tongue of cells in this space in figs. 23 *a*, *b*, I shall refer to later in describing the jaw somite.)

Before proceeding, I may mention the fate of the three divisions of the pseudocœle or permanent body cavity which have so far appeared. The dorsal median cavity (*b. h.*), which is from the first a continuous space, begins at the very front end of the body (Pl. XXXV, fig. 22 *a*, *b. h.*) and extends backwards as far as the ectoderm has separated from the endoderm. It eventually reaches the hind end of the body, and becomes, i. e. all of it, except its very front and hind ends, the heart. The ventral (*b. bc.*), which extends forwards as far as the mouth (Pl. XXXVI, fig. 31), will form the ventral portion of the median chamber of the body cavity of the adult. The lateral cavity, which is at first not a continuous cavity, but eventually becomes so, gives rise in the adult to the lateral chamber of the body cavity (lateral sinus), which contains the nerve-cord and salivary gland.

In Stage E, two new cavities appear amongst the nuclei of the ventral corners of the somites (Pl. XXXV, fig. 25, *b. pc.* and *b. bc'.*). They are first seen at about the level of the seventh somite, and soon (in Stage F) increase in size and extend forwards to the level of the jaw somite, and backwards, eventually reaching the hind end of the body. Pl. XXXVI, fig. 39, shows the typical arrangement of these two additional cavities in Stage F. The dorsal of them (*b. pc.*) has extended dorsalwards as far as the median dorsal pseudocœle or rudimentary heart (*b. h.*), which has in this stage become much smaller. The ventral one (*b. bc'.*) also has extended dorsalwards as far as the ventral wall of the much reduced somite (*d. s.*). The somite, which in the previous

stage extended ventrally as far as the dorsal insertion of the septum (*v. s.*) which separates the lateral chamber (*b. lat.*) from the ventral division of the median pseudocœle (*b. bc.*) (*vide* Pl. XXXV, figs. 23 *e*, 24) has now shrunk, and its space is occupied by the ventral of the two new cavities (*b. bc'.*). This latter chamber, therefore, is bounded dorsally by the ventral wall of *b. pc* and of the somite, internally by the splanchnic mesoderm of the gut wall, and ventrally by a septum separating it from the ventral division of the median pseudocœle (*b. bc.*).

At the hind end of the same embryo the same relations are visible but in a less developed state. Here (Pl. XXXVI, fig. 41) the somites are almost in contact in the middle dorsal line, the heart space (*b. h.*) being very rudimentary. The dorsal division of the somites (*d. s.*) themselves are still well-developed structures with the generative nuclei in their floors. The two new chambers (*b. pc.*, *b. bc'.*) are present, but in a rudimentary form. It is by looking through a series of sections of an embryo of this age, such a series as that from which figs. 33—41 were selected, and comparing them with the previous stage, that it is possible to settle conclusively the fact that in figs. 38 and 39 the space marked *d. s.* and *s.* 4 is the reduced somite—it can be followed backwards, gradually enlarging in successive segments until at the hind end of the body (in figs. 41, 42) it has exactly the relations of the dorsal division of the somite of the earlier stage—and that the spaces marked *b. pc.* and *b. bc'.* are new formations in the walls of the dorsal divisions of the somites and have nothing to do with the true enterocœle or cavities of the somites. They, like the spaces *b. h* and *b. bc*, are, from the first, continuous structures.

The determination of the relations of these cavities in successive stages has been one of considerable difficulty, for this reason, that the dorsal wall of the body often contracts at the death of the animal and obliterates all traces of the complex system of dorsal cavities. They then present merely the appearance, which has been seen by Kennel and has led him into error, of being an irregular system of spaces in the dorsal mesoderm.

Kennel's description of the origin of the heart and pericardium from an irregular system of spaces in the dorsal mesoderm, derived from the broken-up dorsal divisions of the somites, is quite erroneous.

It is easy to see the arrangements which I have described, if embryos be used in which the dorsal ectoderm has not contracted.

Corrosive sublimate solution with a few drops of acetic, or Perenyi's fluid, or 70 per cent. spirit, seem to be the best reagents for obtaining this result, though their action is by no means certain.

With regard to the fate of the two new cavities the dorsal of them (*b. pc.*) becomes the pericardium (Pl. XXXVII, figs. 45, 46); the ventral (*b. bc'.*) enlarges, and by the withdrawal of the gut from the ventral wall of the dorsal division of the somite —which aborts, it will be remembered, in the anterior region (Pl. XXXVII, fig. 46) but gives rise to the generative organs in the posterior (Pl. XXXVII, fig. 43)—becomes continuous with its fellow. It remains for some time separated from the ventral median chamber (*b. bc.*) by the septum, which results from its method of origin. This septum eventually—Stage G—breaks down and the cavity *b. bc'.* becomes continuous with *b. bc* and the two form one chamber, the median chamber of the definite body cavity of the adult.

I have now described the origin of all the parts of the adult body cavity except those in the legs. These arise in Stage F as spaces in the thickened mesoderm of the appendages (Pl. XXXVII, figs. 52, 53, *b. app*).

From the above account it is perfectly clear that, so far as its embryonic development is concerned, the body cavity of Peripatus has, as I stated at the outset, nothing to do with the cœlom. It is a pseudocœle, a space which arises secondarily, i. e. subsequently to the cœlom, partly in the mesoderm masses produced from the walls of the somites and partly as spaces between the ectoderm and endoderm (cf. the vacuoles in the same position in the gastrula stage), which soon become lined by mesoderm cells from the somites. The heart has an origin

identically similar to that of the ventral part of the median chamber of the body cavity. The whole system is probably in communication and functions as a vascular space of which the heart is a specially marked off and contractile tract. The body cavity and pericardium of Peripatus, if comparable with anything in Annelida or Mollusca, must be looked upon as homologous with the vascular system. The pericardium of a Mollusc is, I think (but to these points I shall return later), from its development (Ziegler, No. 36) an enterocœle, and as such has no communication—in this respect resembling the remains of the enterocœle of Peripatus—with the pseudocœle represented by the heart and vascular system and spaces. The chief difference, I take it, between the pseudocœle (body cavity) of Peripatus and the pseudocœle (vascular system and vascular spaces) of a Mollusc is that the latter is usually largely broken up by anastomosing strands of muscle and connective tissue, while in Peripatus the same space is, except in the legs, a fairly continuous and unbroken system.

If the above suggestions are correct, and if at the same time the body cavity and heart of other Arthropoda develope in the same manner as in Peripatus (I shall examine this question later), then that peculiar Arthropod feature, viz. the paired ostia leading from the heart into the pericardium, receives a morphological explanation.

Further Account of the Development of the Somites.

After the foregoing description the various parts of the figures will be intelligible, and I may proceed to give an account of the changes which take place in the other somites. In doing this I shall refer to the changes in the mesodermic tissue generally. The somites, with regard to their development as far as Stage F, may be grouped under six heads:—

1. The somites of the pre-oral lobes, or first pair.
2. The somites of the jaws, or second pair.
3. The somites of oral papillæ, or third pair.
4. The somites of legs 1—17, or fourth to twenticth pair.

5. The somites of the anal papillæ, or twenty-first pair.

6. The rudimentary somites behind the twenty-first.

The heading 4 will have to be further subdivided according as the dorsal divisions contain generative cells or not.

The Somites of the First Pair (somites of pre-oral or antennal segment) take up a position in front of and at the sides of the mouth by the end of Stage B. This position they maintain during the whole of development. Their splanchnic walls are, at first, in close contact with the endoderm of the anterior part of the alimentary canal, and afterwards with the ectoderm of the stomodæum when that is formed (Pl. XXXIV, fig. 8). They grow forward into the antennæ when the latter appear in Stage D, so that the bases of the antennæ are hollow (Pl. XXXIV, fig. 7). Soon—in old embryos of Stage D (Part I, fig. 29)—the cells of that portion of their inner wall which adjoins the ingrown ectoderm, proliferate, and form a mass of cells (Pl. XXXV, figs. 16 *a*, 19 *b*, *ph. m.*) which ultimately give rise to part of the musculature of the pharyngeal wall and tongue.

In Stage E, or possibly late in Stage D, the wall of the posterior external corner of the somite becomes markedly thickened (Pl. XXXV, fig. 19 *b*, *S. o.* 1) and pushed out ventrally into a short pouch. In the later embryos of Stage E, and in young embryos of Stage F, this pouch, which is placed immediately behind the eye and at the level of the origin of the lip from the pre-oral somite, forms a distinct tube lying along the outer side of the nerve-cord (hind end of brain or beginning of ventral cord), and reaching to and fusing with the ventral ectoderm (Pl. XXXV, fig. 22 *b*, *S. o.* 1) immediately in front of the jaw. So far as I can make out, an actual perforation is never formed at the point of contact. The tube persists until the later period of Stage F, being found in embryos in which the cerebral grooves are partly closed (Pl. XXXVII, fig. 5 *o*, *S. o.* 1). It then vanishes without leaving a trace.

There can, I think, be but little doubt that this structure is the rudimentary nephridium of the somite. It presents exactly the same relations as do the nephridia of posterior somites; it

is a development of the posterior, external, ventral corner of the somite; is closely applied to the outer border of the central nervous system, where it fuses with the ventral ectoderm, and, as we shall see in a moment, the part of the somite into which it opens becomes separated from the remainder. With regard to the somite itself, it becomes much reduced in size in Stage E, being encroached upon by the rapidly growing cerebral rudiments (Pl. XXXV, fig. 22 *a*). In Stage F, in consequence of the same process, combined with the great development of the cerebral grooves, the first somite becomes much flattened out (Pl. XXXVI, fig. 33). It lies immediately over the white matter of the posterior lobes of the brain, and becomes divided into two parts posteriorly, viz. an external portion placed close to and immediately dorsal to the eye, and an internal or median portion (Pl. XXXVII, fig. 51, *S.* 1).

The external portion, the walls of which are thicker than those of the median, is continuous with the rudimentary nephridial tube, of which indeed it forms the dorsal end.

The first somite, therefore, behaves exactly as do the posterior somites. It becomes divided into two parts—a median part and a lateral part. The latter sends out a ventral diverticulum, which hugs the outer side of the nerve-cord and fuses with, if it does not open through, the ventral ectoderm.

Kennel has seen the rudimentary nephridium, but he has not appreciated its significance or made out its exact relations to the lateral division of the somite on the one hand and the ventral ectoderm on the other. I should mention that by the time the lateral division of the somite has completely separated from the median, the nephridial tube has nearly vanished. This fact may account for Kennel's omission to notice the connection between the two structures. The suggestion by the same author (No. 14, p. 49) that the eye may possibly be derived from the ectodermal part of the lost nephridium of the first somite, loses, after the discovery of the actual nephridium, any plausibility which it might at first sight have appeared to possess. It is obvious that the nephridium which possesses a rudiment of an external opening behind and ven-

tral to the eye, and within the lip, can have nothing to do with the eye which is derived from the side of the cerebral rudiment.

The Somites of the Second Pair (somites of the segment of the jaws) come to occupy in Stage B their permanent position at the sides of the mouth. In Stage D (the endoderm has separated from the ectoderm ventrally and on each side of the dorsal line, fig. 9) the rudiment of the jaw is laid down, and the somite is prolonged into it. (Pl. XXXIV, fig. 9, which, however, is through the oral papilla, represents quite accurately a section through the jaws at this stage.) The somatic wall of the somite becomes considerably thickened, particularly the ventral portion of it. This is very marked in Stage E (fig. 20, *m. t.*). The somite of the jaw segment is further in Stage E overlapped on the dorsal side by the somite behind (fig. 20). By the close of Stage E (stage of fig. 35, Part I) the portion of the cavity of the somite contained in the jaw has become almost obliterated by the growth of the cells above mentioned. The median portion persists for some time, and furnishes, from its splanchnic wall, cells which apply themselves to the developing stomodæum and assist in forming the pharyngeal and œsophageal musculature.

The median division of the jaw somite, which has from an early period a much less longitudinal extension than the median divisions of the other somites, coalesces in Stage F with the median division of the third somite.

The walls of the ventral portion of the somite, which entirely fill up the jaw, form the muscles of the jaw and are prolonged backwards in the lateral compartment[1] of the body cavity as the tongue of cells, which has been already referred to and is shown in Pl. XXXV, fig. 23 *a—c*, at *m. l.*

In the following Stage (F) the hind end of the jaws becomes enclosed by folds of the dorso-lateral walls of the buccal cavity and constitutes the rudiment (Pl. XXXVI, fig. 36, *le.*) of the internal backward continuation of the inner blade—the so-called lever of the jaw, which is so marked a feature in the adult. The

[1] The posterior end of this tongue of cells lies in the central compartment of the body cavity.

further history of this structure, as well as that of the tongue of cells, which have been already described for Stage E and are shown in sections of Stage F at *m. l.*, Pl. XXXVI, figs. 36—39, will be described in Part IV.

I have not been able to see any trace of even the rudiment of a nephridium of the jaw somite, unless, as Kennel has suggested, the internal prolongation of the jaw be regarded as such.

The Somites of the Fourth to the Fifteenth Pairs, i. e. of the first to the twelfth legs, may be taken together. The development is essentially the same for all, and until Stage F almost exactly the same. The description of the changes of any one of them will therefore serve for all. Like the other parts of the body, they develope in order from before backwards. The dates given in the description below will refer to the anterior somites.

The changes during Stage D are similar to those of the third somite, and the figures 10—12, Pl. XXXIV which were used to illustrate my description of the latter during Stage D, are really representations of these posterior somites. As a result of these changes the thickening of the somatic wall, the leg diverticulum, and the rudimentary septum, which partly separates the latter from the rest of the somites, are established. In Stage E, the somite has become divided into two parts by the completion of the septum,—into an anterior part placed dorsally (fig. 24, *S.* 4) and a posterior part with a very small lumen contained in the leg (Pl. XXXV, fig. 25 *l. s.* 7). These two portions do not overlap, as might be imagined from the earlier stage seen in figs. 13[1] or 21′ *c*, in which the septum is incomplete, the part of the somite in the body on a level with the leg having disappeared. The spaces in the parietal mass of mesoderm, the origin and history of which has already been described, have increased largely in size (fig. 25) and might easily be mistaken for a portion of the true cœlom. It must be carefully borne in mind—as I have already pointed out—

[1] These figures were not taken from the parts here referred to, but inasmuch as they precisely resemble in all particulars sections from these parts, they can be used as illustrations of the text.

that these spaces are, so far as their development in this embryo is concerned, quite distinct from the cavity of the somites, which I regard as the true cœlom. I may, however, draw attention to the fact that the two sets of spaces arise in essentially the same manner, but not at the same stage; the cœlomic spaces arise as vacuoles in the multinucleated bodies called mesoblastic somites, while the parietal spaces arise later as the result of the vacuolation of multinucleated masses derived from the walls of the somites.

The development so far has only differed from that of the third somite in the much earlier separation of the median from the lateral portion of the somite.

In Stage E, the cavity of the lateral portions of the somites becomes extremely reduced in size, in consequence of the enormous thickening of their mesodermal walls (Pl. XXXV, fig. 25, *l. s.* 7), and at the same time confined to the base of the appendage, the whole of the distal part of the latter being occupied by a mass of mesoderm cells.

By the end of Stage E, the cœlomic space of the fourth leg (seventh segment) has acquired an opening to the exterior in nearly the same position as the opening of the third somite, i. e. immediately external to the nerve-cord, and by the same process, viz. a ventral outgrowth from the cœlom, which meets and fuses with the ectoderm (Pl. XXXV, fig. 25). The same process takes place in the three preceding legs (legs 1—3), I think, a little later. If this is so, we have an exception to the prevailing rule of development from before backwards. However this may be, the three preceding somites obviously possess their opening at a slightly later stage (early embryo of Stage F, Pl. XXXVI, fig. 40, *l. s. v.* 6[1]). I should mention that even at this early stage the external openings of somites 7 and 8 have not the same position as in the case of the other legs, but are nearer the periphery of the limb (cf. Pl. XXXV, fig. 25, *o. s.* 7, with Pl. XXXVI, fig. 40, *o. n.* 6).

To sum up, at the beginning of Stage F the lateral portions

[1] The apparent absence of a lumen in the passage in this figure is due to the contraction of the specimen.

of the anterior somites are small spaces in the base of the legs with a ventral prolongation which lies along the outer edge of, and, except in the case of legs 4 and 5, opens to the exterior immediately outside the nerve-cord. In the case of these legs, the opening is a little removed from the nerve-cord and placed on the ventral surface of the leg itself.

At the end of Stage F an important change takes place: the pseudocœle or body cavity of the leg makes its appearance. It arises simply as a space, which is from the first somewhat irregular and traversed by cells, in the mass of mesoderm which occupies the periphery of the appendage (Pl. XXXVII, figs. 52, 53 *a*, *b*, *b.app*). The space almost at once becomes much larger and more conspicuous than the lateral compartment of the cœlom, the outer wall of which—i. e. the wall which separates it from the new cavity—is extremely thin and delicate. So thin and delicate indeed, and so sudden the appearance of the leg pseudocœle, that I was for a long time inclined to the opinion that the latter was derived from a part of the lateral compartment of the cœlom. I have, however, convinced myself, by prolonged and careful study of my sections, that this is not the case, but that the pseudocœle of the leg, both in its origin and subsequent history, has nothing to do with, and is entirely separate from, the lateral or nephridial compartment of the cœlom. The extreme tenuity of the outer wall of the lateral cœlom of the legs is shared by the same structure in the oral papillæ (third somite) (*vide* figs. 38, 38 *a*, *l.s.v.*3, Pl. XXXVI and description above, p. 449).

By the close of Stage F we can distinguish, as in the case of somite 3, two parts in the lateral compartments of the somites, viz. (1) an internal vesicular part, with an internal and dorsal wall in which the nuclei are far apart and separated by a relatively large amount of little staining protoplasm, and an external wall of considerable tenuity separating it from the secondarily developed body cavity of the leg; and (2) a tubular part which leads ventrally to the external opening; and even in this stage, except in the case of the first three legs (somites IV—VI), has begun to become convoluted.

The tubular part, which in the case of the first three legs remains straight even in the adult, becomes the structure which was first described by Balfour (excepting (?) Saenger, whose paper I cannot procure) and called by him the nephridium; while the internal vesicular part, which persists throughout life as a vesicle lying in the leg compartment of the body cavity (pseudocœle) and receiving the internal so-called funnel of the nephridium, has hitherto escaped notice.

The fate of the median compartments of the body cavity of jaws, oral papillæ, and legs 1—15, I have already described. Except in the case of the second and third somites, which coalesce in Stage F, they retain their segmentation until their disappearance. This takes place in Stage F first at the level of about the fifth leg, proceeding backwards and forwards. It is preceded by the diminution in size of the cavity (Pl. XXXVII, fig. 45), and the development of the pericardium.

An indication of the somites remains for a short time as a thickening in the floor of the pericardium, with which thickening the ventral wall of the heart is fused (Pl. XXXVII, fig. 46, *d. s.*). The floor of the pericardium soon, however, separates from the ventral wall of the heart, so that the two halves of the pericardial cavity become continuous. At the same time the dorsal wall of the heart separates from the dorsal body wall, and the heart then forms a tube lying quite freely in the pericardium. The cardiac ostia seem first to appear in Stage F, and are confined in the Cape Peripatus, at any rate, to the posterior part of the heart.

The Development of Somites 16—20 differs from that just described only in so far as concerns the median divisions. The lateral divisions proceed in exactly the same way as in the anterior somites. The median divisions, however, contain in their splanchnic walls the germinal nuclei, and persist throughout life as the generative tubes. Their development therefore requires a special description.

The Generative Organs.

During Stage D a number of especially large (as large as the largest of the ordinary endoderm nuclei), round, granular nuclei appear in clusters in the dorsal endoderm of the hind end of the body. Their anterior limit is the sixteenth somite. They are not therefore seen until the sixteenth somite is formed. When they first appear they are crowded together in a mass in the endoderm at the hind end of the body; but they soon begin to acquire a relation to the cells of the splanchnic walls of the posterior somites. Some of them pass to the surface of the endoderm and project into the somites, pushing the mesoderm cells before them (Pl. XXXV, figs. 26 and 27, *gen.*). I think there can be no doubt that they give rise to the nuclei of the sexual cells of the adult, and I propose to call them the germinal nuclei. With regard to their disposition, I may give the following details:—In an embryo with eighteen somites they were present in the region of the last three, viz. of the sixteenth, seventeenth, aud eighteenth. They were placed in the endoderm near the layer of splanchnic mesoderm, but they did not project, except in one or two cases, into the cavity of the somite.

In an embryo with twenty-one somites these nuclei were present in the region of the sixteenth to the twentieth somite inclusive. They were found in groups in the dorsal endoderm, and a considerable number of them projected into the somite, or, in other words, had migrated from the endoderm into the splanchnic mesoderm (Pl. XXXV, fig. 26).

The same features were presented by an older embryo of Stage E, with the full number of somites. Pl. XXXV, fig. 27, represents a section through the seventeenth somite of such an embryo, and shows very clearly the relations which these nuclei acquire to the splanchnic mesoderm.

The cells of the latter form capsules surrounding the germinal nuclei, which possess but a very delicate (with difficulty visible) protoplasmic investment of their own. By the close of Stage E these germinal nuclei are present in the region of the

sixteenth to the twentieth somite inclusive, lying partly in groups in the dorsal endoderm and partly in the splanchnic mesoderm.

They are of the same size as the larger of the ordinary endodermal nuclei—·014 mm. in diameter. They differ, however, from the latter in their evenly-rounded form, the outline being perfectly smooth, and in the fact that they lie together in groups of three or more.

With regard to their origin, they come, as do all the tissues of the hind end of the body, from the primitive streak; and though there are in the primitive streak in Stage C large round nuclei of their aspect, it is not until Stage D that they can be distinguished in the endoderm with the above-mentioned characteristics.

The median divisions of the somites in the generative region remain continuous with the lateral (nephridial) portions until the close of Stage E. Early in Stage F they have completely separated from the latter (Pl. XXXVI, fig. 41), and now rapidly become reduced in size, so that by the close of Stage F they have the form of somewhat triangular structures placed in the ventral wall of the heart and pericardium (Pl. XXXVII, fig. 43). Inasmuch as the gut has now completely separated from their ventral walls, the generative nuclei have entirely lost their connection with the endoderm—their place of origin. They still lie in the ventral walls of the somites,—a position which they maintain throughout life.

In the next Stage (G, to be more fully described in Part IV of this series) the successive dorsal (generative) portions of the somites of the same side unite with one another, in consequence of the breaking down of the intervening segmental septa, so as to form two tubes (Pl. XXXVII, figs. 47, 48), which are the generative glands. The generative glands separate from the floor of the pericardium, except at their front end, where they remain connected throughout life. They thus have the form of two tubes closely applied together and placed in the dorsal region of the central compartment of the body cavity.

The Somites of the Twenty-first Pair, or somites of the anal papillæ, never become divided into two parts. The median division remains in connection with the lateral (Pl. XXXVII, fig. 44), which, however, as in the case of the other somites, acquires a ventral diverticulum. This hugs the outer side of the nerve-cord, and acquires in late embryos of Stage F an external opening which, however, is much nearer the middle line than in the case of the anterior somites, and, indeed, may be described as being common with that of the opposite side. However this may be, the two openings soon become definitely united to form a single opening,—the generative opening, while the tubes themselves persist as the generative ducts. Whether any large portion of the latter are ectodermal in origin, that is to say, derived from a growth of the lips of the opening at its first appearance, it is impossible to say. Kennel asserts that a large part of the generative duct is so derived, but it is obvious that such a statement, as in the case of the anterior segmental organs, cannot be regarded as anything more than an expression of probability. It is impossible to settle the question by sections, and I know of no other method.

From the above description of the origin of the generative organs and their ducts, which is in the essential points identical with that of my tentative preliminary account (No. 33), it is obvious that Kennel has failed to trace the origin of the germinal nuclei. He also differs from me as to the origin of the generative tubes themselves, which, he asserts, come from the dorsal divisions, not of a series of somites, but of one single pair. If his account is correct, which, seeing that he has not observed the origin of the germinal nuclei, I am inclined to doubt, it would appear that the generative tubes of the Cape species differ from those of the West Indian in this respect. He adopts my account of the derivation of the generative ducts and their openings from the lateral divisions of a pair of somites, though, curiously enough, in another place he stigmatises my description as "falsch."

It thus appears that in Peripatus capensis the nephridial portion of the twenty-first somite does not separate from the

median or generative portion, but remains in connection with the latter and forms the channel by which the generative part of the cœlom communicates with the exterior. The generative ducts are therefore modified nephridia, but it is important to notice that the connection between these structures is not to be compared with the so-called funnel of the normal nephridia. The latter is merely a special portion of the nephridial or lateral portion of the somite, and does not seem to be represented in the twenty-first somite. In the female of the West Indian and South American species, as described by Gaffron and Kennel, the case seems to be otherwise. Both these observers have found between the ovary and receptaculum seminis a diverticulum of the oviduct, which ends in a thin-walled vesicle. This structure is called "Ovarian-trichter" by Gaffron, and "receptaculum ovorum" by Kennel; and the latter observer distinctly states that he does not regard it as homologous with the funnel of a nephridium (No. 14, p. 66), apparently because of the thin-walled vesicle (of which he was the discoverer) which closes up its free end. It seems to me, however, that it is this very thin-walled vesicle which renders it almost certain that the structure in question is homologous with the so-called funnels of the normal nephridia, all of which open into thin-walled vesicles of a nature precisely similar to the receptaculum ovorum. Had the latter been absent and the diverticulum of the oviduct opened directly into the body cavity, as Gaffron at first supposed, then there would have been a very strong reason against regarding the diverticulum as homologous with the nephridial funnels. On my view, then, the receptaculum ovorum would correspond to a nephridial vesicle which had been drawn out of the leg portion of the body cavity and placed in the central compartment.

How comes it that this structure is absent from the oviduct of the South African species? In the neotropical species of Peripatus, in which the receptaculum ovorum is always present, the generative ducts open between a pair of fully-developed legs. In the South African Peripatus, on the other

hand, they never open between a pair of fully-developed legs, but always behind the last pair of such; the legs corresponding to the generative nephridia being more or less completely rudimentary (anal papillæ, &c.); and it seems to me not unreasonable to suppose that this abortion of the appendage has carried with it the abortion or non-development of the portion of the somite which, in the preceding legs, gives rise to the internal vesicle of the nephridia.

It would be of interest in this connection to observe whether the oviduct of the New Zealand species, in which the generative ducts open between a pair of fully-developed legs, possesses a structure corresponding to the receptaculum ovorum.

There are rudiments of two pairs of somites behind the somites of the anal papillæ in Stage E. One of these is just visible in Stage F. They vanish completely at the end of Stage F. No appendages or rudiments of such are developed in connection with them.

General Considerations.

There are four points in the development of Peripatus capensis which appear to me to deserve a more detailed consideration and comparison, with corresponding processes in other types, than it is convenient to give them in a descriptive account. These are: (1) The incomplete segmentation, and syncytial nature of the embryo; (2) the development of the mesoderm; (3) the development of the vascular system, body cavity and cœlom; and (4) the relation of the blastopore to the mouth and anus.

The last point has already been sufficiently considered and its significance pointed out in my paper "On the Origin of Metameric Segmentation" (No. 32). I see no reason to modify the views there set forth on this subject; on the contrary, recent investigations seem to give them additional support.

(1) I have already, in Part II of this series, dealt to

a certain extent with the peculiarities in the segmentation of Peripatus capensis both intrinsically and in relation to other forms. I think, however, that the subject is of sufficient importance to deserve a more detailed treatment than it was possible to give it in that place.

There can be but little doubt that the ovum of this species possessed, at a period relatively not very remote from the present, a large amount of good yolk; that it resembled in fact in this respect the ovum of the species now living in New Zealand, and the ova of Arthropoda generally. The large size, combined with the almost complete absence of food-yolk, can, it appears to me, only be accounted for by supposing that the ovum is passing from the large-yolked to the non-yolked condition, and is intermediate between the ovum of the New Zealand and that of the neotropical species. The ovum[1] of the latter species would on this view have been derived from the large-yolked ovum of some remote ancestor.

There are other instances in the animal kingdom of small ova which there is strong ground for regarding as having been derived from large-yolked ova. The most conspicuous example of this is perhaps that of the Mammalia. Within this class we find both large-yolked and small ova; and the investigation of the former which is now being carried on by Caldwell has particular interest inasmuch as it will show more completely than has been possible hitherto how the development is modified by the loss of yolk. Caldwell's investigations are not yet published, and we do not therefore know whether there is an ovum amongst the lower Mammalia with the property—unique so far as I know—of the ovum of Peripatus capensis, viz. the large size combined with the almost complete absence of yolk.

It is this peculiarity which, while it gives the cleavage of the ovum of P. capensis a great interest, necessitates great

[1] The ovum of the Trinidad species which has been investigated by Kennel (Nos. 13, 14), and of a South American species which I have had an opportunity of examining, is relatively minute (diameter ·04 mm.) and poor in food-yolk.

caution in dealing with the general importance of the phenomenon.

I shall assume, then, to start with, that the ovum of the Cape species has only recently lost it yolk, and that it may be compared to an ovum of the New Zealand form from which the yolk has been almost completely dissolved out by some reagent. As a matter of fact, it is impossible, with our present methods, to effect this complete solution of yolk and leave its protoplasmic framework; but what we cannot effect has been done by nature in the most complete manner, leaving an ovum which is little more than a loose protoplasmic spongework, excepting at one point where the protoplasm is more dense. It is at this point only that the cleavage takes place; for the breaking up of the rest of the ovum into irregular masses cannot be regarded as a process[1] in any way related to cleavage, inasmuch as the nucleus takes no part in it.

The cleavage would appear, therefore, to be meroblastic, and, as in meroblastic ova, the protoplasm round the nuclei at the periphery of the blastoderm is perfectly continuous with that of the main mass of the ovum in which the yolk is contained, but from which it is absent in this ovum; that is to say, we have round the periphery of the blastoderm, and lying in the part of it which corresponds to the yolk of large-yolked meroblastic eggs, a number of yolk-nuclei, or rather of nuclei which correspond to the yolk-nuclei of such large-yolked eggs.

But the cleavage is not only meroblastic, it takes place in the same manner as in centrolecithal ova, i. e. the furrows extend only a short distance into the ovum (*vide* Part II of this series, p. 179); the deeper parts of the segments are continuous with each other. Very soon, however, the loosely reticulated protoplasm extends on to the contiguous surfaces of the segments, from which it was at first absent owing to the fact that the furrows are formed in the densely reticulated protoplasm only. It thus happens that each segment becomes continuous with all the contiguous segments near the surface,

[1] This process is probably identical with the formation of the non-nucleate yolk-spheres found in many Arthropoda.

as well as in the deeper parts (*vide* description of segmentation, p. 179, Part II). By the continued division of the nuclei at the edge of the blastoderm the embryo acquires an external layer of nuclei, which are absent only at one point—the future blastopore.

Of all the forms of Arthropodan cleavage that I know of, this process seems to resemble most nearly that of the mite Tetranychus telarius, as described by Claparède (No. 6). In this form, as in Peripatus, the first segmentation nucleus divides at the periphery of the ovum, and not in its centre, as in most centrolecithal ova. There is, however, the greatest possible variety in the position of the first segmentation nucleus in Arthropoda, and the matter does not seem immediately important. The same cannot, however, be said about the continuity between the segments. This seems to me a matter of the greatest importance at the present moment. It has long been known that the segments of many centrolecithal eggs are at first connected with one another. In proof of this I need only refer to Balfour's summary of the cleavage of centrolecithal ova in vol. i of the 'Comparative Embryology,' and to any of the recent works on Arthropod development (e. g. Patten, No. 25); that is to say, it has for some time been known that the segmentation of centrolecithal eggs is not a complete cleavage, and, indeed, sometimes does not deserve the name of cleavage at all (e. g. most Insecta). But it has generally been supposed that this continuity is soon lost, and that the final result of segmentation is in all cases a mass of completely separate cells (*vide* Patten, No. 25, pp. 565, 567). According to this view the connections which undoubtedly exist between the majority of cells of the adult is purely secondary (*vide* Flemming, No. 7, p. 74).

Two questions now present themselves: (1) Is this view true in fact? (2) Is it genetically true?

In other words, (1) Is it universally true that there is a stage in the embryonic development of the Metazoa in which all the cells of the body are isolated from one another? (2) Has there been such a stage in the evolution of the Metazoa, i. e.

a stage in which the body of the common ancestor consisted of a mass of organically separate cells?

The answer to the first question must be undoubtedly a negative one. The cells arising from the segmentation in Peripatus capensis are at no period of development completely isolated units, but retain a connection with one another throughout life. It is true that some of them break away from the rest, and form blood-corpuscles and generative cells, but the greater number present in the adult a connection with their neighbours—a connection which has been derived directly from the connections between the cells of the segmenting ovum. The same fact, as has been shown by Heathcote (No. 11), holds good for Julus; and it seems to me highly probable that the connections between the various adult cells of other Arthropoda with centrolecithal eggs will be found to be derived continuously from the connections between the cells of the segmenting ovum.

But while we must admit that there are cases in which the cleavage is not complete, yet in a great many animals—in all cases of small holoblastic eggs—it seems to be quite certain that the cleavage is complete. It is true that the spheres always touch one another, and there may be an organic connection at the point of contact; but assuming that there is no such connection, the question naturally arises: which of these two processes—the incomplete or the complete cleavage—is, from a phylogenetic point of view, the more correct?

It has generally been supposed that the complete cleavage is the most primitive process, and that the mass of organically distinct and similar cells, such as is found in the morula of a typical development, represents a colonial Protozoon-like ancestor of the whole of the Metazoa. In short, the general view seems to be that the immediate ancestor of the first Metazoon was a multicellular Protozoon, the separate cells of which were all distinct from one another. Can we find any justification in the animal kingdom, as we know it, for this view? Is there any living form constituted in this manner? The answer is, it is hardly necessary to say, a negative one.

There is no animal composed of a mass of separate and similar cells. All the colonial Protozoa consist of a number of cells connected with one another by protoplasmic filaments; it may be by long contractile filaments, as in colonial Flagellates and Vorticellæ, or it may be by short laterally springing filaments, as in Volvox; and it is by means of these connecting threads that the individuals of the colony effect the little co-ordination of which they are capable.

Further, from an a priori point of view, it seems highly improbable that such a number of disconnected units could have formed a stage in the evolution of the Metazoa. Is it possible, then, that there has not been any such stage, and that the so-called colonial Protozoon stage in the Metazoon ontogeny is purely secondary, and has been produced by the mechanical requirements of individual development? Answering this question for the moment in the affirmative, we come to the alternative view, viz. that incomplete cleavage is the more primitive process. This view, though it possesses, according to our present knowledge, weaker embryological justification than the first, has a far stronger basis of facts derived from the anatomy of living forms. While amongst the Protozoa there is no counterpart of the fully segmented ovum, there is a comparatively large number of colonial forms in which the individuals are connected by irritable protoplasm, and of multinucleate forms, in which the protoplasm, though more (some ciliated Infusoria) or less (some Rhizopoda) differentiated, is without that definite relation to the various nuclei which is characteristic of the colonial forms and of cells in general.

Metschnikoff (No. 22, p. 132), in discussing this very question, contends that the preponderance of complete cleavage, especially in the lower forms, is a strong argument in favour of the colonial Protozoon origin of the Metazoa. Here I differ with him, for in all colonies that we know of the individuals are connected by protoplasmic filaments, which have arisen, not as the result of fusion, but as the result of the incomplete division of the common parent form. A mass of distinct cells, more or less closely applied to one another, is not a colony in

the ordinary acceptation of the term, and it is such a form which, according to what I believe to be the view of Metschnikoff and most morphologists, represents the connecting link between the Protozoa and Metazoa.

But perhaps it will be contended that I am wrong in ascribing this doctrine to them, and that they hold the view that the individuals composing that hypothetical ancestral Metazoon, which is suggested by the cleavage of the ovum, were not completely separate but connected as in living colonies of Protozoa. To this I would reply, that if such be their view, then they can find no justification for it from the development of forms in which complete cleavage occurs. It is rather in such a segmentation as we find in some Sponges (Marshall, No. 21; Sollas, No. 34), in Alcyonarians (Kowalevsky and Marion, No. 17) and most Arthropoda that we shall have to seek the nearest embryological counterpart of the process by which the Metazoa arose from the Protozoa. If this is so, how are we to account for the frequency of the cases in which the furrows, dividing the ovum, completely separate the segments from each other? In the first place, I would ask, are the cases so numerous as is supposed? It seems to me extremely probable that it will be found, on renewed investigation, that incomplete cleavage takes place in many forms in which it has been assumed that complete cleavage is the rule. The complete cleavage of small ova is such a striking phenomenon, and so readily lends itself to speculative suggestions, and has in this form so dominated the views of morphologists (*vide* especially Flemming's remarks above referred to), that I cannot help feeling that it may, in some cases in which perhaps the observation was difficult, have been assumed to occur on insufficient evidence. And this feeling is rather confirmed by the well-known prevalence of the habit of assuming cell boundaries when they cannot be seen. Almost every embryological memoir bears on its plates numerous examples of this habit.

In the second place, it seems possible that the complete cleavage, found so conspicuously in small ova, may be sus-

ceptible of a mechanical explanation. The clean rounded form of the spheres at the moment of division is unlike anything else in the animal kingdom, and is suggestive rather of an intensely active force in the centre of the cell, which compels for the moment the assumption of this form in the protoplasm over which it has dominion, than of a tendency inherited from an adult ancestor. I would refer in this connection to Brook's observations on the total segmentation of Lucifer (No. 5). He describes how, at the moment of activity, the segments round themselves off, touching only at one point, while in the intervals of rest they flatten out against one another, and possibly become partly fused. The same phenomenon is found in other Crustacea (*vide* Balfour, No. 1, p. 112), and it seems fairly generally to happen that at the moments of activity the segments round themselves off, and in the intervals of rest flatten out against each other. These facts seem to me to indicate that it may be possible to find a purely mechanical explanation of complete cleavage. However this may be, it seems pretty clear that the holoblastic segmentation of small ova has not the phylogenetic significance usually ascribed to it.

To sum up, the ancestral Metazoon has generally been assumed to be a colonial Protozoon, and when we examine the evidence for this view we find that the holoblastic segmentation, which really suggested it, is totally opposed to it; and further, that the facts of incomplete cleavage which were thought to be opposed to it are somewhat in its favour, though much more suggestive of another view, which I will now consider.

In Part II of this series I suggested that the ancestral Metazoon was not a colonial Protozoon, but a multinucleated Infusorian-like animal with possibly a mouth leading into a central vacuolated mass of protoplasm, and that evolution of the higher forms has consisted mainly in a definite arrangement of the nuclei and of the specialisation of certain of the vacuoles in the internal protoplasm into the cavities of organs, and of the protoplasmic strands between into the walls of the latter, and into nerves, muscles, &c.

This is not a new view; it is the old view of the origin of the Metazoa, and has been held recently by Saville Kent (No. 28, vol. ii, p. 480) and others. It is entirely in accordance with the facts of the development of Peripatus capensis.

With regard to this development, we have to observe that we cannot speak of cells till a comparatively late period (Stage B), and that the intimate structural change underlying the processes of growth of the young embryo is not an increase of cells, but a multiplication of nuclei. First of all a cortical layer of nuclei, lying in the peripheral layer and entirely surrounding, excepting at one point, the vacuolated spherical mass of protoplasm of which the embryo consists, is differentiated. The central protoplasm, which contains a few nuclei and a great number of large and small vacuoles to which the nuclei have at first no special relation, protrudes from the point at which the cortical nuclei are absent, as though to extend itself in an amœboid manner in search of food. This is the solid gastrula stage (Part II, fig. 20). In it no cell outlines are distinguishable, the whole embryo differing only from, say Vorticella, in its large size, and the presence of a definitely arranged layer of nuclei round its periphery.

From this stage the cœlo-gastrula is derived by the simple process of the confluence of the larger central vacuoles to form a single internal cavity, the establishment of the definite opening of this cavity to the exterior, and the arrangement of the central nuclei in the protoplasm lining it (Part II, figs. 23, 24 *b*). Later the mesoderm appears. It is derived from some of the nuclei already present, which increase in number and arrange themselves in the protoplasm around some of the vacuoles which thus early become specialised into another organ, the cœlom.

Metschnikoff, who has done such important service to biology in drawing attention to the physiological importance of amœboid cells in the organism, has been one of the most prominent advocates of the view that the formation of the gastrula by invagination is a secondary process. He considers

that the animal of which the gastrula is the embryonic reproduction—if indeed it ever existed—was preceded by a form in which the endoderm consisted of a vacuolated mass of protoplasm without any definite enteric cavity. So far most embryologists of the present day are with him. But he goes further; he considers that this parenchymatous gastrula or blastula[1]—or as he calls it, Phagocytella—was preceded by a hollow blastula-like Protozoon form from which it arose by the migration inwards of certain of the cells of the wall of the blastula. This suggestion as to the origin of the gastrula and the form of the ancestral Protozoon has often been criticised, and it seems to me that the facts suggest, with equal strength, quite another view of the matter, viz. that which I have just hinted at.

There are several ways suggested by embryology in which the passage from the Protozoa to the Metazoa may have been effected; and a most admirable and profound analysis of each of these, and a critical review of our knowledge on this subject, is to be found in chap. xiii, vol. ii, of the 'Comparative Embryology.' I cannot do better than quote the words in which Balfour sums up this review of the facts—"Considering the almost indisputable fact that both the processes above dealt with [delamination and invagination] have in many instances had a purely secondary origin, no valid arguments can be produced to show that either of them reproduces the mode of passage between the Protozoa and the ancestral two-layered Metazoa. These conclusions do not, however, throw any doubt upon the fact that the gastrula, however evolved, was a primitive form of the Metazoa; since this conclusion is founded upon the actual existence of adult gastrula forms independently of their occurrence in development" ('Comp. Emb.,' vol. ii, p. 283; the italics are mine).

These words seem to me to express as clearly now, as they

[1] I am not quite sure whether he considers that the cortical layer (kynoblast) of his Phagocytella was interrupted at any point for the protrusion of the central mass (phagocytoblast).

did when they were written five years ago, the state of our knowledge on this subject, and in my opinion neither Metschnikoff's view, nor that which I have just put forward as to the exact method of transition between the Protozoa and Metazoa, can be regarded as anything more than a more or less plausible suggestion without any strong basis of fact.

The gastræa theory, in so far as it implies the existence of an ancestral two-layered organism, is still in accordance with known facts, and no discoveries have been made which decisively settle the mode of transition between the Protozoa and Metazoa. As, however, the subject is an interesting one it seems worth while contrasting Metschnikoff's view with that which I have just put forward. But before doing so, I am anxious to notice one or two points in which Metschnikoff seems to have misunderstood Balfour's views on this subject. Balfour, as is well known, was inclined to the view that the gastræa was preceded by a solid form, such a form as Metschnikoff terms parenchymella, and Metschnikoff himself quotes (No. 23) passages from the 'Comp. Embryology' which show this; and yet Metschnikoff represents Balfour as being opposed to the parenchymella theory. It is not quite clear to me what exactly Metschnikoff means by the parenchymella theory; but if this theory merely postulates the existence of a beast with an outer ectodermal layer and an internal mass of amœboid cells, then I have no hesitation in saying that Metschnikoff is mistaken in regarding Balfour as having been actively opposed to it. It is true that Balfour thought that Metschnikoff's view as to the method of origin of the parenchymella was improbable; but surely one may accept the parenchymella without holding the precise views of Metschnikoff as to its origin, just as one may accept the gastræa theory without pinning one's faith to any particular view of the mode of origin of the gastræa. It appears to me that Metschnikoff, in dealing with both the parenchymella and gastræa theories confuses two questions.

(*a*) Was there an ancestral gastrula, with the characters attributed to it?

(*b*) If so, how did this ancestral form itself arise?

If the answer to the latter question falls within the province of the gastræa theory, Balfour did not accept that theory and Metschnikoff is wrong in saying that he was strongly inclined towards it. If, on the other hand, the gastræa theory simply generalises from a large number of anatomical and embryological facts as to the past existence of an animal with a particular structure, and leaves the question of origin out of consideration, then Balfour undoubtedly did accept the gastræa theory, but did not thereby, as Metschnikoff seems to think must necessarily have been the case, reject the Parenchymella. On the other hand, Balfour expressed himself distinctly in favour of the latter, though he did not call it by that title, for does not he say, and does not Metschnikoff quote him as saying (No. 23, p. 156), that he thought it probable that the ancestors of Cœlenterates possessed a solid endoderm of amœboid cells?

This is not the only point in which Metschnikoff has misunderstood Balfour's views. On p. 141 of No. 23, he ascribes to him the view that an amphiblastula form would represent more nearly than any other the transition between the Protozoa and Metazoa. Balfour maintained no such position, as has been already pointed out by the translator of Metschnikoff's paper on the "Intracellular Digestion of Invertebrates," 'Quarterly Journal of Microscopical Science,' vol. xxiv, p. 107, note.

But to resume: Metschnikoff supposes that the parenchymatous ancestor was preceded by a hollow spherical form, the blastula, the cells of which were all alike; and that the blastula became a parenchymatous gastrula by the migration inwards of cells from its external wall.

I do not understand on what grounds Metschnikoff is so strongly disposed towards the view that the hollow blastula represents a primitive form.

There are many cases, even amongst the Cœlenterates, in which a hollow blastula is not formed and segmentation gives rise to a solid planula, and in the rest of the animal kingdom,

the cases, in which segmentation gives rise to a solid embryo are quite as numerous, if not more so, than those in which the reverse holds.

I would even go further than this, and maintain that Metschnikoff's view, that the ectoderm is primitive and the endoderm secondary—arising from the former by inwandering —is not more in accordance with the facts of embryology than the opposite view, viz. that the endoderm is primary, giving rise to the ectoderm by budding-off cells—outwandering as it may be called. In almost all Invertebrate groups there are instances of the latter process, in which the ovum, before or after division into two or more large cells, buds out a number of small cells which form the ectoderm; either itself, or the large cells produced by it, persisting, and eventually, after the appearance of a central cavity, forming the endoderm.

I do not mean to say that the facts are more in favour of this view than in that of Metschnikoff, but I think they favour the one as much as the other.

But granting the hollow blastula as a possible animal, I agree with Bütschli in thinking that there are great physiological difficulties in the way of accepting the process by which, according to Metschnikoff, it may have become transformed into a solid form. Surely a hollow sphere is in a much more advantageous position with regard to nutrition than a solid one; and yet Metschnikoff supposes that the transformation into the solid form was due to the migration of surface cells into the interior for nutritive purposes. The central part of the animal being empty would require no nutriment, and even if it did, what more convenient arrangement could there be than a layer of cells to pass prepared nutritive substances into it? The ectoderm of Hydra or of Cœlenterates in general is not fed by the migration of cells from the endoderm, and in short we know of no instance in the animal kingdom of food being carried from one part of an organism to another in actively migrating cells. It seems to me much more likely, if the ancestral Protozoon was a hollow blastula, that the first differentiation would have been into locomotive sentient cells

and cells for acquiring food, i. e. the differentiation supposed by the invaginate gastrula hypothesis. For it is clearly necessary on this view that the nutritive cells should be in direct contact with the external medium, and this they are not on Metschnikoff's view.

Then again, what justification do we find in the animal kingdom for the hollow blastula hypothesis? With the possible exception of Volvox, I know of no form at all approaching a hollow blastula in structure.

These difficulties are avoided by the hypothesis that the primitive form was solid, which also suits the facts quite as well. (1) It is no longer necessary to suppose the migration inwards of cells. (2) There are a considerable number of instances in the animal kingdom of lowly organised solid forms which give a certain amount of justification to the hypothesis of a solid ancestor. There is Trichoplax (No. 29), the Orthonectidæ, the whole of the Protozoa, the acœlous Turbellaria, and finally the Sponges, or at least some of them under certain conditions. The latter case is of particular interest, and deserves a little attention here.

Metschnikoff (No. 22, p. 372) states that Halisarca, when overfed with carmine, loses its canals and becomes a mass of amœboid cells containing swallowed food, and surrounded by a common envelope of ectoderm. The same fact has been observed by Lieberkühn in Spongilla (No. 20), in winter. From these observations, and others by Hæckel and Carter (quoted by Metschnikoff, No. 22, p. 361), it appears that under certain nutritive conditions, the flagellated endoderm cells of sponges may lose their flagella and become amœboid, and the whole sponge revert to the condition of the larva of Aplysina (Shulze, No. 30, Pl. 24, fig. 30) of a protoplasmic network with nuclei at the nodes, and a cortical layer of ectoderm.

Metschnikoff (No. 22) has further observed that in many cases the food of the sponge passes directly into the parenchyma and is not found in the collared endoderm cells; while in other cases it is found both in the cells of the endoderm and parenchyma. These facts, as Metschnikoff points out, seem

to imply that the endoderm cells are not mainly nutritive, but that their main function is to cause the currents through the sponge body, and that the food brought by these currents passes into the parenchyma, through the walls of the passages, to be digested by the so-called mesoderm cells.

The collared cells are thus inconstant, and appear to be merely parenchyma cells specially modified under certain conditions and capable of passing back into their original form when the need for them has passed away. When they vanish the canal system also goes and the sponge becomes solid so far as the latter is concerned. Inasmuch as the parenchyma cells, and probably also the cells of the ectoderm, are all connected by their processes (except in the cases in which they break away and become amœboid), it is clear that the sponge in this condition and in the case of Shulze's larva already referred to, is a syncytium, and but little more than a multinucleated Protozoon. It differs from such a Protozoon simply in the greater development of the vacuoles (spaces between the cells) of the central portions, and in the presence of a distinct cortical layer of nuclei.

In some cases this assumption by a sponge of the Protozoon form is much more marked than in the above cases. I refer to the case of the well-known form Haliphysema, described by Hæckel (No. 10) as a sponge with an axial ciliated chamber traversing it, and by Lankester (No. 18) as a multinucleated Rhizopod. It is difficult to believe that either of these distinguished naturalists can have made the mistake implied by these contradictory observations; and the only way of reconciling the latter that I know of, is to be found in the above view, viz. that Haliphysema, like Spongilla and Halisarca possesses under certain conditions, the power of becoming solid: that in certain conditions in which it was found by Hæckel, it approximates to the sponge, while under other conditions in which it has been found by Lankester and Saville-Kent it loses its sponge-like structure and comes to resemble a multinucleated Protozoon. There are certain points in Lankester's description of the soft parts which favour

this view, e. g. the obviously reticulated nature of the protoplasm (see particularly fig. 9 in Lankester's paper), the large number of nuclei, and, finally, the germ cells.

These facts, though not in any sense proofs of the view of the origin of the Metazoa for which I am contending, are at any rate suggestive of it, and, so far as they go, in favour of it. That is to say, they suggest the view that there would be two courses open to a Protozoon after it had reached a size too great for the proper nutrition of its central portion, i. e. a size in which the ratio of surface to mass was unfavorable; it would either divide, in which case it would remain a Protozoon, or it would develop from its vacuoles a system of connected and specialised channels with a definite communication to the exterior at one or more places. In the latter case it would constitute the first stage in the evolution of the Metazoa.

There is no reason to suppose that the protoplasm of such a form, even though partially broken up into areas round the various nuclei, would thereby lose the power of taking in to itself foreign substances which were presented as nutriment; in other words, would not prevent it from discharging the functions discharged by the protoplasm of all Protozoa, and by the parenchyma cells or phagocytoblasts of Metschnikoff.

To sum up, while fully agreeing with Metschnikoff, that the formation of the endoderm by invagination of the wall of a hollow blastosphere is a secondary process, I cannot accept his position that the hollow blastula is a primitive form, or that the formation of the endoderm by migration inwards of the cells is a primary process. It seems extremely probable that the blastula has arisen to provide for the better nutrition of the growing embryo, and that the inwandering and invagination are alike secondary processes, the object of which is, when the proper stage is reached, to get the protoplasm back to its central position and ready for the development of the system of channels which render its maintenance in the inside possible.

(2) The mesoderm in Peripatus arises from certain nuclei in the middle ventral line behind the blastopore. These nuclei may, as I have attempted to show above, fairly be regarded as

corresponding with the nuclei in the lips of the blastopore, intermediate in character as well as in position, between the ectodermal and endodermal nuclei. The multiplication of these nuclei gives rise to a primitive streak, which, as in the Vertebrata, is entirely posterior to the blastopore, and is marked by a longitudinal groove—the primitive groove.

This process resembles, in all essential points, the formation of the greater part of the mesoderm in other Tracheata from the walls of the germinal groove, differing only in this, that whereas in the latter the germinal or primitive streak occupies the greater part of the ventral surface, in Peripatus it is confined to the part of the ventral surface behind the anus.

I have elsewhere (No. 32) stated my reasons for agreement with Balfour's view, viz. that such a method of mesoderm formation is probably to be regarded as a modification of archenteric diverticula, such as are found in Amphioxus, &c. Whether the origin of mesoderm from the walls of archenteric diverticula is a primitive process or not is open to grave doubt.

It seems to me there is a large body of embryological facts which suggest, at any rate, the view that the mesoderm arose as a result of the differentiation and rearrangement of certain of the nuclei of the amœboid central mass of the ancestral parenchymella or gastrula; that is to say, the facts seem to suggest the following as a possible general view of the origin of the three layers of the Triploblastica.

(*a*) Starting with a large multinucleated Protozoon, the first advance consists in the differentiation of a cortical layer of nuclei and of the protoplasm governed by them into a peripheral layer or ectoderm. This layer was possibly of a plastic nature, and allowed the protrusion of the central mass at one or more points. The central mass would, in consequence of its large size, probably be capable of arranging its vacuoles into a series of thoroughfares through itself from one opening on the surface to another, so that the introduction of nutritive matters to its deeper parts would be possible. On the analogy of the Platyhelminth excretory system we may

imagine that the protoplasm of these tracts would acquire the property of throwing out vibratile processes into this system of channels for the purpose of assisting in an effective circulation of the external medium through the body. Such an animal would consist, then, of an ectoderm and a central multinucleate mass which, with Metschnikoff, we may call the meso-endoderm.

(*b*) The next change would consist in the differentiation of the nuclei of the meso-endodermic mass into two kinds: (*a*) those governing the protoplasm lining the differentiated vacuoles; and (*b*) the remainder, which would gradually differentiate into various kinds as evolution progressed. The differentiation of the protoplasm around the nuclei would proceed hand in hand with that of the nuclei; the result being a gradually increasing complexity in the tissues of the animal.

The result would be, if the canal system remained complex, —a sponge; if, on the other hand, the canal system simplified and preserved only one opening, the ancestor of the other Metazoa.

It is beyond the scope of this paper to discuss the evolution of the mesoderm. I merely throw this out as a suggestion, which is supported by the manner and order of development of the layers in many animals (a peeling off, so to speak, from the ovum: (1) of ectoderm; (2) of mesoderm; (3) leaving the endoderm as the remaining central mass), and as a completion of the scheme which I have put forward in discussing the manner of passage from the Protozoa to the Metazoa.

Finally, I would desire to draw attention to the fact (1) that the formation of mesoderm in Peripatus is essentially a formation of nuclei, which pass to their respective positions and arrange themselves in the protoplasmic reticulum there present; and (2) that the primitive streak is the growing point of the animal, from which almost all the tissues of the body of the adult, viz. ectoderm, endoderm, and mesoderm are formed. This is an important point, to which sufficient attention has not been directed. Almost the whole of the embryo, behind

the fifth or sixth somite—not merely the mesoderm, but all the layers—derives its nuclei from the primitive streak. The primitive streak nuclei are therefore not merely mesodermal, but ectodermal and endodermal as well.

3. The last feature in the development of Peripatus capensis which I would desire to notice in its general bearings, is the development of the body cavity and the fate of the cœlom.

The cœlom, as is well known, is the term applied to a body cavity with certain characters—characters which may be summed up in the following terms:—(1) The cœlom does not communicate with the vascular system; (2) it communicates with the exterior by nephridial pores; (3) its lining gives rise to the generative products; (4) it develops either as one or more diverticula from the primitive enteron, or as a space or spaces in the unsegmented or segmented mesoblastic bands (in the latter case called mesoblastic somites).

The vascular space has none of these characters, and is known as a pseudocœlic space: it develops either from the blastocœle or from a system of channels hollowed out in the mesodermic tissue of the body. In the Annelida and Vertebrata these two spaces co-exist, and present a well-marked contrast to one another; while in the two other great groups of the animal kingdom—the Mollusca and Arthropoda—the relations of the two systems has not been thoroughly understood. We will first consider the case of the Arthropoda.

The body cavity in the Arthropoda has generally been regarded as cœlomic, in spite of the fact that it presents none of the ordinary cœlomic characters. It communicates with the vascular system, it does not open to the exterior by nephridial pores, its lining does not, so far as is known, develope the generative cells, for the generative glands are continuous with their ducts, and, so far as is known, have no connection with the body cavity. Neither has the body cavity been traced into connection with the undoubted cœlom of the embryo. In all the groups of the Arthropoda mesoblastic somites with a more

or less well-marked cavity are formed in the embryo; but the fate of these structures has never been followed. We do not know whether their cavities enlarge and unite with one another and give rise to the body cavity and vascular system of the adult, or whether they shrivel up and disappear, their walls only remaining as part of the mesoderm. From what has been said it is also clear that it is impossible to say whether in the Arthropoda the vascular system is nipped off from the cœlom, or whether it arises as a separate set of spaces in the mesoderm, as in Annelids and Vertebrates.

Now, Peripatus is a true Arthropod so far as its body cavity is concerned: thus the heart drives the blood into it, and by means of the paired cardiac ostia sucks the blood out of it; it does not communicate with the exterior by nephridial pores, nor does its lining develope generative cells. We are therefore justified in regarding the body cavity of Peripatus as homologous with that of other Arthropoda. It results from this that the study of the development of the body cavity in Peripatus, which can be traced with comparative ease, must be of extreme interest, as tending to clear up the question of its cœlomic or non-cœlomic nature in Arthropoda generally.

Kennel was the first to trace the body cavity of Peripatus. He showed that it was in part, at any rate, a pseudocœle, but his work was incomplete in that he failed to follow correctly the fate of the cœlom. He thought that the cœlom became merged into the body cavity. If this were correct, it would follow that in Peripatus the vascular system and cœlom would be in communication.

As has been fully shown in the preceding pages, this is not the case. The cœlom of Peripatus can be traced through the whole development, as a system of spaces shut off at all stages of its growth from the system of body-cavity spaces. In the adult Peripatus the cœlom is in the following condition: (1) a series of nephridia ending internally in small thin-walled closed vesicles; (2) two dorsal tubes—the generative glands and the ducts of these, which latter are derived from one pair

of posterior somites. The pericardium, heart, whole of the body cavity (central, lateral, and leg compartments) are exclusively pseudocœlic in origin.

In Peripatus, therefore, the gonads are cœlomic, and their ducts what Lankester would call nephrodinic.

The condition of the body cavity and cœlom of Peripatus will be best appreciated by comparing it with that of the same organs in an Annelid, such as Lumbricus. 1. In Lumbricus the structures corresponding to the nephridial vesicles of Peripatus have swollen up and united with one another in pairs across the middle dorsal and ventral lines, and after some time have become united with one another longitudinally, though the separating walls between successive somites for the most part persist; they constitute the cœlomic body cavity of Lumbricus. 2. In Peripatus the vascular channels, excepting the heart, are swollen out to wide channels, more or less completely continuous with one another, so as to form four or five main vascular tracts, while in Lumbricus they are present as minute, branching, well-defined canals.

On comparing Peripatus with other Arthropoda in this connection we are at once met with these facts: (1) that in no other Arthropod are nephridia, recognisable as such, present; (2) that the cavities of the somites cannot be traced beyond a comparatively early stage of development; (3) that the early stages of the generative organs have not been thoroughly made out.

We may, however, with fair probability predict, from what we know (1) of the development of Peripatus, and (2) of the resemblance of its body cavity to that of other Arthropods, that when the development of the latter has been fully worked out it will be found that the cœlom of the embryo persists as the generative tubes and their ducts, but for the most part vanishes (possibly giving rise to glands of a doubtful nephridial nature), and that the body cavity and vascular system has an exclusively pseudocœlic origin.

In the Mollusca the cœlom and vascular space have not been generally sufficiently distinguished from one another. There seems, however, to be no doubt that the pericardial

cavity of the Lamellibranchiata and Gasteropoda represents the entire cœlom. The reasons for this conclusion are (1) the pericardial cavity is always shut off from the vascular system; (2) it communicates with the exterior by a pair of nephridia.

The generative organs have no relation to the cœlom, so far as is known, in either of the above Molluscan gronps; but in the Cephalopoda the generative cells are developed from the mesoderm lining a certain part of the cœlom. This generative part of the cœlom seems, however, to be shut off in the adult from the viscero-pericardial sac.

This fact, viz. the cœlomic nature of the generative organs of the Cephalopoda, together with the fact that in other Molluscs the generative organs either dehisce into one of the nephridia, which morphologically are part of the cœlom, or possess ducts which open close to or into the nephridial ducts, seems, to say the least of it, in favour of the view that the generative organs of all Molluscs were originally cœlomic and that the present arrangement found in the majority is secondary. The question, of course, can only be settled definitely by embryological investigations, but, unfortunately, embryology does not speak clearly on the point.

There can then, from the point of view of adult anatomy, be but little doubt that the pericardial cavity (and viscero-pericardial and generative sacs in Cephalopoda) alone is cœlomic in the Mollusca, and that the other system of spaces whether simulating a body cavity as in Chiton and other Gasteropoda, or forming a close meshwork of spaces as in Lamellibranchs, are vascular and non-cœlomic spaces; and it is only necessary for embryology to bear out this conclusion to settle the matter definitely. Unfortunately, embryologists have not for the most part sufficiently regarded in their investigations the importance of the point, and, for the majority of Mollusca, we are in ignorance as to the exact method of development of the pericardium as opposed to the heart and vascular spaces.

Rabl (No. 26), Patten (No. 25 *a*), and Ziegler (No. 36) have described mesoblastic bands in Planorbis, Patella, and

Cyclas respectively, arising in the typically Annelidan manner; but Ziegler, so far as I am aware, has alone succeeded in ascertaining what part these bands take in the formation of the pericardium, generative organs and kidney. The generative cells are derived from the mesoblastic bands. The pericardial cavity arises as two cavities—one in each band—which subsequently unite. The kidneys are hollowed out in certain masses of cells of the bands. These results, if generally applicable, appear to confirm absolutely the anatomical proof of the cœlomic nature of the Molluscan pericardium. It is interesting to notice that in Ziegler's figure (fig. 27) the developing pericardial vesicles have exactly the same relation to the primary body cavity or vascular space, i.e. they lie within it, as the nephridial vesicles of Peripatus have to the vascular cavity of the leg.

There are certain animals to which the above general considerations as to the distinctness of the cœlom and vascular system do not apply. I refer more especially to the Nermertinea and Hirudinea. In the Nermertinea, according to Oudemans (No. 24), and in the Hirudinea, according to Bourne (No. 4*a*), structures which it is difficult to believe are not nephridia open into the vascular system. I do not intend to discuss these cases now, because, on the one hand, this paper is already too long, and because, on the other, I do not think our present knowledge is sufficient to enable this to be done with profit. But I venture to submit with regard to them that it is not clear in either case that the vascular system into which the nephridia open is homologous with that of other types. The very fact that there is a communication with the exterior is a strong point in favour of the space being cœlomic; and it should be remembered that very little is known with regard to its development in either group.

In conclusion, I may point out, that whereas in most animals, e.g. Annelida, Mollusca, the vascular space or pseudocœle appears before the cœlom, in Peripatus the cœlom appears first, and that in Arthropods, at least, the vascular space is in the early stages very commonly occupied by yolk, while the cœlom is entirely free from yolk. This latter

fact would seem to imply some connection between the vascular space and the enteric space; and I would also desire to point out that the cœlom, generative glands, and nephridia can, in all animals whose development is at all well known, be traced back to a very early embryonic structure, which appears at the very beginning of development, gives rise to no other structures, and itself arises in very different ways in different animals. The embryonic structure I refer to is in some cases the mesoblastic bands, and in others enteric diverticula. That these two kinds of cœlomic rudiments, as I may call them, are homologous cannot be doubted, but which, if either, of the methods of origin is primitive, cannot in my opinion at present be settled.

Summary of the above Remarks on the Cœlom and Body Cavity.

It is well known that the vascular system of the Arthropoda is in direct communication with the body cavity, and that the vessels are, for the most part, very rudimentary. In fact the blood is driven by the heart or dorsal vessel into the body cavity, and returned directly through the lateral cardiac ostia into the heart. In no other group of animals does this direct communication exist between the heart and the pericardium.

It is therefore important to determine by the study of development, whether or no the blood-containing body and pericardial cavities of the Arthropoda are homologous with the corresponding structures of other types, in which they do not contain blood.

The development of the Arthropodan heart and body cavity is in most cases extremely difficult to follow on account of the large amount of food yolk present in the embryos, and there is not, at present, any completely satisfactory history of it.

The development of Peripatus capensis, which is a true Arthropod, so far as its body cavity and vascular system are concerned, is comparatively easy to follow.

The cœlom appears in the ordinary manner as a series of cavities, one in each mesoblastic somite.

The somites, which are at first ventro-lateral in position,

soon acquire a dorsal extension, and the cavity in each of them becomes divided into two parts—a ventral part which passes into the appendage, and a dorsal part which comes into contact but does not unite with its fellow of the opposite side on the dorsal wall of the enteron.

The dorsal portions of the somites early become obliterated in the anterior part of the body, but posteriorly they persist, and those of the same side unite with each other so as to form two tubes which are the generative glands.

The ventral or appendicular portions persist and retain their original isolation throughout life. They give rise to two structures:

(1) To a coiled tube, which acquires an external opening through the ventral body wall at the base of the appendage and constitutes the nephridium of the adult;

(2) To a small vesicle, which is contained in the appendage and constitutes the internal blind end of the tubular or nephridial portion of the somite. (The opening of the nephridium into the vesicle is funnel shaped, and is commonly known as the internal funnel-shaped opening of the former.)

From the above account it follows (1) that the cœlom of the embryo of Peripatus capensis gives rise to the nephridia and generative glands, but to no part of the body cavity of the adult; (2) that the nephridia of the adult do not open into the body cavity.

The body cavity of the adult consists, as is well known, of four divisions:—(*a*) the central compartment containing the intestine and generative organs, (*b*) the pericardial cavity, (*c*) the lateral compartments containing the nerve-cords and salivary glands, and (*d*) the portion in the appendage.

Of these, without going into details, it may be said that *a* arises as a space between the ectoderm and the endoderm, *b*, *c*, and *d* as spaces in the thickened somatic walls of the somites. The spaces are in communication with each other.

The heart arises as a part of *a* which becomes separated from the rest. Posteriorly it acquires paired openings into the pericardium. It thus appears that the heart and various

divisions of the body cavity of the adult form a series of spaces which have nothing to do with the cœlom. They all communicate with each other and seem to form a series of enormously dilated vascular trunks, of which the heart is the narrowest and alone possesses the property of rhythmically contracting.

To sum up it appears that the cœlom in Peripatus is an inconspicuous structure in the adult, and has no connection with the body cavity; while, on the other hand, the spaces of the vascular system are but little subdivided, and form the heart and various divisions of the adult body cavity.

If these results are applicable to the Arthropoda generally, and there is no reason, from the similarity of the adult anatomy, to doubt that they will be found to be so, the following morphological features may be added to those generally stated as appertaining to the group—cœlom inconspicuous, body cavity consisting entirely of vascular spaces.

In Vertebrates and most Annelids, on the other hand, the parts in question are arranged as follows:—Body cavity entirely cœlomic; vascular spaces broken up into a complicated system of channels (arteries, veins, capillaries).

In most Molluscs, finally, the pericardium alone is cœlomic; the vascular spaces being represented by the heart and the more or less complicated system of spaces in the body.

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EXPLANATION OF PLATES XXXIV, XXXV, XXXVI, & XXXVII,

Illustrating Mr. A. Sedgwick's Paper on "The Development of the Cape Species of Peripatus."

List of Reference Letters.

a. Anus. *am.* Amœboid wandering cells in body cavity (pseudocœle). *a. s. ph.* Septum attaching anterior part of pharynx to dorsal body wall, at junction of somites 1 and 2. *a. v.* Anterior diverticulum of nephridium of oral papilla. *b.* Space formed by withdrawal of endoderm from ectoderm. *b*^{1}. Space formed in the parietal thickening of the somites. *b. app.* Space formed in appendage. *b. bc.* Space formed by separation of endoderm from ventral ectoderm. *b. bc*^{1}. The ventral of the two spaces formed in wall of somites at their ventral corner. *b. h.* Space formed by separation of endoderm from dorsal ectoderm. *b. lat.* Space formed in parietal mesoderm. *b. pc.* The dorsal of the two spaces formed in the wall of the somites at their ventral corner. *br.* Brain. *buc. cav.* Buccal cavity. *c. c.* Cords of cells projecting into pericardial cavity. *c. g.* Cerebral grooves. *c. g.* Groove in brain. *com.* Commissure between the two halves of the brain. *c. o. n.* Circumoral part of central nervous system. *d. n.* Dividing nuclei of endoderm near the lip of the blastopore. *d. s.* Dorsal sheet of somatic mesoderm. *d. s.* 1, 2, &c. Dorsal part of somites 1, 2, &c. *e.* Eye. *ec.* Ectoderm. *en.* Endoderm. *f.* Funnel-shaped opening of tubular part of nephridium into internal vesicle. *F.* 1, &c. Legs. *f. bl.* Line of obliterated blastopore between the mouth and anus. *gen.* Germinal nuclei. *gen. d.* Genital duct. *gen. o.* Generative organ. *g.* and *g. g.* Alimentary canal. *J.* Jaw. *j. s.* Ventral organ of jaw. *L.* Lips. *le.* Internal backward projection of jaw. *l. r.* Limb-ridge. *l. s.* 1, 2. &c. Lateral portions of somite contained in the legs. *l. s. t.* 1, 2, &c. Tubular portion of nephridial cœlom of appendages 1, 2, &c. (segmental organ). *l. s. v.* 1, 2, &c. Internal vesicular portion of nephridial cœlom of appendages 1, 2, &c. *M.* Mouth. *mb.* Mesoblastic band. *me.* Anterior part of thickening of parietal mesoderm of the somites. *m. l.* Muscles of internal projection of jaws. *m. ph.* Muscular wall of pharynx. *m. t.* Posterior part of thickening of parietal mesoderm of the somites. *o. n.* 1, 2, &c. The external opening of nephridium of somites 1, 2, &c. *or. p.* Oral papilla. *o. s.* 3. Opening of somite 3. *p. g.* Primitive groove. *ph.* Pharynx. *ph. m.* Pharyngeal mesoderm from the splanchnic walls of the anterior somites. *p. p.* pre-oral pouch of alimentary canal. *pr.* Proctodæal lining. *p. st.* Primitive streak.

R. Rectum. *S.* 1, 2, 3, &c. The first, second, and third somites, &c. *sal. gl.* Salivary gland. *sep.* Septum dividing the lateral portion of the somite from the dorsal. *sl. g.* Slime-gland. *S. o.* 1. Rudimentary nephridial portion of somite. *st.* Stomodæum or its lining. *T.* Tongue. *v. en.* Ventral endoderm of alimentary canal derived from the cells intermediate between the ectoderm and endoderm when the blastopore was open along its whole length. *v. n.* Ventral nerve-cord. *v. o.* Ventral organ. *v. s.* Ventral sheet of somatic mesoderm. *v. sp.* Vascular space. *w.* White matter of central nervous system.

All the figures are of Peripatus capensis, and drawn with Zeiss's camera, ob. C, oc. 2, unless it is otherwise stated.

Fig. 1.—Transverse section through a late embryo of Stage A (length ·53 mm.), two sections behind the blastopore. The primitive groove is very deep and is hardly to be distinguished from the blastopore. The mesoblastic bands do not extend in front of the hind end of the blastopore. The primitive groove was confined to the front end of the streak. The latter extended through eighteen sections.

Figs. 2 and 3.—Two transverse sections through an embryo of Stage B (length ·65 mm.) with one somite, still solid, and separate from the front end of the mesoblastic band. Primitive streak extended through twenty-one sections.

Fig. 2. Through the single somite which is present. (The section is slightly oblique, passing in front of the somite on the left side.)

Fig. 3. Two sections in front of the anterior end of the somite. The endodermal nuclei at the lips of the blastopore were dividing actively. One such is shown at *d. n.*

Fig. 4.—Section through a late embryo of Stage B (Stage of fig. 25, Pt. I) in front of the mouth. Reduced ½.

Fig. 5, *a—f.*—A series of sections through an embryo (length 1 mm.) of the same age as the last (Stage B, fig. 25, Pt. I). Reduced ½. Four separate somites could be distinctly made out on each side. Thirty sections were obtained through the streak. The groove extends the whole length of the streak.

a. Through the mouth.

b. Between the mouth and anus. The blastopore lips have fused; their line of fusion is marked by a slight groove (*f. bl.*).

c. Through the hind end of the anus. The mesoblastic bands in this region are not yet broken up into somites.

d. Through the front end of the primitive streak, four sections behind the last.

e. Through primitive streak, eight sections behind last.

f. Through primitive streak, nine sections behind the last.

FIG. 6, *a*—*d*.—A series of sections through an embryo of Stage C (fig. 26, Pt. I). Reduced ½.

a. In front of the mouth, through the pre-oral lobes. The anterior wall of the alimentary (*en*.) just touched.

b. Through the mouth.

c. Between the mouth and anus. Wide separation of the somites. Very thin ventral ectoderm.

d. Through the hind end of the body, in the region of the curvature. The embryo is cut in two places, through the anus and through the growing point (primitive streak and groove).

FIGS. 7—12 are from a young embryo of Stage D (fig. 28, Pt. I). The embryos of this age are always much narrower, both dorso-ventrally and laterally, than those older or younger. Reduced ½.

Fig. 7. Section through the roots of the budding antennæ.

Fig. 8. Through the anterior part of the mouth.

Fig. 9. Through the posterior part of the third somite, in the region of the outgrowth of the oral papilla (*or. p*.). The sheets of cells extending from the dorsal and ventral ends of the somites are present (*d. s*. and *v. s*.). The endoderm and ectoderm have separated from one another, excepting along the dorsal middle line. The cavity so formed is marked *b. h*. and *b. bc*.

Fig. 10. Through the anterior part of the fourth somite (in front of the region of the future leg), to show the anterior part of the thickening of the somatic mesoderm (*me*.).

Fig. 11. Through the region of the future leg (posterior part of the somite), showing the position of the thickening on the ventral side of the outgrowth.

Fig. 12. Through the fifth somite. The changes which have produced the parietal mass of cells from the somatic mesoderm have not yet occurred here. The somite is partly collapsed dorsally and ventrally.

FIG. 13.—Through the third somite of an embryo of Stage D, slightly older than the last. Reduced ½. On the left hand side the section passes through the posterior part of the somite, and shows the developing oral papilla and septum tending to divide the cavity of the somite into a part within the appendage and a part within the body. The first trace of the third system of body cavity (*b. lat*.) is visible.

FIG. 14.—Section through an embryo of Stage D, through the pre-oral somite, brain, and eye. The latter (*e*.) has the form of an open pit. Reduced ½.

FIG. 15.—Section through a slightly older embryo, showing a more advanced stage in the brain and eye. Reduced ½.

Fig. 16, *a*, *b*.—Two sections through the mouth of a late embryo of Stage D (fig. 29, Pt. I). Reduced ½.

a. Through the anterior part in the region of the stomodæal ingrowth.

b. Through the posterior part.

Fig. 17, *a*—*d*.—A series of sections through the region of the third somite of an embryo of same age as the last (fig. 29, Pt. I). Reduced ½.

a. Through the anterior part of the somite, in front of the attachment of the parietal thickening (*me*.). The parietal thickening always appears to be free in front; it is attached behind.

b. Point of attachment of parietal thickening to somatic mesoderm.

c. Two sections further back.

d. Six sections further back through the region of the appendage (*or. p*.).

Fig. 18, *a*—*c*.—Three sections through the seventh somite of the same embryo as that from which fig. 17 was taken. Reduced ½.

a and *b* show the leg-ridge, which in fig. 18 *c*—a section through the hinder part of the somite—is enlarged to form the developing fourth leg.

A few sections behind Fig. 18 *c* the cavity of the somite extends into the appendage. The anterior less developed part of the mesodermal thickening lies immediately within the leg-ridge, while the posterior larger part occupies the appendage itself.

Figs. 19, *a*, *b*; 20; 21, *a*—*c* are from sections through young embryos of Stage E. Reduced ½.

a. Through the head and first somite. The optic pit is closed.

b. Through the mouth and first somite. This section shows the developing lip (*L*.). In this and the previous section the mesoderm cells next the stomodæal ectoderm have proliferated to form the commencing pharyngeal and lingual musculature.

Fig. 20. Through the second somite, with the third somite overlapping dorsally.

Fig. 21 *a*. Through the anterior part of the third somite. The limb-ridge (*l. r*.) and the mesodermal thickening with its cavity (*b. lat*.) are well shown.

b. Ten sections further back, through the anterior part of the appendage (oral papilla). The mesodermal thickening is much larger.

c. Through the centre of the appendage. The somite is nearly divided into two parts by the septum (*sep*.). The portion in the appendage sends down a diverticulum, which lies against the outer border of the nerve-cord and reaches the ectoderm.

Figs. 22—25 are through a late embryo of Stage E. Reduced ½.

a. Through the pre-oral region, at the level of the cerebral commissure. The commencing cerebral groove (*c. g.*) is shown.

b. Through the mouth and hind end of first somite, showing the rudimentary nephridium (*s. o.* 1). One half of the section only is represented. (Drawn with Zeiss's D, oc. 2).

Fig. 23, *a—e*. A series through the third somite.

a. The anterior part of the mesodermal thickening and its cavity (*b'*.) is much enlarged. The section passes through the hind end of the jaw (*J.*) and the lip (*L.*).

b. A few sections further back. A tube (*a. v.*) ending blindly in front, and opening behind into the limb portion of the somite, is present.

c. Nine sections behind fig. 23 *b*, through the point of junction of the portion of the somite in the body (*s.* 3), the portion in the appendage (*l. s.* 3), and the anterior diverticulum (*a. v.* of Fig. 23 *b*).

d. Through the centre of the appendage, seven sections behind the last. The anterior end of the fourth somite (*s.* 4) is visible, and rudiment of the slime-gland as an ectodermal ingrowth at the apex the of the oral papilla (*sl. g.*) is present.

e. Nine sections behind the last. The external opening of the third somite covered over by the lip (*L.*), which has grown back to this point, and the mesodermal thickening and its cavity (*b. lat.*) of the wall of the fourth somite are present.

Fig. 24. Between the oral papilla and first leg, through the fourth somite, twelve sections behind fig. 23 *e*.

Fig. 25. Through the fourth leg. The eighth somite overlaps dorsally. The leg portion of the seventh somite opens to the exterior (*o. s.* 7). The great ectodermal thickening, which is so conspicuous in embryos of this stage, is cut through at *d*.

Fig. 26.—Transverse section through the anus and twentieth somite of an embryo of Stage D. The rudiment of the proctodæum with its special lining (*pr.*) is present. The germinal nuclei (*gen.*) are present, both in the endoderm and splanchnic mesoderm.

Fig. 27.—Transverse section through an embryo of Stage E, at the region of the seventeenth somite. The germinal nuclei are present in large numbers. The cœlom has not yet become divided into body and leg portions (see right hand side of section).

Fig. 28.—Longitudinal vertical section through an embryo of Stage C. The section passes through mouth and anus. The hind end of the body is bent round and projects forward, bearing the primitive streak on its ventral surface. The alimentary canal reaches the anterior end of the body, and the transverse commissure (*com.*) connecting the two halves of the cerebral ganglion is visible in front of the mouth. The modified endoderm (*st.*) or

ingrown ectoderm—whichever view of its nature be taken—of the anterior (future dorsal) wall of the stomodæum is present. Zeiss's A, oc. 2.

Fig. 29.—Longitudinal vertical section through an embryo of Stage D. The hind end of the body has grown and become spirally coiled. The primitive streak is still present—but in a rudimentary form—on the ventral surface behind the anus. It is marked by a slight pit. A section to one side of the middle line of this embryo shows a considerable mass of nuclei in connection with it. The anterior end of the body has been drawn back in such a way that no part of the alimentary canal projects in front of the mouth. The anterior wall of the stomodæum is therefore now inclined dorsalwards and slightly backwards. Zeiss's A, oc. 2.

Fig. 30.—Longitudinal vertical section through the hind end of an embryo of Stage E. The anus is now practically terminal, and the primitive streak aborted. A rudiment of the latter still indeed exists, but there are no lateral masses of nuclei. The rudiment of the proctodæum is present (also in the last figure). Zeiss's A, oc. 2.

Fig. 31.—Longitudinal vertical section through the anterior end of an embryo of Stage E. Zeiss's C, oc. 2. Reduced ½. The anterior ectodermic wall of the body has grown forward in the middle line, and separated from the anterior wall of the alimentary canal (cf. fig. 34, Pt. I). The anterior wall of the stomodæum has now become its dorsal wall, and is directed backwards; and an anterior pouch of the alimentary canal lies dorsal to it. The ventral wall of the stomodæum has began to be formed.

Fig. 32.—Longitudinal horizontal section through the anterior end of an embryo of Stage D. Zeiss's A, oc. 2.

Figs. 33—42 are transverse sections of an embryo of Stage F.

Fig. 33. Through the first somite, brain and cerebral grooves. The section passes in front of the region where the two halves of the brain are connected, and the eye (*e.*) is just included in the section on the right side. Reduced ½.

Fig. 34. The section is taken at the junction of somites 1 and 2, and passes through the posterior part of the brain, the anterior part of the permanent buccal cavity, and the anterior wall of the pharynx (*ph.*) The posterior part of the cerebral grooves (*c. g.*) are seen opening into the buccal cavity, the roof (*T.*) of which becomes the so-called tongue of the adult. The jaw (*J.*) is visible on the right side. Reduced ½.

Fig. 35. Through the mouth (*m.*); the opening which leads from the buccal cavity into the pharynx. In consequence of the contraction of the ectoderm, the second somite (*s.* 2) is hardly visible, and the median part of the space *b. h.* is obliterated. Reduced ½.

Fig. 36. Behind the mouth, through the oral papillæ (*or. p.*). The slime-gland (*sl. g.*) is cut through just behind its opening, and the anterior

part of the ventral cœlom of the third somite (internal vesicular portion, *l. s.* 3.) is shown. Reduced ½.

Fig. 37. Immediately behind the junction of the pharynx and mesenteron, through the external opening of the salivary gland (ventral division of somite 3) into the hinder part of the buccal cavity (*buc. cav.*).

Fig. 38. Through the dorsal division of somite 4 and the hind part of the ventral division of somite 3, the opening between the two parts (internal vesicular portion, *l. s. v.* 3, and tubular portion, *l. s. t.* 3) of which are shown. *m. l.* Muscles of internal projection of jaws.

Fig. 38 *a*. One side of a section, a little behind fig. 38, to show the commencing salivary gland (*sal. g.*). Zeiss's D, oc. 2. Reduced ½.

Fig. 39. Between the oral papilla and first leg.

Fig. 40. Through the third leg, to show the ventral division of the sixth somite. The tubular portion of this (nephridium of third leg) is a straight tube (the lumen is not distinct, but this was probably due to the contraction of the specimen), opening externally at *o. n.* 6, and internally into the internal vesicular portion (*l. s. v.* 6).

Fig. 41. Through the twentieth somite, in the region of the generative cells. The differentiation of the various divisions of the body cavity has hardly reached this part of the body, *b. h.*, *b. pc.*, *b. bc'.*, being only present in a rudimentary form. The endoderm is slightly shrivelled up. The generative nuclei are still in the endoderm, though some of them project into the body cavity. Zeiss's D, oc. 2. Reduced ½.

Fig. 42. Through the rectum and anal papillæ (rudimentary eighteenth leg). The dorsal and ventral divisions of the somite are in communication.

Figs. 43—46 are from old embryos of Stage F. Zeiss's D, oc. 2. Reduced ½.

Fig. 43. Through the seventeenth somite, to show the dorsal division of the somite (*d. s.*), which may now be called the generative gland. The gut has separated from the latter, so that the two divisions of the part of the body cavity marked *b. bc'.* communicate. The dorsal part of the section only is drawn.

Fig. 44. Through the anal papilla (rudimentary eighteenth leg) and twenty-first somite. The two parts of the somite are in communication, and the ventral has almost acquired an opening to the exterior. This opening will be the generative opening.

Figs. 45 and 46.—Dorsal parts of two transverse sections from the middle region of the body in front of the generative region; 46 is the anterior. In 45 the dorsal division of the somite (*d. s.*) is not yet obliterated; in 46 it has entirely vanished, and is represented only by the thickened layer of cells which form the ventral wall of the heart.

Figs. 47 and 48.—Through the generative organs of an embryo of Stage G,

in the region where they are detached from the pericardial floor. Zeiss's F, oc. 2.

Fig. 49.—Longitudinal vertical section through the anterior part of the body of an embryo of Stage F. Reduced $\frac{1}{2}$.

Fig. 50.—One side of a transverse section through a young embryo of Stage F. To show the latest stage of the rudimentary nephridium of the first somite (*s. o.* 1), in close contact with the outer side of the hind part of the brain (a few nuclei of the latter are indicated). Zeiss's D, oc. 2. Reduced $\frac{1}{2}$.

Fig. 51.—One side of a transverse section through the brain of a late embryo of Stage F. To show the two separate parts of the first somite. The cerebral grooves are closed. Reduced $\frac{1}{2}$.

Fig. 52.—Transverse section of the sixth leg of an old embryo of Stage F. To show the funnel-shaped opening (*f.*) of the tubular portion of the nephridium into the vesicular internal portion (*l. s. v.*), and the relation of the latter to the body cavity (pseudocœle) of the leg (*b. app.*). Zeiss's D, oc. 2. Reduced $\frac{1}{2}$.

Fig. 53, *a* and *b*. Longitudinal horizontal sections of two contiguous legs of an embryo of same stage as last. Reduced $\frac{1}{2}$.

Morphological and Biological Observations on Criodrilus lacuum, Hoffmeister.[1]

By

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With Plate XXXVIII, figs. 1 to 8.

In Vejdovsky's very complete work, 'System und Morphologie der Oligochæten,' Prag., 1884, which bears the character of a useful text-book on the morphology of the Oligochæta, I find only a scanty and incomplete account of the very interesting terricolous form, Criodrilus. On pages 16 and 58 he says: "Lage der Hoden, Eierstöcke, Samenleiter und Samentaschen, sowie Gurtels unbekannt." "Leider weiss Man sehr wenig von dem Leben eines so merkwürdigen Oligochæten." I think that these assertions by this well-known investigator justify me in publishing my own observations, incomplete though they are, relative to this worm.

Criodrilus lacuum was discovered by the well-known German zoologist Fritz Müller, in 1844, in the so-called "Tegel-see," near Berlin, and in the following year was described and figured by Hoffmeister.[2] It is almost incredible that fully thirty years should have elapsed since its discovery without its being found again. In 1876 this worm, found in a branch of the Danube near Linz, was again mentioned by

[1] Translated from the MS. by Wm. B. Benham, B.Sc.

[2] 'Die bis jetzt bekannten Arten aus der Fam. der Regenwürmer,' Brunswick, 1845, p. 41.

Hatschek[1] in his work, which furnishes contributions to the knowledge of the development and morphology of the Annelids.

Two years later Hatschek[2] recognised this worm, described its development, and provided Vejdovsky with material for his researches.[3]

Like Hatschek, I found Criodrilus in the neighbourhood of Buda-Pesth, and described it in a communication to the Hungarian Academy of Science.[4] Recently it was found by Rosa[5] in Italy, where it lives in the basin of the Po; somewhat earlier, too, it was noted by Panceri.[6]

I have no doubt that it exists in other parts of Europe, and that only its habit of concealing itself has placed it amongst rare and hitherto little known Earthworms. The following description ought to lead to the discovery and to the better knowledge of it.

Criodrilus lacuum. Hoffm.

1845. 'Die bis jetzt bekannten Arten d. Faun. d. Regenwürmer.'

A mudworm 4—12 cm. in length, and about 5—10 mm. in breadth, of a dark brown or greenish colour dorsally, with lighter, sometimes reddish colouration ventrally, with rusty-yellow areolæ, and milk-white, horn-like spermatophores near the male genital pore.

The body is quadrangular (though this is less noticeable

[1] 'Sitzungsber. der Kais. Akad. d. Wiss. in Wien,' Bd. 74, pp. 442—459.

[2] "Studien z. Entwick. d. Anneliden," 'Art. Zool. Inst. Wien,' Bd. 1.

[3] (*a*) 'Monograph. d. Enchytræiden,' Prag., 1879. (*b*) "Ueber der Entwick. des Herzens bei Criodrilus," 'Sitzungsb. k. böhm. ges. der Wiss.' Prag., 1879. (*c*) 'System und Morphologie der Oligochæten,' Prag., 1884.

[4] (*a*) Amagyar. 'Oligochæt. Fauna,' Buda-Pesth, 1881. (*b*) 'Revisio et distributio specierum terricolarum regionis palæarcticæ,' Buda-Pesth, 1885.

[5] "Nota sui Lombrici del Veneto," 'Atti del R. Inst. Ven. di Sci. lett. ed. arti,' t. iv, s. vi.

[6] "Catalogo degli anellidi d'Italia," 'Atti d. Soc. Ital. d. Sci. Nat.,' 1875, xviii, p. 201.

anteriorly), gradually narrowing posteriorly, and ending in a pointed, yellowish, and often regenerated tail. When the worm is contracted the dorsal surface is usually depressed.

The number of somites is 200—250, or more. The somites are well defined, obscurely triannulated, and somewhat pressed together towards the tail. There is no dorsal pore. The last or anal somite is longer than those just in front. The anus itself is dorsal. There are rounded swellings on the somites x, xi, xii, and xiii.

The prostomium is moderately elongate and as long as the buccal somite, from which it is distinctly separated, without having a prolongation dorsally or a furrow ventrally (Hoffmeister's description—"Die Lippe ist mit dem Mundsegment verwachsen"—is incorrect). The prostomial pore is indistinct.

The four rows of setæ extend along the corners of the body. The distance between the rows is nearly equal. The setæ of each pair are somewhat apart; they are not prominent, and are slightly curved with rough ends.

The genital organs are on the same plan as in the Lumbricinæ, and present no peculiarities. The seminal reservoirs, with their lateral cæca, extend through the somites ix to xii. The true testes last for only a short period, during which they early break up into spermatogonia, so that I could recognise the two pairs, which lie in the somites xi and xii, only by the remnants. The two pairs of ciliated rosettes have an obscure plate-like structure; those of the first pair lie on the septum between the somites x and xi; those of the hinder pair on that between xi and xii, so that they project into the somites xi and xii respectively. The sperm ducts are spirally coiled at the base of the rosettes, unite with one another at the level of the somites xii and xiii, and thence a wider, tortuous, common canal extends on each side to the external pore on the ventral surface of somite xv, between the two couples of setæ. The termination is simple; without an atrium there is only a large gland ("vulva" of Hoffmeister), which probably serves for the construction of the cocoon. There are two pairs of

spermathecæ, which appear to open on the ventral surface between the somites IX, X and X, XI respectively.

The ovaries lie in somite XIII, one on each side of the ventral blood-vessel, attached to the hinder face of the septum between the somites XII and XIII; they contain many ripe eggs, which are chiefly found at the free end of the ovary. I have not found a pointed prolongation at the distal end of the ovary. The oviducts lie opposite each ovary between the somites XIII and XIV; their plate-like funnels project into the former somite, and their very diminutive canal opens to the exterior on the ventral surface of somite XIV.

I have not found separate yolk and cement glands. The horn-like spermatophores (Hoffmeister's "penis-formige Körpchen"), 6 to 8 mm. in length, are found in the neighbourhood of the male pore; their number is variable, and they are usually placed ventrally, although exceptionally they are to be met with on the dorsal surface. As a rule only two are situated on the neural side of somite XIII, close to the ventral setæ; though very often they are some distance from them. They are always in pairs, from two to six in number; only once have I found eight spermatophores, which were arranged irregularly round the male pore. These structures are products of copulation, and appear only during this operation; whether they are formed in the sperm-duct, or by the swellings in front of the genital pore, I am unable to say with certainty. The spermatophore, the shape of which is exactly rendered by fig. 7 (Pl. XXXVIII), consists of an homogeneous, hyaline, mucous substance, in which are embedded numerous fine, enlongated filaments. The lumen is fairly wide and deep, open at the end, and filled with bundles of spermatozoa, which are massed together in a spiral fashion. The fibres in the wall can scarcely be the product of the epiderm cells; moreover, the spermatophores vary so much in number and position that one can scarcely admit that they are formed by the swellings. I think it more likely that they are formed in the spermathecæ, there filled with spermatozoa, and that they are then attached in position during mutual copulation. The broad basal portion clings fast to the

cuticle, but never grows closely with it, so that the spermatophore very easily falls away. That the great areola round the male pore and the swelling in front of it play an important part in copulation cannot be doubted, for, after the laying of the eggs, these structures immediately decrease in size. In specimens which I collected at the end of June I could find neither the swellings nor the areola, and in some even the male pore also had become indistinct.

As to the time of sexual maturity of Criodrilus nothing positive is known. According to Vejdovsky the maturity seems to be attained in the months of June and July, since Hatschek found the cocoons with segmented eggs and embryos in the middle of June, whilst Hoffmeister mentions the worms furnished with "pseudo-spermatophores" at the beginning of July; Vejdovsky himself has not studied mature worms. My researches, however, extending over many years, show that the embryos escaping from the egg in summer may attain sexual maturity as early as February or March in the following year; indeed, in the most favorable seasons copulation may even take place in these months. Copulation and egg laying take place almost certainly in June, since I have found at the beginning of July of this year no cocoons with embryos. The best sign of maturity are the large and very striking spermatophores, which are to be found regularly from March to the end of May, certainly not later. The embryos escape from the cocoons in May, June, and July; at the end of the latter month I have collected only empty egg cases. At first the young worms are to be found amongst the thick roots of aquatic plants, only later in the mud, where they pass the winter and attain maturity. The clitellum, so very characteristic of the Lumbricinæ, is, as Hoffmeister rightly insisted, absent. I have for many years collected these worms at all seasons, yet I have found no trace of a clitellum, nor of the so-called "tubercula pubertatis;" the great glandular areola of the male genital pore appears to replace the clitellum.

The egg cases of the Lumbricinæ are known as roundish-oval chitinous capsules with pointed appendages, and are

presumably secreted by the clitellum. The cocoons of the Criodrilidæ, however, are spindle-shaped, parchment like structures with a colour that changes; they are about 5 cm. in length, rapidly diminishing towards each end. One end, drawn out into strongly fibrous threads, serves for attachment to the roots, or more rarely to the leaves and branches of water plants; the other end truncated, with a dentate edge, allows the embryos to escape. As is the form, so also is the colour different. The perfectly newly laid cocoons are nearly transparent, horny yellow in colour, but after a time they become darker, and towards the time of hatching of the embryos they are blackish in colour. This change in colour, which reminds me of the egg cases of Shark embryos,[1] may here too be traced to chemical changes.

The substance of the egg cases is not wholly chitinous; at any rate a large portion is dissolved in caustic potash; on the contrary, a sort of coagulated yolk and mucus take a large share in their constitution. The substance itself is very easily wetted, so that liquids and gases can diffuse through it.

The inside is filled with a fluid albuminous substance, allied in density to white of egg; in this from eight to twenty eggs are embedded, and in it are found the remains of the substance of the spermatophores as well as innumerable spermatozoa, which are to be met with especially round the developing eggs; their appearance is reproduced in fig. 8, Pl. XXXVIII. The number of eggs is very variable; usually only one third of the fertilised eggs develop; the largest number of embryos in a cocoon was eight, the smallest two.

The structure of these egg cases is especially well shown, if freshly laid eggs, preserved in alcohol, are placed in water in order that they may swell up. When such cocoons are carefully examined the swollen part is found to be banded; these bands appear to correspond with the somites of the anterior part of the body. This correspondence, as well as the fibrous structure of the outermost layer (fig. 2) and the remains of the

[1] L. Örley, "Zur Physiol. der Haiembryonen," 'Termeszet. füzetek,' ix, 1885, Buda-Pesth.

spermatophores in the cocoon, allows one to suppose that the moulted skin of the anterior part of the body takes a share in the formation of the cocoon, just as in Lumbricinæ and Nephelidæ, the egg case probably owes its origin to the moulting of the clitellum. Since, however, the number of bands in this egg case exceeds twenty, it is probable that the somites lying behind the genital pores also take part in the formation of the cocoon.[1] The tough secretion which builds up the chief portion of the cocoon, is probably furnished by the large swellings around and in front of the genital pores, and by the inner lining of the sperm duct. The process of formation of these egg cases, which alone would lead to positive results, I have unfortunately been unable to watch. Worms which I kept in my aquarium always hid themselves under cover of the roots of Sium latifolium, so that I was unable to overlook their operations.

A transverse section through this cocoon shows three layers; an inner yellowish and homogeneous layer, an outer strongly fibrous, and a middle layer of interlaced strands (fig. 4). The fibrous layer is most easily seen at that end of the cocoon which is drawn out into threads (fig. 2), where they are collected together into strands and finally separate out into elastic fibres; the latter serve for attachment to aquatic plants. Towards the swollen portion the fibrous layer becomes thinner at the expense of the middle layer.

The middle layer (fig. 5) consists of innumerable interwoven bundles which are not separated into fibres. The network is densest below and becomes looser above. It looks so very much like a plant tissue, that a young botanist of this country at first disputed as to the substance of the tissue. Some thought it of vegetable origin.

The lowermost or basal layer is made up of very many extremely delicate strata (fig. 6); these show a striated structure, and contain here and there fibrous elements. This layer projects from the free end of the cocoon (fig. 3), is strongly folded, and serves to close the egg case.

[1] See the following paper, in which the Clitellum is described.—Translator.

The young are of a reddish colour, about 2—3 cm. in length, when they leave the cocoon. They escape from the free end of the egg case by the separation of the two "lips," which at first, owing to their elasticity, were closed.

Hatschek supposes a Criodrilus to lay several cocoons, because the number of worms was very small in comparison with the cases which he found. I placed a Criodrilus amongst the roots of Sium latifolium, and in a few days found two quite transparent, and therefore fresh, cocoons. It appears to me, therefore, that a Criodrilus will lay two cocoons, in correspondence with the number of the sperm ducts. It is natural that more cocoons than worms should be found, since empty cocoons appear throughout the year attached amongst the roots; one sometimes finds old, black, very much frayed cocoons in certain places by thousands; of freshly laid cocoons, on the contrary, I have never collected more than double the number relatively to the mature worms.

Habits. — In isolated branches of large rivers, e. g. the Danube, as well as in flowing streams with muddy beds, there are places where the bottom is very nitrogenous owing to the decomposition of organic matter. In such places there are usually many aquatic plants with dense roots, which (at any rate here in Buda-Pesth, in the streams flowing into the Danube) are met with in great abundance. Amongst these plants I found a very large quantity of Sium latifolium, L., the favourite plant of Criodrilus.

If these plants with their roots are taken out in the spring, and the "covert" carefully examined, one finds the long spindle-shaped cocoons and Criodrili engaged in egg-laying, so closely interlaced with the roots that they can only be separated with difficulty. The egg cases are at first sight so very like the Enteromorpha, that young botanists might dispute as to whether they are of vegetable or animal origin. It is only during the breeding season that the worms are to be found amongst the roots, where copulation and egg-laying takes place. After the completion of these operations they return to the mud, where their genital organs commence to

degenerate. I have never been able to study the copulation, though I have looked at many worms. The swellings, around and in front of the male genital pore, are, however, so very swollen during the breeding season, and secrete so much mucus, that I presume the copulation takes place as in the Lumbricinæ. The worms found in the mud are very active, they burrow deep into the mud; I have even met them at a clay bottom, wherever the penetration of the water through the deeper layers renders their passage possible. In very shallow water, areas regularly and finely perforated are to be seen at the sides and bottom of the channel, which disclose their presence; these perforated places can frequently be used as a guide to their discovery. They only live scattered over a territory: as they can swim in a peculiar serpentine way they wander to different places, and settle where the necessaries of their life are present. Their food consists of rotting and decaying vegetable matter, which they swallow mixed with mud. Their size varies according to their habitat and local circumstances, as the statements of other observers affirm. However, even under the same circumstances, very great differences in size exist, so that, I think, in the first place individuality, and in the second place environment must be considered as factors in their varying size.

In the economy of nature they appear to do good service by their destruction of organic matter; their fæces, as in the case of Earthworms in general, increases the goodness of the mud, as is proved by the settlement of many plants in the places where Criodrilus lives. The mud of such a bottom is very rich, and on the overflowing of the stream it will be carried over the fields where it is of further use for the nourishment of plants.

In winter these worms burrow very deep in the mud, so that one can dig them out only from very great depths. Their tenacity of life is great, yet after this season they are very much changed. In winter they soon perish in tanks with pure water, but in autumn they can be kept for a week. In the tanks they twine themselves into a knot and are then very difficult to separate. Their power of regeneration is astonishing. A

worm, cut through the middle, forms a new tail with shortened somites. In autumn more worms with regenerated tails are found than in the spring. Once I found, in October, out of fifty specimens, thirty with regenerated tails. The tail is very brittle, and the reason is very likely to be found in the irregular arrangement of the muscle-bundles.

In company with Criodrilus there lives a very interesting Earthworm, Allolobophora dubiosa, Örley, which has nearly the same habits.

Amongst the Hirudinea, species of Aulostoma and Nephelis are their greatest enemies; these swallow three or four Criodrili at a time.

[For the explanation of figures 1 to 8, Plate XXXVIII, illustrating Dr. Örley's paper, see p. 570.]

Studies on Earthworms.
No. III. Criodrilus lacuum, Hoffmeister.

By

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With Plate XXXVIII, figs. 9 to 19.

Thanks to the kindness of Dr. Örley, who, at Professor Ray Lankester's request, sent him a bottle containing a large number of Criodrilus preserved in spirit, and including both sexually mature and young specimens, as well as cocoons, I have been enabled to make a study of this interesting worm. The specimens are all in a good state of preservation, and I have been able to add several new facts concerning its anatomy. This is the first time that figures illustrating the general anatomy of Criodrilus have been published. Hoffmeister[1] gives a coloured figure of the worm, and of the cocoon, showing their natural size and appearance, but with no details as to setæ, pores, &c.; Vejdovsky[2] has already published excellent figures of various portions or organs of the worm; e. g. the ovary, nephridial funnel, setæ, transverse section of the body, so that I have not repeated these. Dr. Örley[3] added drawings of the prostomium, as well as of that of another worm which he described as Criodrilus dubiosus; but in his paper, published in the present number of this Journal, he makes no

[1] 'Die bis jetzt bekannt. Art. aus d. Fam. d. Regenwürmer,' Brunswick, 1845.
[2] 'Systeme und Morph. d. Oligochæten,' Prag., 1884.
[3] 'Mathemat. u. Termeszt. tudomanyi Közlemenyek,' Budapest, Bd. 16, 1881.

reference to this worm. He, however, mentions Allolobophora dubiosa as occurring with C. lacuum, so that, presumably, they are one and the same animal. But with all these figures no general view of the worm has been given.

My thanks are due to Professor Ray Lankester, not only for these worms, but also for allowing me to translate Dr. Örley's paper, so that I could corroborate or comment on his observations, and fill in details which he has left untouched. I am quite aware that a great deal more still remains to be done in reference to the anatomy and histology of Criodrilus, but I think the following, taken with the description of the previous observers, forms a fairly complete account of its anatomy.

External Characters.—I have nothing to add to Örley's statements as to the length and number of somites of the worm; my specimens are all preserved in strong spirit, and are therefore greatly contracted; they are much coiled and twisted and had to be soaked in weak alcohol before they could be conveniently dissected. A deep groove traverses the dorsal surface posterior to somite L; the ventral surface is rounded, and the sides are more or less vertical (Pl. XXXVIII, fig. 12).

The prostomium is distinct, and its terminal pore has been figured by Vejdovsky (loc. cit., pl. xiii, fig. 12). The anterior somites are longer than the posterior ones, and are not so prominently quadrangular in section. On the ventral surface of somites IX, X, XI, XII, and XIII, there are prominent rounded papillæ, in which the ventral setæ are inserted.

The structure of the epidermis is to a certain extent figured by Vejdovsky (loc. cit., pl. xiv, fig. 3). It consists of narrow columnar cells with oval nuclei; their inner ends seem to diverge and between them are seen small rounded cells with rounded nuclei (Pl. XXXVIII, fig. 17, *c*.), which Vejdovsky considers as young epiderm cells. Goblet cells are very rare; they are narrow cells filled with granular matter, with the protoplasm and nucleus at the inner ends. As the worm lives in water the necessity for secreting mucus would not be so great as in Earthworms, properly so-called, and hence the mucous cells are few and far between. The capillary loops of

the blood-vessels pass between the cells of the epidermis (fig. 17, *d*), as in the Leech, and as Beddard has shown to be the case in Perionyx and in Perichæta.

The longitudinal muscles are arranged irregularly, as in Microchæta, Allolobophora, and others. Connective tissue is abundant, and forms a fairly thick layer between the muscular layer and the coelomic epithelium.

Previous writers have denied the existence of a clitellum; even Örley, who expressly looked for it, says that he has found no trace of it; yet in all my specimens, which are sexually mature, a considerable difference in appearance is noticeable behind somite xv, and extending to about somite xlvii. The worm is here nearly cylindrical, though slightly concave on the ventral surface, where the intersegmental grooves are not distinctly marked, but tend to run into one another across the middle line as shown in fig. 10. The colour, at any rate in spirit specimens, is rather darker over the dorsal and lateral surfaces of this region than elsewhere. Noticing this, I cut a series of transverse sections through the body, and I then found that behind the somite xv the epidermis gradually changed its character.

In addition to the columnar cells forming the epidermis of the general surface, a layer of elongated, club-shaped cells, of various lengths, is present (fig. 18, *c*), so that the epidermis is here some four or five times deeper than elsewhere, and deeper at the sides than on the dorsal surface. These cells have a very similar appearance to those in the clitellum of Lumbricus and Microchæta, though they differ slightly in detail. Each cell is filled with numerous highly refracting, small spherical globules, and the protoplasm with the nucleus is confined, apparently, to the inner, swollen end of the cell. As the cells vary in length, the appearance presented is that of three or four layers of such cells, as in Lumbricus; but in the latter worm these club-shaped cells contain a granular substance, and the rounded, refracting globules are confined to narrow, elongated cells, intermediate in length between the club-shaped and columnar cells and which are absent in Criodrilus.

Another point of difference is presented in the absence of the strands of connective tissue, which in Lumbricus separate the club-shaped cells into more or less distinct groups. I think that there can be no doubt that the clitellum is present; but as it commences and ends gradually, and since, from Örley's remarks and from Hoffmeister's drawing, there is no difference in colour in the living worm, it may easily be overlooked in this condition.

The anus is situated quite dorsally (fig. 11), on an enlarged somite, which Vejdovsky considers as representing some seven or eight fused somites, as indicated by the ganglionic swellings figured in pl. x, fig. 21, of his work.

The pore of the sperm-duct is placed on a large hemispherical papilla, or swelling, on somite xv, between the ventral and dorsal setæ, which Örley speaks of as "der Hof," and which I have translated as "areola." It is, in the sexually mature worm, very conspicuous, and has caused, in spirit specimens, the lateral swelling shown in Pl. XXXVIII, figs. 9, 10.

The pore of the oviduct is similarly placed in somite xiv, but on a much less prominent papilla. Both these pores are visible from the side (fig. 13); and near them are usually one or more white spermatophores. These are fully described in the preceding paper, but whereas Örley states that they are generally fixed close to the ventral setæ, the specimens examined by me show them nearer the dorsal setæ; at the same time I do not intend by this, nor by my figure, that it should be inferred that Örley is in error: he has had much greater opportunity for observation than I have, and my figure was drawn some weeks before I saw his paper.

I have been unable to see the nephridia pores, and there are no dorsal pores.

The four couples of setæ are set at the corners of the animal, as shown in fig. 11, and are perfectly evident throughout the body, including the clitellum. They are usually broken off short, so that I was unable to extract them; but in sections they are seen to have the ordinary shape (Vejdovsky, pl. xiii, fig. 13).

Internal anatomy.—The alimentary tract differs from

that of other earth-worms, with the exception of Pontodrilus,[1] in the absence of a gizzard.

The pharynx extends to the hinder boundary of somite IV (fig. 14), the walls are very muscular, and the usual radiating muscles pass to the body wall, some going as far back as somite VI. In transverse sections I found numerous glandular-looking cells amongst the muscles of the dorsal and lateral wall, but I was unable to find any duct leading to the lumen of the pharynx. There are similar groups of cells in the anterior somites, through which the œsophagus passes; these lie on each side of the subintestinal blood-vessel, but I could find no duct. The œsophagus is a narrow, simple tube, the walls of which are fairly thick and very vascular. In somite XIII the œsophagus enlarges, and in somites XIV to XVIII the diameter is some three or four times greater than in front. This "crop" has a whiter appearance, due to its thicker muscular walls, than the rest of the œsophagus; it is deeply constricted as it passes through the septa, and the wall is greatly folded internally. I almost expected to find that this was a gizzard, but the structure is quite the same as that of the œsophagus. In the nineteenth somite the crop narrows and becomes the intestine, the walls of which are fairly thin, so that the dark food-material is seen through.

Vejdovsky states that there is no typhlosole, but on slitting open the intestine along one side, and examining its interior, a moderate-sized typhlosole is seen on the dorsal wall. Series of sections confirmed this observation, and showed that the epithelium covering this in-pushed dorsal wall differs somewhat from the rest of the lining in that the cells are here longer and more regular in size. The typhlosole then is present, and in it a small typhlosolar vessel or irregular blood space, into which vessels from the intestine wall enter, and from which small vessels pass vertically into the dorsal blood trunk, just as is the case with Lumbricus. How far back the typhlosole extends I am unable to say.

[1] Perrier, "Études sur l'organisation des Lomb. terrestres," 'Arch. de Zool. Exper. et Gen.,' ix, 1881.

The absence of a gizzard, both in Criodrilus and in Pontodrilus, is probably related to the soft nature of their food-material. Both are aquatic in habit. Pontodrilus, as Perrier tells us, lives on the seashore, and its food consists of decaying vegetable matter thrown up by the sea. Criodrilus lives entirely in the water, and obtains its food, according to Örley, by swallowing the mud which contains decomposing vegetable matter. In both cases the food is soft, and already more or less finely divided, and can be easily digested, so that the necessity for a gizzard does not exist: in Lumbricus and other worms, however, which live on land and burrow and swallow the hard soil, some crushing apparatus is needed before the digestive fluid secreted by the wall of the intestine can act on the food.

The vascular system I have not traced to any extent. The dorsal blood-trunk is large, and has the usual ampullate appearance up to somite xv. In the next preceding somite it is bent slightly to one side, and gradually gets narrower till it divides up on the wall of the pharynx. In each of the somites vii to xi a pair of large and long moniliform hearts unite the dorsal to the ventral trunk; and there are lateral vessels in each of the somites posterior to the hearts.

In the neighbourhood of the anus the dorsal trunk divides into two (Vejdovsky; pl. xiv). A subneural vessel is present and a typhlosolar vessel, but neither latero-neural nor intestino-tegumentary vessels exist.

The nervous system presents no points of difference from the usual arrangement. The three "great fibres" are present.

The nephridia are not present in front of somite xiii. A series of sections confirmed the results derived from dissection. In and behind this somite they are large and fairly conspicuous organs, having a slight muscular vesicular portion. Vejdovsky states that they open exteriorly in front of the ventral setæ: he also figures a nephridial funnel (pl. xiii, 21), which somewhat resembles that of Lumbricus.

Pontodrilus agrees with Criodrilus in that there are no

nephridia in the anterior somites, the first nephridium being apparently in somite XIV, so that both these approach the Limicolæ in having no nephridia in those somites in which the spermathecæ and ciliated rosettes lie, though they are present in the same somites with the oviduct and the posterior part of the sperm-duct.

The Genital Organs.—I have succeeded in finding all the usual organs connected with the genital apparatus, with the exception of spermathecæ. The seminal reservoirs or sperm sacs are constructed on the plan of Allolobophora, and not on that of Lumbricus, as Örley seems to indicate, since there is no median portion connecting the sacs below the intestine (fig. 15). The worms which I dissected are sexually mature, one of them having spermatophores attached to somite XIV. There are four pairs of pouches, as in Allolobophora,[1] one on each side of each of the somites IX, X, XI, and XII; they vary in size in these somites, and in different individuals. Each is an irregular loose mass, which is easily torn on opening the worm, and in sections the lobation is seen to be carried to a great extent, the cavity of the sac being subdivided by long, narrow inpushings of the wall of the sac, whilst loose separate masses of developing spermatozoa are seen in the somites in which the reservoirs lie. Those in somites IX and X are formed as anteriorly directed saclike outpushings of the hinder septa of these somites, whilst those in somites XI and XII are posterior outgrowths of the anterior septa of these somites. Each is connected to a septum by a short pedicle (Pl. XXXVIII, fig. 15, e^1 to e^4).

The testes (which Örley states lie in somites XI and XII) are in reality in somites X and XI, attached to the anterior septa, very close to the ventral body wall, near the nerve cord (fig. 15, *a*). They have a digitate form, like the testis of Allolobophora turgida, figured by Bergh.[2] (Pl. XXXVIII, fig. 16). Owing to their deep position they are very difficult to

[1] R. S. Bergh, "Untersuch. über d. Bau u. d. Entwickl. d. Geschlectsorgane d. Regenwürmer," 'Zeit. für wiss. Zool.,' 1886, p. 303.

[2] Ibid., fig. 1.

find at first, but my dissections are confirmed by transverse sections.

Close behind each testis is a ciliated rosette, lying, therefore, in somites x and xi, and close to the posterior septum of the somite. (Örley wrongly states that they are attached to the anterior septum of somites xi and xii, into which they project.)

The sperm-ducts from the two ciliated rosettes of one side unite at the level of the septum behind somite xi, and the single duct passes to somite xv, embedded in the connective tissue which exists between the coelomic epithelium and the longitudinal muscles of the body wall; hence it is practically impossible to trace it except by means of sections, unless it happen to be filled with spermatozoa, when it will appear whiter than the surrounding tissue. In somite xv is a large and conspicuous hemispherical gland, which may be called a prostate; the sperm-duct passes to the dorsal surface of this gland, dips down through its mass and opens to the exterior by the pore mentioned above, which is situated on a prominent rounded papilla, which seems to be merely the outer half of the prostate. This gland itself consists of cells similar to those forming the epidermis of the clitellum, and quite continuous with them; the muscular layers of the body wall are here thin, and pass over the inner surface of the prostate, so that the gland appears to be formed merely by an hemispherical thickening of the epidermis over this area.

The ovary is a flattened rounded disc attached to the anterior septum of somite xiii, close to the nerve cord (fig. 15, *f*). It resembles the ovary of Perichæta in shape, and is without the tail-like prolongation of the ovary of Lumbricus (fig. 19). It is figured in Vejdovsky's work,[1] but I have added a figure here, as he does not show the delicate membrane surrounding the organ.

The ovisac (which seems to be a better name than Bergh's "receptaculum ovorum," since the word "receptaculum" has been applied to a spermatheca) is a botryoidal sac-like

[1] Loc. cit., pl. xiii, fig. 23.

protrusion of the posterior septum of somite XIII, and thus lies in somite XIV. It is filled with ripe ova and has a goodly supply of blood capillaries on its wall. It is very conspicuous in the specimens dissected by me, much more so than the ovary, for which I should probably have mistaken it had not Bergh's paper appeared;[1] and it is curious that Örley makes no mention of it.

The funnel of the oviduct (fig. 15, *g*) projects into somite XIII, close to the point where the ovisac is attached; and the edge of the funnel is more prominent than is usual. The external pore has already been mentioned, as being on somite XIV (fig. 10, *c*).

Örley states that the spermathecæ "appear to open to the exterior between the somites IX and X, and X and XI." I can find no trace of spermathecæ, though I have searched for them in some half a dozen specimens, of various stages of maturity; nor is any trace of them presented in a series of sections through this region of the body. I must therefore conclude that this is an error of observation on his part; he says no more of them than the above quotation. Can he have mistaken the ciliated rosettes for these organs, and mistaken the testes for the rosettes? It seems to me quite probable from his description of the relation of these structures that such is the case; a portion of a ciliated rosette, removed, teased, and examined, would show mature spermatozoa, which might lead an observer to conclude that he was dealing with a spermatheca. Again, the shape of the testes, as seen with a lens, might without difficulty be mistaken for ciliated rosettes, which he places in the position occupied by the testes, though he has placed these in the wrong somites.

The cocoon and spermatophore are so fully described and figured by Örley, that I have nothing to add to his description of these structures.

His interesting observations on the habits of Criodrilus

[1] It is probable, as Mr. Beddard has remarked in a recent number of the 'Proc. Zool. Soc.,' that the structure figured and described by me as the ovary of Microchæta (see this Journal, vols. xxvi and xxvii) is really the "ovisac;" and that I have overlooked the true gonad.

will, I hope, enable this form to be discovered in England and similar observations on the habits of other forms are a great desideratum.

Parasites of Criodrilus.—My attention was first attracted to certain curious elongated structures attached to the ovary, and I found them afterwards in various parts of the body. These are narrow bodies, about one tenth of an inch in length, and of a white colour (in spirit). Each is invested by a well-defined cuticle, which encloses a very granular dark medullary protoplasm, in which is a clearer space, probably the nucleus. The shape varies to a great extent; some consist of an elongated ovoid body drawn out at each end into a much narrower portion; others are just the reverse, consisting of two ovoid swellings connected by a narrower portion.

They are apparently Gregarinæ, which have been killed in various states of englenoid movement, such as is exhibited by Monocystis lumbrici; the worms had been killed in corrosive sublimate, judging from the white deposit on their surface, and this would cause the various states of movement to be fixed. At one end the cuticle is thickened and presents somewhat the appearance figured by Professor Lankester in vol. 3 of this Journal, Pl. VII, for M. aphroditæ.

EXPLANATION OF PLATE XXXVIII,

Illustrating Dr. Örley's Paper "Observations on Criodrilus lacuum," figs. 1 to 8, and Mr. Benham's Paper "Studies on Earthworms," figs. 9 to 19.

Criodrilus lacuum, Hoffmeister.

Fig. 1.—Cocoon of Criodrilus lacuum. Natural size. *a.* The end by which it is attached. *b.* The free end.

Fig. 2.—The attachable end more highly magnified. (× 300.)

Fig. 3.—The free end more highly magnified. (× 300.)

Fig. 4.—A transverse section through the middle portion of the wall of the cocoon. (× 300.) *a*. Outer layer. *b*. Middle layer. *c*. Inner layer.

Fig. 5.—A detached piece of the middle layer. (× 300.)

Fig. 6.—Strata of the inner layer. (× 300.)

Fig. 7.—A spermatophore. (× 60.)

Fig. 8.—Spermatozoa from the spermatophore. (× 500.)

Fig. 9.—The anterior extremity of the worm viewed from above. (× 2, spirit specimen.) *a*. The prostomium. *b*. The dorsal setæ. *c*. Enlargement occupying somites XIV to XVIII, due to the large papillæ on the ventral surface. *d*. The clitellum. Behind somite XLV the quadrangular shape of the worm is shown.

Fig. 10.—The same worm from below. *a*. The prostomium. *b*. The mouth. *c*. The pore of the oviduct. *d*. The pore of the sperm-duct, situated on a large rounded swelling causing the enlargement in this region. *e*. The ventral setæ. *f*. The clitellum.

Fig. 11.—The last few somites of the body, showing:—*a*. The anus, situated dorsally. *b*. The region regarded by Vejdovsky as representing seven or eight fused somites.

Fig. 12.—A diagrammatic outline of a section through the body in the posterior region of the body, showing its quadrangular shape, with the dorsal setæ (*a*) and the ventral setæ (*b*) placed at the corners.

Fig. 13.—The side of the body, showing a spermatophore (*a*) attached to somite XIV. *b*. dorsal setæ. *c*. The ventral setæ. *d*. The sperm-pore, on its enlarged papilla. *e*. The ovipore.

Fig. 14.—General view of the anatomy of Criodrilus when opened in the usual way. (× 2.) *a*. The pharynx. *b*. The œsophagus, swelling out at *c* to form a strong muscular crop. *d*. The intestine. *e*. The dorsal blood-vessel. *f*. *f*. The lateral hearts. *g*. *g*. The seminal reservoirs. *h*. The ovary. *k*. The ovisac (Bergh's "receptaculum ovorum"). *l*. The hemispherical glandular swelling or prostate around the terminal portion of the sperm-duct. *m*. Prostomium. *n*. Supraphary ngeal ganglia. *o*. The nephridia.

Fig. 15.—The genital organs of the left side greatly enlarged. A portion of the œsophagus is represented on the right of the figure. *a*. *a'*. The two testes. *b*, *b'*. The ciliated rosettes. *c*. The sperm-duct, which dips into the hemispherical prostate, *d*. The seminal reservoirs, e^1, e^2, e^3, e^4, are represented as relatively rather smaller than their true size; they are seen to be attached to the various septa. *f*. The ovary. The septum between the somites XIII and XIV is turned back so as to show the funnel of the oviduct, *g*, and the nephridial funnel, *l*. *h*. The ovisac. *k*. Nephridium.

Fig. 16.—A testis attached to the septum.

FIG. 17.—A small portion of a section through the epidermis, to show a capillary loop, *d*, passing between the columnar cells, *b*, and the small cells, *c*. *a*. The cuticle. *e*. The circular muscles.

FIG. 18.—A portion of the epidermis from somite XVIII, in order to show its clitellar structure. *a*. Cuticle. *b*. Columnar epithelial cells. *c*. Elongated club-shaped clitellar cells. *d*. Circular muscles.

FIG. 19.—An ovary.

Notes on the Chromatology of Anthea cereus.

By

C. A. Mac Munn, M.A., M.D.

With Plates XXXIX and XL.

The colouring matters of Anthea cereus were first examined by Sorby,[1] who found several present in this Actinia. Among others he found chlorofucin, the bands of which had been observed by Mr. Charles Horner, and the position of which led Mr. Horner to think that the supposed chlorophyll was different to that of land plants. Sorby had previously found chlorofucin in fresh-water algæ and subsequently in Fucus and other olive marine algæ; and in his paper on "Comparative Vegetable Chromatology" he gave directions for its separation from other pigments. Prof. Lankester, in the list of chlorophyll-containing animals in the English edition of Sachs's 'Botany,' includes Anthea cereus and puts "chlorofucin" after it, thus accepting Sorby's statements.

Among those animals which have been proved to contain symbiotic unicellular algæ Anthea is now, I believe, included;[2] and it becomes of interest to find out whether chlorofucin is due to the presence of these symbiotic algæ or whether it is a pigment belonging intrinsically to the animal; whether, also, the other colouring matters associated with the chlorofucin

[1] 'Proc. Roy. Soc.,' No. 146, vol. xxi, 1873, p. 454.

[2] Hertwig, O. and R., "Die Actinien," 'Jena'ische Zeitschrift. f. Naturwis.,' Bd. xiii, 1879, S. 495—500; and Geddes, 'Proc. Roy. Soc. Edin.,' vol. xi, 1881—1882.

belong to the animal or the algæ. I have already[1] proved this point almost completely, as I found that in Anthea cereus, in Bunodes Ballii, and Sagartia Bellis, "yellow cells," or symbiotic algæ, are present, that these animals all contain chlorofucin, all contain the same accompanying colouring matters, and that these colouring matters are evidently due to the "yellow cells" with which the tentacles are stuffed; for there is no essential difference in the spectra of the solutions of the tentacles in which the colouring matters are derived entirely from the "yellow cells" and those obtainable from other parts of the Actinia.

Moreover, I have also proved that in anemones possessing yellow cells there is more or less suppression of the respiratory proteids found in other Actiniæ.

But I had not repeated Sorby's experiments in which he applied Stokes's "fractional" method for the separation of the chlorofucin from the other colouring matters. In the present paper I have given the results of this examination, and, as will be seen, the statements of Sorby have been verified. This is of importance, as Krukenberg[2] has omitted to mention in the account of his experiments the results arrived at by Sorby, although, as I shall show, he had evidently chlorofucin before him in some of the solutions whose spectra he has mapped.

In the paper referred to above[3] I have shown that the mixture of colouring matters obtained from the Actiniæ therein mentioned contain chlorofucin, and that the bands of this correspond to the chlorofucin bands in a similar solution of Fucus serratus. Sorby has figured in a diagram the bands of this pigment, but he does not give their wave-length measurements, and only figures the dominant bands of "blue" and "yellow chlorophyll" in the same diagram for the sake of comparison; consequently some confusion is caused when one endeavours to find out what

[1] 'Proc. Roy. Soc.,' No. 235, 1885, and 'Philos. Trans.,' 1885, Part II, 641, and seq.

[2] 'Vergleichend-Physiol. Studien.,' 1ste Reihe, 5te Abth., 1881, S. 38, and Ibid. 2te Reihe, 3tte Abth., 1882, S. 72.

[3] 'Philos. Trans.,' loc. cit.

Sorby means by "blue" and "yellow chlorophyll" and "chlorofucin," and the object of this paper is to clear up this confusion as regards chlorofucin more especially, also to prove that the chlorofucin and its accompanying pigments are due entirely to the "yellow cells."

It is necessary here to recall the experiment of Geddes[1] to mind. Geddes found that by exposing Anthea cereus to sunlight he got as much as 32—38 per cent. of oxygen, and he found starch and cellulose in the "yellow cells." He tries to reconcile this fact with the statement made by Krukenberg, who failed to get any evidence of the evolution of oxygen, by supposing that Krukenberg must have examined a different variety of Anthea; and he further observes: "Thus, then, the colouring matter of Anthea, described as chlorophyll by Lankester, has really been mainly derived from that of the endodermal algæ of the variety plumosa, which predominates at Naples, while the Anthea-green of Krukenberg must mainly consist of the green pigment of the ectoderm, since the Trieste variety evidently does not contain algæ in any great quantity. But since the Naples variety, contrary to the opinion of the brothers Hertwig,[2] does contain a certain amount of ordinary green pigment, and since the Trieste variety is tolerably sure to contain some algæ, Heider having indeed shown the presence of yellow cells in Sagartia, both spectroscopists have then been operating on a mixture of two wholly distinct pigments—one vegetable, the other animal—diatom-yellow and Anthea-green." In other words, Prof. Lankester's pigment would be "diatom-yellow" and Krukenberg's "Anthea-green." But I believe this theory will not account for the above-mentioned discrepancy, as I find a chlorophyll as well as a chlorofucin in extracts of the "yellow cells," and I shall endeavour to show that out of these yellow cells one extract may contain more of one coloured constituent, another more of another; and this result does not prove that one extract contains

[1] 'Nature,' 26th Jan., 1882, pp. 303—305, and 'Proc. Roy. Soc.,' Edin., vol. xi, 1881, 1882, p. 377—396.

[2] Loc. cit.

an intrinsic Anthea pigment and another an algal pigment, but simply this: that the colouring matters of the algæ are several, for we find at least one chlorophyll, one chlorofucin, and certain lipochromes, and perhaps other pigments, all of which belong to the "yellow cells."

The Physiological Proof is not wholly reliable. The evolution of oxygen in the presence of sunlight in the case of Anthea must to a large extent at least depend on the situation of the "yellow cells," for it is evident that if in a given species these are shut up in the tentacles the oxygen given off has not much opportunity of escaping out of them so as to make itself evident in a vessel containing the anemone, moreover a large amount of the oxygen will in such a position be largely absorbed by the tissues of the animal, and the same manner in which the cells are packed, as shown in the accompanying drawing, prevents the deeper lying cells from being acted upon by the rays of light. If on the other hand the ectoderm of the animal were studded with algæ there would be a considerable development of oxygen perceptible, and there is certainly such a variation in the distribution of the "yellow cells," for in some Actiniæ I have observed rows of algæ embedded in the ectoderm, while in others they may be mostly confined to the tentacles. This point should not be lost sight of, and may account for the discrepancy to a great extent.

The Morphological Proof that the "yellow cells" are parasitic algæ has been so well discussed that I need not here "treat of it," but I may observe that Krukenberg's idea as to their hepatic function must, so far as their microscopic character is concerned, be completely negatived. In no invertebrate liver or answering organ are such bodies found. On the contrary, the microscopic characters of liver chlorophyll or entero-chlorophyll at once separate it from that of the "yellow cells;" for, in the case of entero-chlorophyll, it is easy enough to see that it occurs mostly dissolved in oil, or in granules, or diffused through the protoplasm of the lining cells of the liver tubes.[1] The spectroscopic reasons for the same conclusion are considered below.

[1] 'Proc. Roy. Soc.,' No. 237, 1885, and 'Philos. Trans.,' 1886, Part I.

The Chemical Proof is no less convincing, as the presence of starch within the "yellow cells," and of a cellulose wall surrounding them, is easily proved, especially after, as Geddes has shown, the usual botanical precautions have been taken, namely, steeping the "yellow cells" in alcohol, then in caustic potash, and neutralising with acetic acid before applying the tests with iodine and with Schulze's fluid. The same tests applied to liver chlorophyll entirely fail.

It is not necessary here to describe the differences between the "yellow cells" and the chlorophyll corpuscles of Hydra and Spongilla, as Professor Lankester[1] has shown that the latter are not parasitic algæ, and I have[2] lately studied the chlorophyll corpuscles of Stentor polymorphus, Paramœcium, and Ophrydium, and compared their morphological characters with those of the "yellow cells," and have concluded that they too are not parasitic algæ, although in some corpuscles I have found traces of an amyloid substance, and the presence of a cellulose wall. Miss Jessie Sallitt[3] has also studied the morphology of the chlorophyll corpuscles of certain Infusorians, but she has not found starch. This, however, is of no importance, for there is no reason why starch should not appear in the protoplasmic contents of an animal chlorophyll corpuscle containing chlorophyll. Nor would the absence of starch within such cells justify us in concluding that it is not formed there, as it may be, and probably is, rapidly removed elsewhere as soon as it is formed (Lankester). Granting that starch is built up by the agency of chlorophyll from carbon dioxide and water, we may not always meet with it, for botanists teach that some "non-nitrogenous[4] organic substance is first formed in the chlorophyll corpuscle from carbon dioxide and water," which is "not starch, but a sub-

[1] "On the Chlorophyll Corpuscles and Amyloid deposits of Spongilla and Hydra," 'Quart. Journ. Mic. Sci.,' vol. xxii, N.S., p. 229.

[2] 'Proc. Birm. Philos. Soc.,' vol. v, Part I, pp. 177—218.

[3] "On the Chlorophyll Corpuscles of some Infusoria," 'Quart. Journ. Mic. Sci.,' vol. xxiv, 1884.

[4] Vines, 'Lectures on the Physiology of Plants,' 1886, p. 145.

stance (possibly allied to formic aldehyde) which goes to construct proteid, by combining either with the nitrogen and sulphur absorbed in the form of salts from the soil, or with the nitrogenous residues of previous decompositions of proteid. The starch deposited in the corpuscle is, however, the first visible product of the constructive metabolism going on within it; for, unless protoplasm is being formed, no starch can be produced: it may be regarded as a temporary reserve material." The fact that such "reserve material," while, being of great service in a vegetable cell, and not being of much service in an animal cell, may lead to the metabolic process stopping short of its actual formation; for it appears to me that the principal use of chlorophyll in the animal cell may be to supply the animal with oxygen by the decomposition of the animal's waste carbon dioxide, and the formation of starch would be, to a great extent, a superfluous advantage. In that case the formation of starch would be more accidental than of actual necessity. This view of the function of animal chlorophyll is very much strengthened by the recent experiments of Regnard,[1] which, if confirmed, will tend to support the view that chlorophyll, even separated from the "chlorophyll corpuscles," is of use in the respiratory processes of animals. Whatever the rôle of the intrinsic chlorophyll of the animal may be, there can be little doubt as to that of the "yellow cells" of Anthea, which all proofs, morphological, physiological, chemical, and spectroscopic, point out as being distinct organisms, having an independent life from the animal, although, of course, benefitting by their position not only themselves but their host.

If sections of the tentacles are made after hardening in alcohol, it will be seen that the masses of yellow cells are packed in the tentacle at random as it were, and as the water is absorbed from them by the alcohol, radiating cracks appear in the mass of cells which are merely laid in apposition to each other, and not connected in any way as they would be if part of the animal's structure. I have endeavoured to show this in

[1] 'Compt. rend.,' CI, 1293—1295, and 'Journ. Chem. Soc.,' March, 1886, p. 254.

the accompanying drawing, an inspection of which alone will convince most people that these bodies are not "secreting cells" (Pl. XXXIX).

Results of Spectroscopic Examination.—The chlorophyll of Anthea differs from other chlorophylls in its remarkable instability towards caustic alkalies; this I have already described,[1] and the chlorofucin which accompanies it is also remarkably unstable. I propose first to describe the results of an examination of the solutions of these pigments, and then to compare them with plant chlorophyll.

In comparing my results with those of Krukenberg, the difficulty at once is encountered of attempting to find out what bands of his correspond with mine, as in all his early maps the Fraunhofer lines occupy the wrong position, and none of his measurements have been given in wave lengths. Still it is not difficult to see that the pigments met with by him do not differ from those here described, and if this be the case it is easy to say which are the bands of chlorofucin and which of chlorophyll, &c., in his drawings, as I shall show further on.

I now proceed to describe the results of an examination of the colouring matters of Anthea. All the specimens which I examined have been of a dull greenish colour and therefore are of the same colour as those examined by Sorby.

The tentacles were removed from several specimens and put into absolute alcohol after washing with water, this I may call solution (1). The other parts without the tentacles were cut up small, washed with water, and also put into absolute alcohol; this I may call solution (2). In both cases the absolute alcohol was left in contact with the parts for three days or longer in a dark place.

The colour of solution (1) was greenish yellow, and it had a red fluorescence and gave in a certain depth sp. 1, while in a shallow layer a band became detached in the violet end of the spectrum, which I have tried to represent by sp. 2; but it must be remembered that this band may not be exactly represented owing to the difficulty of seeing it, even by the help of

[1] 'Philos. Trans.,' loc. cit.

good daylight. The presence of the second band (commencing from the red end) at once stamps this spectrum and distinguishes it from the usual chlorophyll spectrum. These bands read approximately:

1st band	. . .	λ 674·5 to λ 653.
2nd „	. . .	λ 641 to λ 625.
3rd „	. . .	λ 595 to λ 575.

While the 4th band was guessed to be λ 467 to λ 443, but its edges were so ill-defined and it was so encroached upon by the general absorption of the violet end that this measurement may require to be corrected.

The colour of solution (2) was a deep orange and it had also a red fluorescence, but not so well marked as that of the former solution. The spectrum is shown in sp. 3, and it is seen that while the same bands are present as those of sp. 1, yet the two first are of relatively different intensity of shading. This points to the obvious conclusion that two colouring matters are present, the one indicated by the band in red, the other by the second band (and the third, as will be shown further on), there is more of the former present in the solution of the tentacles, of the latter in that of the other parts. The readings of these bands were the same as the last. In a thinner layer of this solution (2) there are two bands nearer the violet, that nearer the red being less shaded than the other. Sp. 4 is an attempt to show this, but it only approximately represents the position of these bands, of which one is coincident with the lipochrome band of sp. 2. This difference of spectrum explains the difference of colour of these solutions, for the latter solution probably contains an additional yellow colouring matter probably derived from the animal, but it is not an additional green but a yellow.

Solution (1) (of the tentacles) was now agitated in a separating funnel, after dilution with water, with carbon bisulphide, when the bisulphide fell to the bottom of a yellow-green colour leaving the alcohol layer orange. This bisulphide solution on separating possessed a red fluorescence and gave sp. 5, and the following readings:

1st band . . . λ 685 to λ 656.
2nd „ . . . λ 647 to λ 612·5. (?)
3rd „ . . . λ 597 to λ 573.

Two other bands in the green were also seen which doubtless are obscured in the alcohol solution by the shading produced by the yellow constituents absorbing the violet end; and two others in the violet end were also visible, which, as far as I could judge, measured approximately from λ 479 to λ 460, and perhaps λ 453 to λ 436.

A second bisulphide extract of the same solution, after the first had been removed, was not nearly as green as the first bisulphide extract, and contained less of the constituents giving the bands in the red half of the spectrum, but showed a dark band in the blue which was coincident with the λ 479 to λ 460 band just mentioned. This spectrum is shown in sp. 6.[1] In every other respect the bands of sp. 5 and 6 agree.

A third bisulphide extract of the same solution was hardly coloured, and gave traces of the same bands. The alcohol solution, which had been thrice extracted with bisulphide, was of an orange colour and contained a good deal of bisulphide, and now this solution no longer showed the first band in red, but did show the second and third bands of the original spectrum; these latter are evidently the bands of Sorby's chlorofucin, as can be seen by a comparison with his diagram. They are shown in sp. 7; in a thinner layer there are other bands nearer the violet measuring approximately λ 511 to λ 488, and λ 477 to λ 457, as shown in sp. 8.

To see whether these two latter were the bands of Sorby's fucoxanthin, a couple of drops of ammonia were added and a little water; the bisulphide layer was now turbid and gave one broad band in green, the solution having an amber tint. (It is noticeable that in this and in a second similar bisulphide extract the dark bands in the blue and violet of sp. 6 and 8 were not present; but the blue colour with hydrochloric acid

[1] Possibly the band may not belong to a lipochrome, as I found a pigment in Anthea's tentacles soluble in glycerine, with a band in this part of the spectrum. See below.

described by Sorby was not produced, because this pigment was not fucoxanthin.) The chlorofucin bands of sp. 7 after the bisulphide had removed as much colouring matter as possible was (approximately) :

1st band . . . λ 644 to λ 627·5.
2nd „ . . . λ 595 to λ 581.

There were, however, other bands present—one in blue green and one in violet. This solution was a deep yellow colour, due, probably, partly to the incomplete separation of the yellow pigment giving the bands in the violet end.

On evaporation of this solution some brown-yellow flocks separated out; the residue was extracted with absolute alcohol. In this it formed a fine deep yellow solution, and gave sp. 7 again, the bands reading:

1st band . . . λ 641 to λ 627·5.
2nd „ . . . λ 595 to λ 579.

What the alcohol left undissolved was nearly all taken up by distilled water, forming a yellow solution, giving some shading at the blue end of green and absorbing the violet end. Hydrochloric acid did not seem to affect this solution.

According to Sorby,[1] the alcohol solution of chlorofucin is changed by hydrochloric acid, and on adding this reagent to its alcohol solution a new spectrum appears, namely, sp. 9, whose bands read approximately:

1st band . . . λ 607·5 to λ 597.
2nd „ . . . λ 585 to λ 573.

Hence there is no doubt that this colouring matter was chlorofucin.

Solution (2) (i. e. the alcohol solution of the parts of Anthea without tentacles) also contained chlorofucin, as I proved, by a similar method. It was stated above that the solution (1), from which the bisulphide had removed as much of the colouring matter as it could take up, had been treated with ammonia (two drops) and agitated afresh with bisulphide, and that this

[1] Loc. cit., p. 455.

bisulphide gave a band in the green; such a solution was evaporated to dryness, and the residue tested by the lipochrome tests. With iodine and iodide of potassium it remained unchanged, with nitric acid it gave a transient blue, with sulphuric acid a green and blue; hence it is a lipochrome.

A second absolute alcohol extract having been made from the tentacles only was of a greenish-yellow colour with a red fluorescence, and gave the following bands:

1st band . . . λ 681·5 to λ 650 (dark part = λ 678 to λ 653).
2nd „ . . . λ 636 to λ 601.
3rd „ . . . λ 593 to λ 573. (See Sp. 10.)

In a thin layer there may have been some shading between green and blue, and perhaps some in violet. To this solution water was added, and it was then agitated with carbon bisulphide. On separating the latter it was greenish, leaving the alcohol yellow, and in both these solutions certain bands in the violet could now be seen, which were not visible in the original alcohol solution (before separation). I have tried to show all the bands in sp. 11 and 12. These read as follows:

1st band . . . λ 686 to λ 656.
2nd „ . . . λ 644 to λ 612·5.
3rd „ . . . λ 599 to λ 575.
4th „ . . . (a shading before the next).
5th „ . . . λ 523 to λ 496.
6th „ . . . λ 479 to λ 460.
7th „ . . . λ 453 to λ 436.

Now, on comparing these bands with the bisulphide extract from the first alcohol extract of the tentacles, they are found to be practically almost the same. The yellow alcohol solution from which the bisulphide had removed the pigment giving these bands, no longer contained any chlorofucin, and showed hardly a trace of a band in red in a deep layer. It did show the shadow of a band in green, and perhaps another in the violet.

If we compare a second absolute alcohol extract of the parts of Anthea free from tentacles with the second absolute extract of the tentacles, we find that no great difference exists between

them. Such a solution was greenish yellow; it possessed a fine red fluorescence, and its bands read as follows:

1st band	. . .	λ 681·5 to λ 650.
2nd „	. . .	λ 638·5 to λ 605.
3rd „	. . .	λ 593 to λ 573.

This solution, diluted with water, was agitated with bisulphide; the latter fell down of a yellow colour, and possessed a red fluorescence, and gave a spectrum the same as sp. 11, as will be seen by the following readings:

1st band	. . .	λ 685 to λ 656.
2nd „	. . .	λ 644 to λ 612·5 (?).
3rd „	. . .	λ 599 to λ 577.
4th „	. . .	?
5th „	. . .	λ 523 to λ 499.
6th „	. . .	λ 479 to λ 460.
7th „	. . .	λ 453 to λ 436.

The alcohol solution from which this solution had been removed was of a yellow colour, and gave a feeble band at the end of green, and one in violet; on evaporation it left a dirty yellow residue, which gave no reaction with iodine and iodide of potassium, was slightly green with nitric acid, and brownish with sulphuric acid.

A third absolute alcohol extract of the tentacles only, which had a green colour and a red fluorescence, gave sp. 13. The only difference between this and sp. 11 is in the shading of the second band, as in 13 it is one single band, whereas in 11 there is a distinct narrow part over the broad, less shaded one. The wave length readings, too, show a close agreement; thus the bands read:

1st band	. . .	λ 678 to λ 647.
2nd „	. . .	λ 633 to λ 601.
3rd „	. . .	λ 591 to λ 571.

There was also a band at the blue end of green, and perhaps one in violet.

A third extract of the parts of Anthea without the tentacles contained the same colouring matters. It was of a

greenish colour with a blood-red fluorescence, and gave the following bands:

1st band . . . λ 678 to λ 647.
2nd „ . . . λ 633 to λ 601.
3rd „ . . . λ 591 to λ 573.

There was also a band at the blue end of green, and perhaps one in violet. Hence, then, the third extract of the tentacles and the third extract of the parts without the tentacles contain the same colouring matters. Now, the colouring matter in these latter extracts, which gives the bands in the red end of the spectrum, is almost, if not altogether, free from chlorofucin; at least the second and third bands of the spectrum give different measurements. So far as I can judge this, then, must be the pigment which Krukenberg would have belonging to Anthea, which he calls "Antheen," although I cannot prove this assertion, owing to the way his spectra are represented. Krukenberg says that the corresponding pigment withstands saponification, but in this case, as I have previously shown,[1] the addition of a little caustic soda alters the spectrum, as shown in sp. 14.[2]

A fourth absolute alcohol extract of the tentacles gave the same spectrum, the bands reading:

1st band . . . λ 678 to λ 650.
2nd „ . . . λ 633 to λ 601.
3rd „ . . . λ 591 to λ 573. Others uncertain.

A fourth similar extract of parts without tentacles read:

1st band . . . λ 678 to λ 650.
2nd „ . . . λ 633 to λ 601.
3rd „ . . . λ 591 to λ 573. Others uncertain.

And a fifth and sixth alcohol extract of the tentacles, and a fifth and sixth of the parts without them, gave the same readings. Hence we have precisely the same colouring matters present in the tentacles as in the rest of the animal, and as those in the tentacles are due to

[1] 'Philos. Trans.,' Part II, 1885.

[2] NaHO causes precipitation, but after filtering, the solution is green, and gives sp. 14.

"yellow cells," it is fair to conclude that those of the latter are also due to "yellow cells," and do not belong intrinsically to the animal, which is the point to be proved.

So far, then, there are several pigments present, three at least; one represented by a chlorophyll-like spectrum, characterised by the dominant band in red, and by others when the chlorofucin is separated out, chlorofucin represented by sp. 7, and a lipochrome, or lipochromes, represented by bands in the violet half of the spectrum. The chlorophyll is, however, more decomposable by caustic alkalies than is that of land plants; and it is now necessary to see whether it agrees with Sorby's "blue" or "yellow chlorophyll." Sorby's yellow chlorophyll is found in Ulva latissima.[1]

In the following experiments it must be remembered that a relatively large quantity of chlorophyll was present, and that consequently the dominant chlorophyll band was much broader than that in any of the above spectra. I did not boil the Ulva, as Sorby directs, but merely extracted it in the cold with absolute alcohol for three days in the dark. The resulting solution was a fine sap green colour with a blood-red fluorescence, and gave in a suitable depth the following measurements:

1st band	. . .	λ 681·5 to λ 641.
2nd ,,	. . .	λ 625 to λ 599.
3rd ,,	. . .	λ 591 to λ 566.
4th ,,	. . .	λ 549 to λ 532.

There were also two other bands, one in blue green and one in violet. But in order to compare this solution with the chlorophyll constituent of Anthea's colouring matters, it is necessary to agitate with bisulphide of carbon and examine the latter solution. Putting now the measurements of the bands of this solution, and those of the corresponding extract of Anthea side by side, we get:

[1] Sorby, loc. cit., p. 453. Besides the lipochromes there is in Anthea's tentacles a pigment soluble in glycerine which gives bands in the violet, which I have described in my paper on the "Chromatology of the Actiniæ," loc. cit.

	Ulva.	Anthea.
1st band . .	λ 688·5 to λ 659 and shaded to λ 641.	λ 686 to λ 656.
2nd „ . .	λ 633 to λ 612·5.	λ 644 to λ 612·5.
3rd „ . .	λ 597 to λ 571.	λ 599 to λ 575.
4th „ . .	Uncertain.	Uncertain.
5th „ . .	λ 520 to λ 496.	λ 523 to λ 496.
6th „ . .	λ 485 to λ 466.	λ 479 to λ 460.
7th „ . .	λ 455 to λ 438.	λ 453 to λ 436.

The discrepancy in the measurements of the 2nd band of Ulva and Anthea may be due to a trace of chlorofucin in the bisulphide extract of the latter. The bands in the violet may be neglected as they belong to the lipochrome constituents. Hence it would appear that the chlorophyll constituent in Anthea is closely related to, if not identical with, "yellow chlorophyll." A comparison of sp. 11 and 12 with sp. 15 and 16, teaches that there is a remarkable resemblance between the pigments of Ulva and Anthea not only as regards the chlorophyll bands in the red half of the spectrum, but also as regards the lipochrome bands in the violet half.

I have not here considered the identity of the chlorofucin of Anthea with that found in Fucus serratus, as I have done so already, but I may add that I have lately separated chlorofucin by Sorby's method from other olive algæ, such as Fucus nodosus, and Laminaria digitata, and find that the bands are identical with those of the chlorofucin of Anthea.

We may now conclude that the pigments of Anthea are the pigments of certain marine algæ, and are therefore the pigments of the "yellow cells," which are known to be unicellular algæ.

With regard now to Krukenberg's results; taking his first paper[1] and examining the plate attached to it, we find that in the first spectrum he figures the double band in the red, the second of which I have shown to belong to chlorofucin; the second spectrum represents the effect of NaHO on "Anthea-green," but it really represents the effect of NaHO on

[1] 'Vergleichend. physiol. Studien.,' 1te Reihe, 5te Abth., 1881.

chlorofucin and yellow chlorophyll mixed; the third spectrum also evidently represents the bands of both pigments, and the fourth shows a chlorofucin spectrum. In his second paper, which deals with Anthea viridis var. plumosa, the plate (Tafel iv)[1] shows clearly enough the presence of the same colouring matters, thus in sp. 2 the bands of chlorofucin are visible; in sp. 3 the chlorophyll constituent is present, perhaps mixed with the former, and so in all the others the presence of the same pigment with the lipochrome or lipochromes can be detected. Hence there is no essential difference between the pigments of Anthea plumosa and Anthea cereus, and doubtless, in the former, they are all due to the "yellow cells" also. Krukenberg seems to lean to the opinion that these colouring matters are allied to what he calls "hepatochromates," but such a theory is easily controverted, because (1) enterochlophyll is not nearly so easily decomposed as are the pigments of Anthea, and (2) the "fractional" method distinguishes them at once: in the case of enterochlorophyll, the bisulphide takes up more of the lipochrome, the alcohol retaining some chlorophyll. There are other reasons[2] which I have given elsewhere, and the morphological differences are so well marked that it is no longer possible to maintain such a view.

The present paper contains only a preliminary account of the subject, which I hope to study more thoroughly again.

I have gone over most of Sorby's experiments during the last ten years, and I am more and more convinced of the soundness of his deductions and the accuracy of his experiments; but owing to his diagrams not giving all the bands, and his bands not having all been described in wave-lengths, one has great difficulty sometimes in following the descriptions. This remark more especially applies to the chlorophylls, as I am unable yet to distinguish "blue" from "yellow chlorophyll," and I think one is safe in concluding with recent observers that the green constituent of chlorophyll gives four bands in the red half of the spectrum, and the yellow those in the violet half;

[1] Ibid., 2te Reihe, 3tte Abth., 1882.

[2] E. g., the spectra are totally different.

and hence I am inclined to think that the band represented in Sorby's diagram in the case of "blue" and "yellow chlorophyll" and chlorofucin occurring in the violet end, really belongs to the yellow constituent, which has been incompletely separated by the "fractional" method.

EXPLANATION OF PLATES XXXIX and XL.

Illustrating Dr. C. A. Mac Munn's "Notes on the Chromatology of Anthea cereus."

PLATE XXXIX.

Transverse section of a tentacle of Anthea cereus which had been kept in alcohol for some time; it was stained with picrocarmine, and mounted in Canada balsam. The relationship of the "yellow cells" to the endodermal lining of the tentacle is well shown. The specimen is supposed to be magnified 80 diameters, and was drawn by means of Swift's erecting camera lucida, the paper being fourteen inches distant from the eye-piece.

The radiating cracks in the interior of the mass of "yellow cells" are clearly shown, as well as the absence of any connecting structure.

PLATE XL.

Spectra of the colouring matters of Anthea cereus, &c.

Sp. 1.—Absolute alcohol extract of tentacles of Anthea, deep layer.

Sp. 2.—The same shallow layer.

Sp. 3.—Absolute alcohol extract of other parts without tentacles.

Sp. 4.—The same shallow layer.

Sp. 5.—The alcohol solution of tentacles was diluted with water and agitated with bisulphide of carbon, which gave this spectrum.

Sp. 6.—Second bisulphide extract from the alcohol solution; note the dark band in violet end, which does not belong to the pigment giving bands in the red end.

Sp. 7.—The original alcohol solution after having been three times extracted with bisulphide; these are the bands of Sorby's chlorofucin.

Sp. 8.—The same shallow depth.

Sp. 9.—Action of hydrochloric acid on the same solution, showing changed chlorofucin as Sorby describes it.

Sp. 10.—Second absolute alcohol extract of the tentacles; this shows mainly the chlorophyll bands, possibly the narrow band belongs to the chlorofucin ?

Sp 11.—This was diluted with water and agitated with bisulphide of carbon which on separating gave this spectrum.

Sp. 12.—The same shallow depth.

Sp. 13.—Third absolute alcohol extract of tentacles (the second and third extract of the parts without the tentacles gave the same bands as the second and third extract of the tentacles, therefore they have not been mapped). Note in 13, the narrow (second) band is gone, showing that now the chlorophyll constituent is probably alone present.

Sp. 14.—Action of caustic soda on the last solution.

Sp. 15.—For comparison with the chlorophyll bands of Anthea. An absolute alcohol extract of a green Ulva, was diluted with water and agitated with carbon bisulphide, which after separation gave this spectrum; this solution appears to contain Sorby's "yellow chlorophyll." Compare with 5, 11, and 13.

Sp. 16.—Thin layer of the same. Compare with 12, and it will be seen that probably a similar lipochrome is present in Anthea and in Ulva.

On Ctenodrilus parvulus, nov. spec.

By

Robert Scharff, B.Sc., Ph.D.

With Plate XLI.

The genus Ctenodrilus, which was first established by Claparède, is represented by two species, viz. Ct. pardalis and monostylos.

A new species of this small and most interesting Annelid was recently discovered by Mr. Bolton, of Birmingham, in his seawater aquarium, and forwarded by him to Professor Ray Lankester for identification. Professor Lankester had the kindness to place the whole material at my disposal, and gave me the opportunity of pursuing my investigations at the zoological laboratory of University College. Mr. Bolton states that he does not know the exact locality whence the worm originally came. It is certain, however, that it was sent to him from some place along the British coast, where to my knowledge it has not been met with before. I kept about a score of them in a small bottle for some weeks in November and December, 1884, in order to study their peculiar mode of fissiparous reproduction, and if possible to detect their sexual means of propagation. As regards the first item I was successful, but shared the same fate as my predecessors, Kennel and Zeppelin, in not finding any generative organs.

The worms were at first not very lively, crawling slowly along the glass near the surface of the water, but soon began to divide rapidly. The new species, which I propose to name Ctenodrilus parvulus, differs in several respects from

Ct. pardalis, and more so from Ct. monostylos. Its smaller size is the most striking feature, for while Ct. pardalis has from twelve to fourteen segments and a length of 6—7 mm., Ct. parvulus has only from seven to ten segments and averages about 4 mm. in length. The most important difference, however, lies in the nature of the bristles, which are not pectinated as in Ct. pardalis, but approach in shape one of the two kinds found in Ct. monostylos (fig. 2).

In the anatomical details, as well as in all the other external characters, Ctenodrilus parvulus agrees very closely with Ct. pardalis. Ct. monostylos has characteristic distinguishing features in its peculiar tentacle and in the form of the bristles. In that animal, recently described by Zeppelin,[1] two different kinds of bristles have been found, the one straight and needle like, the other slightly bent at the apex. Both occur in the same bundle except in the anterior segments.

General Characters.—As already mentioned, Ct. parvulus (fig. 1) agrees in general with Ct. pardalis, and exhibits the same characteristic dark green or violet spots in the skin, which are due to some colouring matter filling up part of the epidermal cells. The colour of the worm itself is of a greenish yellow, which sometimes allows of the internal arrangements being plainly visible in the living animal. On the other hand, very frequently the view is obscured to such an extent by the dark spots in the skin as to render its study very difficult.

The segmentation of the body is clearly indicated. It is made up of a prostomium, with the head segment bearing the mouth, followed by a number of equal body segments and the tail segment with the anus.

There are two rows of bristles on each side, and, as in Ct. pardalis, their number in the various metameres is subject to great variation. Sometimes I found only one bristle in the dorsal row and several in the ventral row of the same

[1] Max. Graf. Zeppelin, "Ueber den Bau und die Theilungsvorgänge des Ctenodrilus monostylos," 'Zeitschrift f. wissenschaftliche Zoologie,' vol. xxxix.

segment and vice versâ. In all probability the bristles become worn out in time and are then replaced by new ones. This shows that we are not always justified in using the number of bristles for diagnostic purposes.

Ctenodrilus parvulus is covered by a very thin cuticula and the under surface of the prostomium as well as the under part of the mouth segment are ciliated as in Aelosoma. The whole of the œsophageal cells and those of the intestine also bear cilia. Under the lens the animal has a reddish appearance, due to the bright red colour of the large stomach (fig. 1, *st.*), which will be dealt with later on. The œsophagus reaches to the commencement of the third segment, the stomach occupies generally three or four, and the remaining segments contain the intestine.

With regard to segmental organs there is only one pair of them, viz. in the head segment (fig. 1, *so.*). In some of the more transparent specimens they are readily seen, but the study of their histological structure offers great difficulty.

The vascular system, consisting of a dorsal (*d. v.*) and a ventral vessel (*v. v.*), is not always distinctly visible. The blood is colourless and does not contain any blood-corpuscles. There is a dorsal vessel, existing only in the three anterior segments. It takes its origin at the junction of the œsophagus and stomach and its lumen is gradually narrowed down as it reaches the head segment. In the first segment it divides into two branches, which surround the œsophagus, and, joining again below into one, forming the ventral vessel.

It now remains to say something about the movements of the animal. When isolated it quickly draws itself together and remains in a curved position for some time. It then protrudes the large bifid lower lip, which is no doubt used as a "point d'appui," in order to draw the body forward. But to do this the longitudinal muscles are gradually shortened, causing the body to swell up. The swelling is first seen near the hinder end, but soon travels towards the head, which is thus moved onward. Having hereby changed its position, before it ventures

on a new move it feels its way first by means of the prostomium, which seems to act as a tactile organ, and then repeats the same performance. Sometimes it coils itself round in a snake-like fashion, continually using the thick lower lip, which, I believe, may also be useful in nutrition although I have not actually seen it taking up food with it.

The Skin.—This is composed of an epidermis covered by a very thin homogeneous cuticula. The outlines of the component cells are not very readily made out in cross sections, and they vary in size according to the different parts of the skin in which they are found. At the sides of the body their height is about equal to their breadth, towards the ventral side. On the other hand, the cells increase in height and decrease in width. Their appearance here will be referred to again in connection with the nervous system, which lies in its entire length in the epidermis; suffice it to say that it appears to consist of several layers in the ventral part of the body.

I said before that the under surface of the head segment is ciliated. The same has likewise been noticed in the other two species of Ctenodrilus, as well as in a few more kindred forms.

A surface view of the skin under very high power reveals a large number of the dark violet pigment spots, and among them a few much lighter ones. The intervening spaces are partly covered by exceedingly dark minute specks, contrasting with the light green or yellow ground. According to Kennel[1] the dark pigment is dissolved out by alcohol, a fact which I can testify by my own experience. The same author says, p. 380, that if this fatty matter be withdrawn from the cells by the action of alcohol and turpentine, the colouring material remains behind in shape of irregular stains. The dark pigment lies in the epidermal cells, and appears to be of a fatty nature. In the prostomium and around the anus it is largely developed, and in these parts almost fills up the whole of the cells. This condition does not obtain towards the middle of the body, where the pigment only enters to a small extent into the

[1] J. Kennel, "Ueber Ctenodrilus pardalis," 'Arbeiten aus d. Zool. Institut, Würzburg,' 1882, p. 380.

interior of the cell. Prof. Ray Lankester[1] makes mention of "clear cells or vesicles" as occurring in the skin of Chætogaster limnæi, which appear to be identical structures. He tells us further that the clear vesicles in the integument of many Planarian worms likewise belong to the same order of structures.

Projecting from the skin we have a certain number of bristle-bundles in every segment, viz. one pair in each. I have already called attention to the fact that the quantity of bristles in each bundles is not the same, but varies to a considerable extent. A characteristic structure of the Oligochætes, the small involutions from the skin in which the bristles take their origin, is not present here.

According to Zeppelin, Ctenodrilus monostylos has never less than two bristles in a bundle, while in parvulus I frequently found only one. The shape of the bristle in Ctenodrilus parvulus in distinction to the closely-related Ct. pardalis is that of a spear, the apical part being slightly bent (fig. 2). In the latter species the bristles are pectinated, and in Ct. monostylos there are two different kinds, one of them being long and slender, and the other somewhat resembling the one I just described as occurring in Ct. parvulus. Another point of difference in respect to the bristles of Ct. monostylos is that they project much further from the skin than in Ct. parvulus.

The Muscles.—Beneath the epidermis we find one very thin muscular layer. There is no perceptible division into a transverse and longitudinal part, the layer consisting merely of the primitive longitudinal fibres, which stretch without intermission from head to tail. The few bristles in each bundle are moved by some fibres attached to the general body wall.

A powerful muscle inserted at the front part of the œsophagus causes the protrusion of the lower lip. No special structure can be assigned to the muscular fibre, and, as Zeppelin has pointed out, Ctenodrilus shows great resemblance to the

[1] E. R. Lankester, "A Contribution to the Knowledge of the Lower Annelids," 'Trans. of the Linnean Soc.,' vol. xxvi, p. 634.

Polygordius group with regard to the muscular system. We also find in this group the same primitive arrangement of a simple longitudinal layer of muscles, and in this simple muscular structure we see no doubt represented the original condition among the Annelids.

The Alimentary Canal.—Three parts may easily be distinguished in the alimentary canal of Ctenodrilus parvulus. The ventral mouth, which has the form of a longitudinal slit, opens into the ciliated œsophagus. This, in its turn, is followed at the beginning of the third segment by the red stomach, and the intestine constitutes the remainder of the alimentary canal. The latter, like the œsophagus, is ciliated, and generally commences at the end of the sixth or the commencement of the seventh segment. In Ct. pardalis the cells of the whole of the alimentary canal are ciliated,[1] while in Ct. monostylos we find a similar condition as in Ct. parvulus, viz. ciliated cells in the œsophagus and intestine only. The cells of the ventral side of the alimentary canal are considerably higher than those of the lateral or dorsal sides, so as to cause that part to appear from two to three times as thick. The reddish cells of the stomach gradually merge into the ciliated cells of the intestine, that is to say, they become lighter and lighter in colour, and assume the ciliated condition on approaching that part of the alimentary canal. At the commencement of the stomach we do not see anything of that kind, but an abrupt change from the ciliated to the non-ciliated condition.

The œsophagus (fig. 1, *œ*) is much narrower than the stomach, and attains its smallest diameter close to the mouth, just above the hinder end of the lower lip ("Unterlippe" of Kennel, "Rüssel" of Zeppelin).

This lower lip is situated beneath the mouth, and consists of a broad muscular plate, the anterior part of which being split. I mentioned before that a strong muscle, acting as protractor to this organ, is inserted into the lower portion of the œsophagus.

[1] Kennel, loc. cit., p. 383.

Claparède,[1] who gave the first description of Ct. pardalis, represented the lower lip of this form (pl. xv), as if it were the swollen front portion of the œsophagus, and described it as a "tonnenförmiger muskulöser Schlund." Professor Ray Lankester pointed out some time ago that O. Schmidt's Parthenope serrata was of the same genus as Ct. pardalis, and this too has been figured by Schmidt[2] with a muscular œsophagus similar to Claparède's. If low powers are used the real nature of the lower lip might easily be mistaken, and I think it quite probable that on re-examination the "proboscis" of Parthenope serrata would be found to be an organ corresponding to that of the genus Ctenodrilus.

I refer to Zeppelin's and Kennel's papers for further details on the subject of the lower lip. Whether the organ is used as a means of taking in food I have not been able to observe, but it probably is. At any rate, it plays an important part in the act of locomotion, as I had the opportunity of mentioning before.

The Vascular System.—We have a dorsal and a ventral blood-vessel in Ctenodrilus parvulus, just as in the other species, and a communication between the two in front. The dorsal vessel (fig. 1, *d. v.*) divides into two branches in the first segment, which, uniting again below the œsophagus, forms the ventral vessel (*v. v.*). The vessels are not readily visible in the living animal, except the dorsal, which contains a peculiar organ known as the "räthselhafte" organ (fig. 1, *en.*), also as "corps cardiaque" among French authors. Due to this organ the dorsal vessel is comparatively conspicuous. It is likewise found in the other species of Ctenodrilus, but neither Kennel nor Zeppelin give a satisfactory answer as to its origin or function. I shall say some more about it presently.

The dorsal vessel takes its origin at the anterior portion of the stomach, where it is attached to the wall of that part of the

[1] Claparède, 'Beobachtungen über Anatomie und Entwicklungsgeschichte wirbelloser Thiere d. Küste von Normandie,' Leipzig, 1863.

[2] O. Schmidt, 'Zur Kenntniss d. Turbellaria rhabdocoela und einiger anderer Würmer d. Mittelmeers Sitzungsberichte d. Kais. Akademie, Wien., vol. xxiii, 1857.

alimentary canal. It begins here with a wide mouth, its diameter decreasing rapidly as it reaches the first segment. The above-mentioned enigmatic organ takes up a central position in the vessel. It, too, attenuates towards the anterior part of the animal and disappears in the head segment. A little more light has quite recently been thrown on the function of a similar hitherto problematical structure by Horst.[1] He looks upon it as a gland in connection with a blood-sinus of the alimentary canal, the latter being a continuation of the short dorsal vessel. The blood circulating in this sinus is supposed to pass through the glandular organ into the dorsal vessel. As there does not appear to be a sinus in the wall of the alimentary canal of Ctenodrilus we may regard the so-called enigmatic organ as a remnant from some more ancient type, where the organ was of more use in connection with the blood system, and that it has in time undergone retrograde development.

Segmentation and Segmental Organs.—The outer segmentation corresponds to the inner as in other Chætopods. This, however, is not the case throughout, and the alimentary canal especially shows numerous constrictions quite independent of those of the body. There are from seven to ten segments in Ctenodrilus parvulus, one of the distinctions between it and the closely related Ct. pardalis and monostylos.

The dissepiments are loose and easily moved forward and backward with the alimentary canal. Between the septa are seen a number of small nucleated cells, which float about the body cavity. Zeppelin is of the opinion that they serve as stores for nutritive material in those individuals which are still unable to provide food for themselves. As these cells appear to be more numerous in specimens that are just undergoing division, this view is probably the correct one.

The small segmental organs are not always clearly discernible. In no case are they distinct enough to admit of their true structure or parts being well recognised. Like Ct. pardalis

[1] Horst, "Ueber ein räthselhaftes Organ bei den Chloraemiden," 'Zoologischer, Anzeiger,' Januar, 1885.

and monostylos there is here only one pair of them. The segmental organs lie in the head segment, one on each side of the œsophagus. They are in form of two minute coiled tubes. I could only see the ciliated canal and the internal opening; an external opening described by Kennel was not visible, but it undoubtedly exists.

No segmental organs are found in the other segments. We do not meet with this very peculiar condition in any other fully grown Annelid. The larval form of Polygordius, on the other hand, exhibits in the so-called "Kopfnieren" a condition not unlike that of Ctenodrilus. Hence we may compare the latter in this respect with the Polygordius larva. Kennel[1] even goes further by saying that "in Ctenodrilus the excretory organ of larval Annelids has remained as the permanent organ of excretion."

The Nervous System.—The nervous system agrees in position and structure with that of the other two species of Ctenodrilus. It lies in its entire length in the epidermis, and consists of a cerebral ganglion sending two commissures towards the ventral surface, where they unite to form the nerve-cord.

A similar condition of the nervous system obtains in a few other Annelids mentioned by Semper,[2] as well as in the Gephyrea, Priapulus and Halicryptus.[3]

The parts are too small to allow of a more minute examination of the structure of the nervous system. As in Halicryptus and Priapulus epithelial and ganglionic cells seem to merge into one another, and near the cerebral ganglion, for instance, it is difficult to say where the former end and the latter begin. The epithelium apparently becomes several layered near the nerve-cord, as well as close to the cerebral ganglion. Peripheral nerves were not to be seen.

[1] Kennel, loc. cit., p. 392.

[2] Semper, "Die Verwandtschaftsbeziehungen d. gegliederten Thiere," 'Arbeiten aus. d. Zool. Institut, Würzburg,' vol. iii. According to this author the following species have the nervous system lying in the ectoderm either wholly or partially:—Terebella sp. of Heligoland, Terebella zostericola, Hyalinæcia tubicola, Maldane sp.

[3] Scharff, "On the Skin and Nervous System of Priapulus and Halicryptus," 'Quart. Journ. Micr. Soc.,' vol. xxv, 1885.

The only sensory organs are two small ciliated pits, one on each side of the cerebral ganglion. They are not very conspicuous, and may quite easily be overlooked. At any rate they are very much smaller than those indicated in Claparède's[1] figure of Ct. pardalis.

Fissiparous Reproduction.—It has been mentioned that no traces of reproductive organs could be discovered, but many of the animals kept were either young ones or were just undergoing division. A lengthy report of the division of Ctenodrilus pardalis has been published by Kennel.[2] The general plan of the division in Ct. parvulus is the same as that in Ct. pardalis. The want of sufficient material prevented me from investigating the histological changes going on during the act of fissiparous reproduction. Hence I will merely state the general characters as far as I made them out, and which agree with those reported by Kennel as occurring in Ct. pardalis. At the same time it will not be out of place to give a short summary of the division in Ct. monostylos in comparison with that of the two other forms. A few diagrammatic drawings (figs. 3 and 4) will help to make the statements clearer.

We find a peculiar, but at any rate primitive, condition with regard to the division of Ct. pardalis and parvulus. The great characteristic of these two Annelids is that almost every segment becomes a zooid which rapidly develops into the multisegmented form. This is brought about in the following manner: —A bud appears between two segments, and, in distinction to Naids and kindred forms, the buds are produced in the same order as new segments are, viz. from before backward (fig. 3, *b*). In Naids the production of buds takes place in a similar way to that of the tapeworm, a condition which has been termed "strobilation." Hence Semper's[3] "proglottidentheorie," which he thought was applicable to all Annelids, does not hold good in the case of Ctenodrilus.

In order to make the terms "strobilation" and "segmenta-

[1] Claparède, loc. cit., pl. xv, fig. 28

[2] Kennel, loc. cit., p. 395.

[3] Semper, loc. cit., p. 290.

tion" clearer I will quote the following formula from Kennel. Supposing we call the oldest segment 1, the second oldest 2, and the youngest x, we obtain:

Strobilation, 1, x, $x-1$, $x-2$ . . . 4, 3, 2.
Segmentation, 1, 2, 3, 4 . . . . . $x-2$, $x-1$, x.

Previous to the act of division the animal generally remains in a curved position, the posterior segments exhibiting independent motions which were not before noticed.

The appearance of buds is the next feature, preceding division. The first bud makes its appearance dorsally immediately behind the third segment, the second behind the fourth, and so on. In Ct. parvulus we never find more than three or four buds (in Ct. pardalis there may be six or more). The first three segments never show any indication of budding, nor do the last two or three.

An accumulation of pigment appears in the buds as soon as they show themselves. The red colour of the stomach at the same time becomes materially darker. The part between two segments is now very much constricted, and the bud grows rapidly forward, so as to assume the shape of the head segment. The previously red portion of the alimentary canal appears almost black just before division, except at the constrictions, where it takes the colour of the intestine.

Zooids have the peculiarity, as long as they remain united, never to develop new dissepiments. Only after the division, after the breaking up, as it were, of the animal do new segments appear. In the true Naids, on the other hand, a differentiation of the budding zones goes on to such a degree that when division occurs each zooid may already be regarded as a fully-developed animal.

The first zooid in Ctenodrilus needs only to regenerate the anus, and the last zooid the head. As soon as division has actually taken place the zooids very soon develop into the adult condition. The alimentary canal is closed at those parts where it was ruptured by the constrictions, and a new mouth and anus are formed in the two or three middle zooids. The

animals I had under observation all completed the act of fisssiparous reproduction within forty-eight hours.

Comparing the division of Ct. monostylos (fig. 4, *a*—*c*) with that of the two forms I just described, the most striking difference lies in the fact that there is no appearance of buds. In Ct. monostylos we have merely a breaking up of the animal into two almost equal parts, each of which may again break up into two or more parts. The regeneration only takes place after the division has been completed.

Summing up the result of Zeppelin's researches on the division of Ct. monostylos into a few words, we should have the following:

In the fully-grown normal individuals a constriction takes place about the middle of the body, which gradually becomes deeper and deeper. At the same time the intestine becomes rounded off in the two parts, and ultimately the mother animal breaks up into two daughter animals of about the same size, one of which bears the head along with some segments, the other the anus, also with a few body segments. The regeneration always begins after division has taken place.

Both daughter animals are capable of subdivision into parts, all of which contain a portion of the original animal's intestine. Those parts are either—

(1) Parts which have neither head nor anus, consist only of one to three segments, and are incapable of division; or

(2) Parts without head or anus, possessing more than three segments, which may divide again.

The daughter animal which contains the primary head has been noticed to constrict off a part provided with the secondary anus. Whether a corresponding constriction also takes place in the other daughter animal has not been observed.

This constitutes the main difference in the mode of fissiparous reproduction of Ct. monostylos, on the one hand, and Ct. pardalis and parvulus on the other.

EXPLANATION OF PLATE XL,

Illustrating Dr. Robert Scharff's paper on "Ctenodrilus parvulus," nov. spec.

Fig. 1.—View of Ctenodrilus parvulus (highly magnified, somewhat diagrammatic). *mo.* Mouth. *a.* Anus. *s. o.* Segmental organ. *d. v.* Dorsal vessel. *v. v.* Ventral vessel. *œ.* Œsophagus. *st.* Stomach. *br.* Bristles. *en.* Enigmatic organ. *int.* Intestine.

Fig. 2.—One of the bristles of Ct. parvulus (highly magnified).

Fig. 3.—Fissiparous reproduction of Ct. parvulus (Ct. pardalis is quite similar; see text). *a.* First stage. *b.* Second stage. *c.* Third stage.

Fig. 4.—Fissiparous reproduction of Ct. monostylos. *a.* First stage. *b.* Second stage. *c.* Third stage. (Copied from Zeppelin.)

The Relation of the Nemertea to the Vertebrata.

By

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Professor in Utrecht.

With Plate XLII.[1]

In venturing at the close of my Report on the Nemertea, collected by H.M.S. "Challenger," to leave the region of demonstrated facts and actual observations, and to enter upon that of speculation and suggestion, I gladly availed myself of the permission for so doing granted to me by the editor, Mr. John Murray. I thought it necessary to ask for that permission, because general speculations on the ancestry of the Chordata hardly appeared to me to fit into the framework of those Reports. My desire in this case to deviate from a rule which I held to be salutary, was due to the fact that of late these speculations have been conducted along very varying channels, an entirely new one having only very lately been opened by Bateson's important series of papers on Balanoglossus. An attempt to give more depth to one of these channels, and thus to lead into it the attention of a greater number of my fellow-workers, especially commended itself to me, since it was my conviction that the lines laid down by myself in former publications derived considerable support from the "Challenger" material, and were thus entitled to a renewed and full consideration.

[1] Published by permission of the editor of the 'Zoology of the Challenger Expedition,' Mr. John Murray, F.R.S.E.

I would formulate the proposition, to the further development of which this memoir is to be devoted, as follows:

More than any other class of invertebrate animals, the Nemertea have preserved in their organisation traces of such features as must have been characteristic of those animal forms, by which a transition has been gradually brought about from the archicœlous Diploblastic (Cœlenterate) type to those enterocœlous Triploblastica, that have afterwards developed into the Chordata (Urochorda, Hemichorda, Cephalochorda, and Vertebrata).

It will be seen that this statement excludes the idea of any direct ancestral relations between Nemertea and Chordata. If any such relation were proposed, it might with good reason be asked—considering the very extensive variation which is met with amongst Nemertea—which species or which genus was more particularly pointed to. The question in itself condemns the proposition which leads to it.

It will, moreover, be seen that this statement accepts the outcome of Bateson's researches and speculations, in so far as the points of agreement between Balanoglossus and Amphioxus are fully recognised. A provisional link between these two, and an arrangement of Balanoglossus as amongst the Chordata, appears to be quite as justifiable as the elevation of the Urochorda to their new dignity in zoological classification.

There is, however, a great difference between looking at Balanoglossus as a low type amongst the Chordata (in which I fully agree with Bateson) and rejecting the significance of the Nemertean type as one of transition in the way above indicated.

There is no doubt that the Nemertea represent a more primitive phase than the Enteropneusta (Hemichorda). They have no gill-slits; but their nervous system shows certain unexpected analogies with that of the higher Chordata of more intrinsic value than those that obtain between Balanoglossus and the Chordata in general. Also for the important question,

which is so vital in any consideration of the ancestry of the Vertebrates, viz. the origin of metameric segmentation, it appears to me that the Nemertea offer points very worthy of consideration. The question of the proboscis and its sheath, as comparable to hypophysis and notochord was fully treated by me in another paper,[1] and will here only be very briefly touched upon. In my opinion, this comparison is all the more forced upon us, now that in other respects (nervous system, &c.) new evidence of genetic relationship is here brought forward.

The first point I wish to consider is that of metameric segmentation. It has been specially treated of late years by various authors of renown, with whom I do not wish to enter at this moment into any lengthy controversy, but will briefly state what may be gathered for the theory in general, from a careful consideration of the incipient metamery of the Nemertea.

If we start from a more or less radiate ancestor of the earliest diploblastic type, in which neither a radial nor a serial repetition of organs or organ systems has yet come about, and which may indifferently be considered to resemble either a more flattened Trichoplax or a more spherical gastrula, we may assume that in the course of the development of other internal organs (towards the formation of which the secondary accumulation of cells between the two primary layers often so largely contributes) the radial symmetry may either be further accentuated or may be replaced by a tendency towards bilateral symmetry. In the latter case we are inclined to ascribe the first impulse towards this bilateral symmetry to a preference, which slowly establishes itself in the animal mechanism, for moving in one direction rather than in any other, i. e. for generally stretching forward, when moving about, one particular portion of the body.

One impulse of this sort will suffice to lead us to understand, or rather to deduce, a very considerable number of consequences, which cannot fail to make their appearance under the

[1] "On the Ancestral Forms of the Chordata," 'Quart. Journ. Micr. Sci.,' vol. xxiii, 1883.

influence of natural selection acting upon the organisms that have inherited this tendency in different degrees. Thus we may understand the narrowing and lengthening of an animal that moves in one direction in preference to any other; and similarly the development in the nervous system of a centralisation not far away from the anterior extremity.

All this has already been stated by Balfour in clearer terms in his 'Comparative Embryology' (vol. ii, pp. 308, 311), where he describes the gradual steps by which a radiate medusa-like animal may have passed into a bilateral worm-like form, with two longitudinal nerve-stems, which are regarded by Balfour as the stretched nerve-ring of the Medusa.

I fully endorse these views; only, with respect to the nervous system, I hold it to be safer to leave out of comparison the already specialised nerve-ring of the Medusa, and rather to go back to the Cœlenterate nervous system as primitive as that of the Actinia, where the plexus, both of the epiblast and the hypoblast, with an increase in density in the region of the mouth and the tentacles, may be said to be the fair representative of one of the lowest starting points. In this the plexiform arrangement predominates.

Now we find in all the lower invertebrates various though distinct nerve tracts that are being specialised in this plexiform nerve-tissue according to the modes of motion of the animal, and according to the general shape of the body.

Thus in the Medusæ, which move about in the water by annular contractions of the lower portion of the bell-shaped body, one of the nerve-rings already alluded to was demonstrated by the Hertwigs to innervate the musculature by which this is brought about.

In the Ctenophora the nerve system is less satisfactorily known, but still Lang[1] does not hesitate to bring them into genetic relationship with the Polyclada. Among the latter, Gunda, with its two longitudinal lateral stems, may be looked upon as an extreme term in this series.

[1] A. Lang, 'Monographie der Polycladen,' Leipzig, 1884.

Another series may indeed be supposed to have derived longitudinal stems from a ring which became extended to form lateral cords, as the animal passed from the radial to the bilateral symmetry, in the way suggested by Balfour. Still, even in this case, a nerve-plexus may be expected to be coexistent with or to have preceded the nerve-ring. The longitudinal stems originating from the anterior thickenings of the plexus that innervate the sense organs and the tip of the head (specially sensitive in connection with the forwardly directed movements of the body), would all the more probably be preserved and increase in development, as during this forward movement they form a right and a left centre for the reception of outward stimuli. In the same way those of the radially arranged stems of the Polyclada that are parallel to the longitudinal body-axis, and mark out right and left, are more strongly developed than the others, presumably on account of their importance in connection with the well-directed movements of the body in response to external agents.

In the ancestral Mollusca I think we may assume with great probability the presence of four longitudinal stems —two latero-dorsal and two latero-ventral ones; in the ancestral forms of Annelids and Arthropods two, which have gradually coalesced ventrally, as was first suggested by Gegenbaur. Again, in Nematodes differently situated longitudinal stems in what was originally a uniform plexus are preserved; whereas in ancestral Nemertea two lateral longitudinal trunks in the plexus were undoubtedly characteristic features.

That one medio-dorsal stem in this plexus, in which all the impressions made by outward agencies on both halves of the body might be concentrated, and from whence the corresponding movements might be regulated, will more fully answer the purpose than two lateral stems, however they may be united by an intervening plexus, is a priori probable, and would explain the first impulse towards the formation of such a longitudinal concentration in the uniform plexus.

And when once such a dorso-median stem is present, in addition to two lateral ones, a struggle for supremacy, presided

over by natural selection, may lead to a diminution of the lateral stems, and to an increase of the dorso-median one.

This, in my opinion, as will be more fully developed below, was the case in the ancestors of the Chordata, traces of this struggle and of the competing structural elements being duly preserved.

If we suppose the bilateral symmetry to be established in one of the lower representatives of the Metazoa, and the type to go on increasing in length in the course of generations, then this increase indeed exposes it to very different and perhaps more numerous dangers and enemies than would threaten it were the same bulk concentrated in a spherical or radial circumference. And if, even in the latter case, injuries to the individual might prove fatal were it not provided with strong powers of regeneration (cf. Star-fishes, Ophiurids, Crinoids, &c.), still it needs no comment that, when bilateral symmetry and increase in length so considerably enlarges the surface which is open to attacks, and so enormously facilitates the rupture of the individual, or the severing of parts by rapacious enemies preying upon it, similar regenerative powers are none the less required to insure the persistence of the type.

These dangers, continually threatening the existence of the individuals, and thus injurious to the species, counteracted as they are by regenerative processes (power of reproduction of lost parts), I hold to be at the base of all those cases of metamery in the animal kingdom which do not fall under the head of strobilation, the latter being comparatively rare with respect to the former. Incipient metamery, once established by this cause, may further differentiate in the most diverse directions (heteronomous segmentation, &c.), even after the absolute cessation of the causes that in the first instance have provoked it.

The explanation has, moreover, the advantage of being applicable to radial as well as to serial metamery.

These propositions must now be more fully developed. The

power of reproduction of lost parts comes, without doubt, under the general laws of formation and growth. We find it even in the lowest Protozoa. If the material which heredity has accumulated, either in such a unicellular being or in the egg of a Metazoon, and out of which the elements of the different organ systems will gradually develop, is hereditarily so disposed that a compensation for the loss of important parts is facilitated, this will, of course, constitute an advantage. Such a compensation may, e.g. be obtained where the generative products are developed in very many separate centra, and not in one closed sac. Injury to the latter will, ceteris paribus, be more fatal than an equivalent injury destroying one or more of the former. The same holds good for diffused instead of concentrated nervous centra, for the case of liver saccules to the intestine, instead of one compact liver, for numerous apertures and deferent ducts to the nephridial system instead of one, &c. And all this is still more evident when we have before us a long, bilaterally symmetrical animal, which is easily snapped in two. In this case it must be of pre-eminent importance, that the remaining halves, which may in their turn be severed by the same cause into smaller parts, should possess sufficient power of reproduction to repair the damage. Now, it cannot be doubted that an equal distribution of the important components of the organism (nervous centra, generative organs, nephridia, intestinal appendages, &c.) throughout the whole length of the animal meets this requirement. Any severed portion will then be provided with these more important parts, and will be more or less adapted for a separate and individual existence.

The formation of a new mouth and of new brain-lobes in a fragment of this description remains, of course, quite as wonderful and inexplicable as before, but still we cannot fail to see that such an arrangement as here indicated must somehow be beneficial to the species, and that we need not stop short with Bateson,[1] when he says that "the repetition of various struc-

[1] Bateson, "The Ancestry of the Chordata," 'Quart. Journ. Micr. Sci.,' vol. xxvi, pp. 545, 546, 1886.

tures is one of the chief factors in the composition of animal forms. . . . . The reason for their appearance is as yet unknown, and the laws that control and modify them are utterly obscure." Obscurity is not exchanged for broad daylight, but something is gained when we can see that a growth of the principal organ-systems in separate and more or less independent batches, which in an elongated and bilaterally symmetrical animal insensibly passes into the phenomenon of incipient metamery, may be of the highest value for the persistence of the species.

Now this is actually the way in which we find the important organ-systems distributed in the lower Nemertea. And out of this more irregular distribution a gradual metamery, in some incipient, in others more complete, is seen to evolve within the boundaries of the class. Even the nephridial system, in the primitive forms provided with only one opening to the exterior, participates in this tendency towards metamery, and acquires a greater number of apertures, serially arranged in pairs, thereby also tending towards a diminution of damage when artificial division into two takes place in the nephridial region. The metamery, the regular and serial repetition of parts, is thus seen to be of great importance in aiding towards repair after damage to a lengthened bilateral form, in the same way as the radial repetition of parts facilitates repair in the Echinodermata. In both cases the destruction is only partial, the other homonodynamic portions temporarily ministering, thanks to their more independent relation to the injured region.

When the faculty of repair of damage, occasioned by the severing of the animal into two or more portions, has in the course of generations become more and more complete, it can be readily understood that it becomes at the same time a defensive instead of being only a curative process. An animal that at the approach of danger can separate in two or more parts, each of them capable of reproducing an entire new animal, evades this danger very effectively by doing so; whereas another that is attacked in the same way and does not possess this faculty, is laid hold of, shaken about, and wholly or partly

swallowed. So in the Nemertea there is indeed a very strong faculty of spontaneous division combined with the faculty of repair;[1] and anybody who has observed a fresh and living Cerebratulus, with its extremely delicate sense of touch, commence to rupture into two, in preference at the spot where it was grasped with the forceps, cannot fail to see in this a defensive action.

This mode of self-defence may in quite another respect be useful to the species, because at the same time it serves for propagation. Thus we see that the passage of this defensive process to that of reproduction by fission is so gradual, that it would be impossible to decide in every case what name should properly be applied to it. It cannot well be denied that in all probability ours is only a special case, in which the power of reproducing the species by a process of fission, reaching down as far as the unicellular ancestors, has come to be regulated by other motor forces than growth, and—if it may not be called voluntary fission—still may be regarded as sudden and spontaneous fission, brought about by external influences, of a threatening nature to the further existence of the specimen. This regulation is no doubt a consequence of selection. Schizogony having once been established, it must have been further beneficial to the species, on the grounds that were developed above, that the internal organs should be present in multiple numbers, and this having once come about it is easy to understand that a regular, rigorously metamerous arrangement of this multiple material, still more fully answers the same purpose, and is gradually evolved under the influence of selection.

Thus we may be said to be able to follow the appearance of metamery in a bilateral animal, along all the gradual steps by which it is evolved, and many of these steps have remained fixed and permanent in different Nemertean genera.

[1] Both M'Intosh and Barrois have observed and described very peculiar cases of repair in Nemertea, where the head, brain, side-organs, &c., were reproduced on a headless trunk-piece. These experiments are well worthy of careful repetition. It may be that only those fragments in which a portion of the œsophagus was retained were capable of repair of the head.

The last system that will participate in this metamery is the muscular system, and a rash conclusion—such as is not rare in these days of ontogenetic fetichism—might lead to the rejection of the views concerning metamery here developed, on the consideration that it is exactly the metamery of the muscular system which appears first of all in the ontogenetic development of Vertebrates. I will not circumstantially refute this argument, but will only remark that in Polygordius and other Chætopods, which are representatives of a group of animals in which segmentation reaches such a very high degree of perfection, the longitudinal muscular layer of the body-wall is as yet continuous in the adult, and not divided into metameric sections, as it is in certain Arthropods and in Vertebrates. Now let us consider contractions of the inner muscular layer of the Nemertea, the only layer that is common to all of them, from Carinella to Cerebratulus and from Cephalothrix to Pelagonemertes. This layer also corresponds with the longitudinal muscular layer just alluded to of other lower worms, such as Polygordius, and, as was noticed in our paragraph on the muscular system, its contraction is sometimes very distinct in favorable sections.

We then see the contraction marked out as so many successive blocks of contracted, thickened fibres, separated by intervening parts of non-contracted fibrous tissue. The sections demonstrate that the phenomenon persists throughout the whole breadth of the animal, i.e. that successive rings of contractile tissue alternate with intervening rings in which no contraction is observed. This phenomenon is thus in a certain degree comparable to an arrangement in distinct myomeres.

It is not unimportant that it was especially noticed in the fundamental muscular layer, and it may at the same time be remarked that it appears, from what I have as yet been able to observe myself, that the number of these rings in a given length of the animal is the same, or a multiple of the number of intestinal cæca and transverse nerve-tracts in the plexus; in other words, that the incipient metamery of the internal

organs is in a definite relation to these phenomena—which might also deserve the name of incipient metamery—in the muscular layers.

For the present the fact is, however, not yet definitely demonstrated that these successive blocks are indeed present as such in the living animal. The possibility is still open that they may be waves of contraction which have been fixed at the moment of the immersion of the animal in the preserving fluid. For this reason I will not lay any undue weight on this observation.

The ideas concerning the origin of metamery here expressed, and advocated for several years in my university lectures, differ from those of Lang (loc. cit.) and Sedgwick,[1] in so far as they do not recognise the primary importance of the so-called cœlomic sacs—the paired archenteric diverticula of Amphioxus—for the solution of this question.

The question of the Vertebrate cœlome, so full of obscurities and difficulties, is purposely left out of consideration here, where the relation to archicœlous ancestral forms is discussed, and where an attempt is made to show that it is indeed probable that the impulse towards the establishment of metamery is due to forces for which the archenteron was not the only, nor perhaps the most important part of the organism to act upon.

Still more different are they from those advocated by Perrier[2] and Cattaneo,[3] who have adhered to and extended the idea already held by others, but by them most actively defended, "that the metamery of Arthropods, Vertebrates, and a great many Vermes, has originated out of the multiplication by transverse fission of very simple primitive worms which were not metamerous. The products of this transverse fission remaining connected together have then formed a chain of individuals, or a linear colony; later on the unity of the chain has become more definitely established, the single individuals at the same time becoming different both in

[1] A. Sedgwick, "On the Origin of Metameric Segmentation," 'Quart. Journ. Micr. Sci.,' vol. xxiv, p. 43, 1884.

[2] E. Perrier, 'Les colonies animales,' Paris, 1881.

[3] G. Cattaneo, 'Le colonie lineari e la morfologia dei Molluschi,' Milano, 1883.

form and in function, and the foremost individual thus becoming the head of the series. Each segment (metamere) thus represents a reduced individual; a metameric (segmented) animal is the result of the more or less complete fusion of single individuals into an individual of higher order."

Emery, from whose paper[1] I have translated the foregoing sentence, has very successfully combated these propositions. This author, however, adheres to Lang's views in ascribing to the archenteric pouches, the "gemmation" as Emery calls it (loc. cit., p. 18) of the intestine, the most important and initial significance for the first origin of metamery, "the sexual glands and excretory canals being in relation to the intestinal diverticula," and following the lead. I have above explained why I cannot adhere to this argumentation, which brings the cœlome and the sacculated intestine too strongly into the foreground, and why I rather suppose incipient metamery to have been antecedent to either of these (e.g. Carinella). On the other hand, many views contained in Emery's important paper coincide with my own. Thus he writes (loc. cit. p. 11), speaking of that interesting marine Triclade, Gunda segmentata:

"The metamery of Gunda is thus manifestly the consequence not of the 'symbiotic' fusion of a colony of equivalent 'parts' (meridi), but of the 'autobiotic' differentiation and perfectioning of one 'part' (meride);" and further on (p. 15): —"When I consider the facility with which certain worms break into one or more pieces even spontaneously, it appears to me that this capacity for rupture may well have been the origin of the reproductive purpose of transverse scission in similar elongated organisms. The rupture, in the first instance accidental, could have contributed to the more rapid multiplication of the organism, being followed by the regeneration of the parts that were deficient in the separate fragments. This process of rupture might further have been so perfected that the spot best adapted for rupture, with a view to the best condition of the fragments, was prepared in advance. In the

[1] C. Emery, 'Colonie lineare e metameria,' Napoli, 1883.

more perfect evolutional phases of the process, which are at the same time those that have till now been more carefully investigated, the new head is formed anteriorly to the rupture, or at least its essential parts are pre-established."

My own views emphasize the presence of a peculiar process of development of the internal organs, running parallel to this predisposition for rupture in a particular spot—the spot which will correspond to the outwardly visible demarcation between the future segments. They thus go one step further—and, in my opinion, a very essential step—in the attempt to explain the origin of metamery in the lower Platyelminthes, these bilateral descendants of radiate Cœlenterata, and at the same time predecessors of both Chordata and Appendiculata.[1]

This view of the origin of metamery also affords an explanation for the very different degrees in which we find metamery or segmentation expressed in the different divisions of the animal kingdom. The incipient metamery which we have traced (and which we have pictured to ourselves as arising through natural selection amongst those forms, which, while developing in length, find metamery to be a protective peculiarity) immediately creates, by the fact of its existence, new and variable material for selection, again to be acted upon. And whilst metamery develops in one direction in one line of descendants, the other line brings to the foreground a different set of advantageous combinations, each of them again the stock of new and varied forms. In other words, metamery once established in its most primitive form, and intimately connected with spontaneous fission under the influence of external agents, has been of very great moment in the bringing

[1] Gegenbaur, in his 'Grundriss der Vergleichenden Anatomie' (1878), hints at similar explanations to those advocated by Emery and myself, when he says (p. 64):—"Die Metamerie . . . lässt Zustände des Beginnes und der nicht ausgeführten Beendigung mannichfach erkennen . . . . In dem Maasse als ein Metamer die Abhängigkeit vom Gesammtorganismus durch die Ausbildung seiner eigenen Organe aufgiebt emancipirt er sich vom Ganzen und gewinnt die Befähigung freier Existenz." Further on he speaks of incipient metamery as "eine stellenweise, für den Organismus praktisch werdende Ausbildung" of the different organ systems.

about of new and endless variations of animal life. And it is irrational, when we have before us, say one of the lowest Vertebrata, in which nobody will deny the presence of distinct metameric segmentation, to conclude that this metamery must necessarily be in many respects reduced, and that in the ancestral forms it must have been far more complete, must have stretched forwards along the whole of the head, must have been more forcibly expressed than it is now—in all the cephalic nerves, in the nephridia, the gill-slit, &c.;—all this on the presumption of the existence of an ancestor so completely and exemplarily segmental as to throw no light on the origin of segmentation and metamery, unless by the aid of Perrier's and Cattaneo's exaggerations. Such conclusions must, however, necessarily be made by those who follow Dohrn's and Semper's lead concerning the phylogeny of the Chordata.

Bateson, in taking Balanoglossus as his starting-point, finds the acknowledged points of resemblance in the metamerous gill-slits, &c., and adds to them important data concerning the metamerous cœlomic diverticula. Still, for a general view on the origin of metamery, Balanoglossus offers no points that we do not find more strongly represented and more forcibly expressed in the Nemertea. It certainly deserves mention that long before Bateson drew renewed attention to the numerous points of agreement between Balanoglossus and the Chordata, M'Intosh[1] had done the same for Balanoglossus and the Nemertea, a separate paragraph of his monograph being devoted to the discussion of these homologies.

Sedgwick (loc. cit.) holds the unsegmented worms to be wholly "negligeable quantities," at any rate superfluous links in the chain that connects the Chordata with the antecedent Diploblastic stages. In my idea both these authors, valuable as certain of their suggestions are, have not been thoroughly aware of the necessity that, in all discussions on the origin of metameric segmentation, we must attempt to grasp at data

[1] W. M'Intosh, 'A Monograph of British Annelids,' "A. Nemerteans," Ray Society Publications, 1873, 1874.

that give a clue to the possible action of natural selection in the gradual evolution of metamery. This clue appears to me to be far more distinctly contained in the views here advocated than in the other hypotheses.

It may further be remarked, now that we have once more alluded to Bateson's phylogeny of the Chordata, that even this naturalist does not feel justified in wholly rejecting the Nemertea from the Vertebrate pedigree. Whilst in the text of his article (loc. cit. p. 566) he does seem to prefer this negative alternative; still, in the subjoined diagram of the general relationships of Urochorda, Hemichorda, Cephalochorda, and Vertebrata, the Nemertea are introduced—with a point of interrogation, however—as a side branch lower down on the common parent stock. Now, this being concordant with my own views of the Chordate phylogeny,—the point of interrogation excepted,—it is necessary to inquire why there is this discrepancy between Bateson's speculations in the body of his treatise and the hypothetical pedigree at the end of it. It appears to me that this is due to his hesitation (loc. cit. p. 555) in accepting the views hitherto entertained and advocated by myself as to the phylogenetic connection between the Nemertean and the Vertebrate nervous system. For this hesitation Bateson has good reasons, and while I appreciate the soundness of them, I hope in the remainder of this chapter to remove the reluctance of him and others to accept the phylogenetic significance of the Nemertea, thanks to new light that may be thrown on the evolution of the central nervous system of the Chordata by the observations recorded by me on the nervous system of the "Challenger" Nemertea.

It is to these speculations on the nervous system that we now have to turn our attention.

As will be seen from the terminology introduced in the paragraph on the nervous system in my Report on the "Challenger" Nemertea, and as it is now time more fully to develop, I am inclined to attach considerable morphological importance to the arrangement of the different constituent parts of the nervous system in the Nemertea. In former publications I

have repeatedly insisted on the significance of certain points in the anatomy of the Nemertea, when considering the general question of the relationship of the Chordata to their unknown invertebrate ancestors, and I have insisted not only on the possibility of the homology between the Nemertean proboscis and the hypophysis cerebri of the Vertebrates, but I have, even earlier still, attempted to show that the nerve-system of these two groups might be considered in a common light, as was first indicated by Harting in his 'Leerboek van de Grondbeginselen der Dierkunde,' of the year 1874. Further reference to the hypothesis here alluded to is found in Balfour's Monograph on the Elasmobranch Fishes (pp. 170—172), in my own publications[1]—, and in Balfour's 'Comparative Embryology'[2] (vol. ii, p. 258). I will not here enter upon this hypothesis more fully, but will briefly state that it attempted to consider the central nervous system of the Vertebrates as a possible median coalescence of two nerve-trunks, that were lateral in the primitive ancestors of the Vertebrates, in the same way as the coalesced ventral nerve-cord (Bauchmark) of Annelids and Arthropods may be considered with Gegenbaur as having arisen out of a double lateral trunk, which in certain, still more highly differentiated, forms have fused ventro-medially.

A strong argument against the first-mentioned hypothesis is the fact that the spinal cord ontogenetically always makes its appearance as a median unpaired plate or thickening, a very faint trace of a possible double origin of this plate being hitherto only observable in one species of Amphibia, Triton tæniatus; whereas in all other Vertebrates, Amphioxus

[1] "Zur Anatomie und Physiologie des Nervensystems der Nemertinen," 'Verhandel. van de Koninkl. Akad. van Wetenschappen,' Amsterdam, 1880, vol. xx. "The Peripheral Nervous System of the Palæo- and the Schizonemertea, one of the layers of the body-wall," 'Quart. Journ. Micr. Sci.,' vol. xx, 1880.

[2] It may here be remarked that Balfour has omitted to mention that Harting was the first to bring forward this hypothesis; it is well to be reminded of this when Beard, Bateson, and others similarly ignore this claim to priority of my venerated predecessor.

and the Cyclostomata not excepted, the unpaired origin is most evident. The bilateral symmetry of the full-grown brain and spinal cord is a much later feature, and can hardly be regarded as the expression of a primary coalescence of two separate halves to form a median whole.

I am the more inclined to abandon this hypothesis because I will attempt to show that we can establish phylogenetic comparisons between the Chordate and the Nemertean nervous system on a much more simple basis; comparisons which at the same time cover a far more extensive ground than did those of Harting, Balfour, and myself, which I have just alluded to.

Since in the nervous plexus of all the Nemertea a median longitudinal tract, sometimes of comparatively large size, has now been detected, since even in the Hoplonemertea, where the plexus has disappeared, the same medio-dorsal nerve-tract has in most cases been preserved, and, finally, since from this dorso-median stem metameric and paired nerve-tracks may be seen to emerge in Palæonemertea and Schizonemertea, we must inquire in how far the direct comparison of this medio-dorsal nerve-stem with a primitive spinal cord may be said to hold good.

In order to do this we must first consider the relation of this stem, to which we have given the name of medullary nerve or medulla, to the rest of the nervous system, more especially the brain-lobes.[1] In a former publication,[2] where the medullary nerve was for the first time noticed and described as the proboscidian-sheath-nerve, I traced its origin to the dorsal commissure between the two lateral halves of the brain (loc. cit., pl. i, fig. 1). Thanks to certain very favorable specimens in the Challenger collection, I have now been able to add new data to this statement. Sections through the brain of Cerebratulus macroren, Cerebratulus corrugatus, and

[1] In the course of these considerations a certain amount of repetition of facts already noticed in the paragraph on the nervous system cannot well be avoided.

[2] Verhand. Kon. Akad. v. Wetensch., Amsterdam, vol. xx, 1880.

Cerebratulus angusticeps ('Zool. Challenger, Exp.,' Part 54, pl. xii, figs. 1, 7, 8; pl. xiii, fig. 1) show that the condition of things is indeed less simple than this original statement would imply,—that the medullary nerve is not an eminently fibrous cord springing at right angles from the eminently fibrous upper brain-commissure, but that the nerve-tissue constituting the foremost and uppermost portions of the upper brain-lobes spreads out over a far more considerable surface than the fibrous tract which is known as the dorsal commissure. This expansion of nerve-tissue, in which the cellular elements are no less conspicuous than the fibrous, is posteriorly directly continuous with the plexus above described, laterally with the brain-lobes, anteriorly with the cephalic nerves springing from these lobes. It attains its fullest development just before and behind the region where a transverse bundle of fibres uniting the fibrous core of the lateral brain-lobes forms the well-known dorsal brain-commissure. This commissure is a transverse fibrous tract forming part of a more extensive nerve-plate. To this expansion of nerve-tissue the presence of nerve-cells gives a more primitive, at any rate a less specialised, character. These nerve-cells and nerve-fibres are directly continuous with those of the medullary nerve and (backwards) with those of the nerve-plexus, of which this nerve is only the median longitudinal thickening. There is even more reason to look upon the fibres of this medullary nerve as a tract of the general fibrous stroma not necessarily connected with the fibres of the brain-commissure. In other cases a more direct continuity between the commissural and the medullary nerve-fibres was, however, observed.

In order clearly to understand the relative importance of the different parts of the nervous system here noticed, the primitive Palæonemertea offer the best starting-point.

Thus in Carinella we find the brain-lobes not yet separated into distinct upper and lower lobes, nor do we find a posterior lobe (side-organ). The brain is a double lateral and anterior thickening in the nerve-plexus, situated like it and like the lateral nerve-stems outside the muscular body-wall in the

deeper strata of the integument. The only difference between the medio-dorsal medullary nerve in this species and the lateral nerves with their anterior enlargements (the brain-lobes) is its position and its greater tenuity (Pl. XLII, fig. 1), which, however, does not prevent its being very clearly observable in every transverse section. Its connection with the brain-commissure was already described and figured by me (loc. cit., p. 25, pl. iii, fig. 31). It must, however, be remarked that in these most primitive Palæonemertea the anterior dorsal brain-commissure is less significant than in the Schizonemertea, and hardly anything else than the foremost of those numerous transverse metameric tracts in the plexus (*dvr*, Pl. XLII, fig. 1) which connect the lateral stems with the medullary nerve (dorsally) and with each other (ventrally).

These important metameric nerve-pairs are most distinctly observed in Carinella. Here, as in the Schizonemertea, the medullary nerve is also continued forwards in front of the brain thickenings. This continuation sometimes shows a short bend just on the level of the commissure, so that both the medullary nerve and its anterior continuation may be seen in one section. Posteriorly the medullary nerve can be followed down to the hindmost extremity of the body. In Eupolia and the Schizonemertea the arrangement remains the same, the metamery of the transverse stems is perhaps more clearly expressed, the whole plexus and the longitudinal stems are no longer in the integument, but between the muscular layers. Still, the whole of the nervous system also answers to the general type as represented in the diagrammatic fig. 1 on Pl. XLII.

We have now seen enough of it to understand that a comparison with the central apparatus of the Vertebrate nervous system cannot indeed be called a strained comparison. On the contrary, the comparison is much less artificial than was the one which Balfour was inclined to adopt, and which, as noted above, rendered necessary the acceptance of the phylogenetic development of the Vertebrate medulla of a double cord.

And so I do not hesitate to proclaim the medullary nerve of the Nemertea to be a very important link in the phylogenetic

chain, of which the Vertebrate spinal cord is the outcome. Like the Nemertean medulla, the Vertebrate spinal cord is median, unpaired, and composed of nerve-cells and nerve-fibres; like the Nemertean medulla, it is a thickening in a nervous plexus, originally wholly epiblastic, of which, among Vertebrates, the Amphibian embryos offer such a striking example. This instructive and suggestive case was known to Remak and Stricker (as the "Nervenschicht" of the frog embryo), it was more carefully studied and elaborately described by Goette (his "Grundschicht" of the epiblast, in his 'Entwickelungsgeschichte der Unke'), and it has been again recently brought into the foreground by Baldwin Spencer, in his latest paper on the subject.[1] The latter author compares the Amphibian plexus with that of Palæonemertea and Schizonemertea (loc. cit., p. 134), as had already been done before him by my friend Professor Ray Lankester, with whose suggestion I at that time (1880) did not yet venture fully to associate myself.

The numerous data that have since been accumulated for a direct comparison of Nemertea with lower Vertebrates appear, however, now to fully justify that comparison which was first expressed in a footnote to a former paper of mine.[2] There can hardly be any doubt as to the existence, consequent upon natural selection, of a constant tendency in the different component parts of living organisms towards simplification and increased efficiency (Roux's 'Kampf der Theile im Organismus'). This fact enables us to understand the gradual supremacy of the median cord in the Nemertean plexus over the two lateral ones. It seems as if it were mathematically demonstrable that for the delicate adjustment of the impressions from the exterior to the co-ordinated movements thereby occasioned, one longitudinal central stem in bilateral, lengthened animals would be more efficacious than two lateral ones. And if we ask if, at the final stage of this struggle for supremacy

[1] Baldwin Spencer, "Some notes on the early Development of Rana temporaria," 'Quart. Journ. Micr. Sci.,' vol. xxv; Suppl., p. 123, 1885.

[2] 'Quart. Journ. Micr. Sci.,' vol. xx, 1880, p. 438.

between three longitudinal stems, any remnants of the lateral cords are yet detectable in the Vertebrate embryos, perhaps even in the adults, I am inclined to answer in the affirmative. Here I must be allowed to insert a reference to the three figures on Pl. XLII, which will facilitate the exposition of the further consequences of the hypothesis I am here developing Fig. 1 represents the chief points in the nervous system of the Nemertea. The brain-lobes are simple lateral swellings of the longitudinal stems, as in Carinella; plexus, medulla, and transverse stems, together with brain-lobes and lateral stems, may be considered as forming part of the integument (cf. Carinina). A double innervation of the respiratory portion of the intestine is indicated; one due to visceral branches (*vi. sy*) springing from the plexus (or from its transverse tracts), the other to the more specialised nerve (*v*), which has above been indicated as the Nemertean vagus nerve. The plexus and its innumerable radial fibres, both sensory and motor, are not indicated in this figure, nor are the nerve-stems which, when present, pass from the lateral stems directly to the integument.

This figure must now be compared with the two others. Of these, Pl. XLII, fig. 2, diagrammatically represents the chief points that may be considered as characteristic of the nervous system of a lower Vertebrate, in which the dorsal and ventral roots of the spinal nerves (*dr* and *vr*) are as yet separate nerve-tracts, in which the sympathetic nerve system is as yet only represented by visceral branches given off by these dorsal roots (*vi. sy*), and in which the polymerous character of a primitive vagus (*Vag*) is established.

Pl. XLII, fig. 3, stands for Amphioxus, as far as we know its nervous system, more particularly through the researches of Rohon and others. It differs from the foregoing by the absence of a distinct brain swelling and of a polymerous vagus. A number of spinal nerves are considered as homologous with the vagus of Vertebrates by Rohon. The commissural connections between the successive spinal nerves form a plexus, which is peripherally even much more elaborate, according to

Rohon's figures. This plexus does not reveal the presence of any distinct lateral longitudinal nerve, nor any ganglia of spinal or cephalic nerves. The latter (*cn*) may be said to be three in number. Visceral branches (*vi. sy*) are given off by the dorsal nerves (*dr*). The ventral ones, springing from the lower edge of the medulla, are here represented as shorter stems (*vr*).

The opposite half of the system, seen in transparent perspective, as given in the two other figures, is purposely omitted here, because of the asymmetry of Amphioxus in this respect.

Now a glance at these figures will convince us that the situation of the Nemertean medullary nerve in its plexus, and with its set of transverse nerves, is directly comparable to the Vertebrate medulla and spinal nerves. The nerve-plexus filling up the intervening spaces in Nemertea is present as a transitory structure in Amphibian embryos.

The ulterior appearance of an anterior enlargement forming the Vertebrate brain; the higher complication attained by the brain and spinal cord when its mass increases, but not its dorsal expansion, by the appearance of medullary ridges; and the formation of a neural canal by infolding of the neural plate, all these are important developmental facts which do not in any way weaken the grounds for comparison of the two structures. They may be looked upon as adaptations to the much more considerable efficiency and concentration that is gradually attained by the central nervous system as we ascend higher in the scale of the animal kingdom.

The fact that the neural ridge in so many Vertebrata precedes the appearance of the spinal nerves, and is inserted along the top of the folds that bend together to form the neural tube, may be thus interpreted, that during the phylogenetic process of infolding the transverse nerve-tracts (dorsal spinal roots) remain attached in the same way to the mediodorsal collecting trunk as they did in the ancestral forms, and are dragged upwards by the infolding process. The ventral roots must be phylogenetically linked to the plexus as well;

inasmuch as the musculature originally lies inwards of the nervous plexus, their deeper situation is not surprising.

In the points hitherto enumerated there is entire coincidence between Amphioxus and the other Vertebrata, as far as their comparability with the Nemertean diagram goes. Another point of coincidence is the way in which the foremost position of the intestinal canal and adjacent blood-vessels are innervated by visceral nerve-stems, indicated in all the three diagrams by *vi. sy.*

The claims to validity of the comparison here made between the spinal nerves of the Chordata and the transverse stems of the Nemertea should again be insisted on, now that the researches of Rohon,[1] Freud,[2] Schneider, Ransom, and d'Arcy Thompson[3] have established for the lower Chordata (Cephalochorda and Cyclostomata) that the typical chordate spinal nerve is not originally provided with a double root, but that this double root appears to have arisen by the coalescence of what were primitively in the groups just mentioned separate and alternating dorsal and ventral nerve-tracts. With these so much simpler spinal nerves the transverse nerve-stems of the Nemertea undoubtedly offer points of comparison. These Nemertean nerves specially differ from the Vertebrate spinal nerves in two respects: (1) they give off nerve-fibres in different directions, which are probably motor as well as sensory and visceral, according to the different organ systems they

[1] V. Rohon, "Untersuchungen über Amphioxus lanceolatus," 'Denkschr. d. k. Akad. d. Wiss. Wien,' Bd. xlv.

[2] S. Freud, "Ueber Spinalgangliën und Rückenmark des Petromyzon ('Sitzungsb. math.-nat. cl. k. Akad. Wiss. Wien,' Bd. lxxviii, Abth. 3, 1878). This author says (p. 154):—"Ich kann wenigstens von den letzten Wurzeln des Caudalmarks sagen dass ihre Selbständigkeit so gross ist, dass man von vorderen und hinteren Spinalnerven, anstatt von vorderen und hinteren Wurzeln reden könnte"; and Wiedersheim in the 2nd edition of his 'Lehrbuch der Vergleichenden Anatomie' (p. 321):—"Vieles spricht dafür dass die Vorfahren der heutigen Wirbelthiere getrennte dorsale und ventrale Nervenwurzeln besessen haben müssen."

[3] W. R. Ransom and d'Arcy W. Thompson, "On the Spinal and Viscera Nerves of Cyclostomata," 'Zool. Anzeiger,' No. 227, July, 1886.

terminate in; and (2) they go round ventrally, each of them forming a loop all round the body. As to the first point of difference just alluded to, it is the expression of a low and primitive degree of differentiation, and when a step forwards is made differentiation of labour will tend to develop certain tracts more particularly containing sensory and visceral nerve-fibres, which are more especially directed towards the epithelia (the primitive dorsal or posterior roots), and others more particularly containing motor nerve-fibres, and more especially directed inwards towards the muscles (the primitive ventral or anterior roots), because the musculature, as was already mentioned, is originally situated internally to the nervous system.

For the present we can only hold it to be established that the fibres of these three categories are blended in the Nemertean plexus, without being able to determine in how far the specialisation therein observed, of the appearance of transverse metameric nerves, may at the same time be accompanied by a commencement of differentiation, such as has just been alluded to. We may, in other words, not yet assume that among these metameric stems there is already a tendency to an alternation between such as have sensory and visceral and such as have motor predispositions.

Only in a few cases may we be justified in saying that certain nerve-tracts belonging to the Nemertean peripheral system are more especially sensory or visceral, and these no doubt offer important analogies in their situation and connections to similar nerve-tracts of the Vertebrata

The second point of difference, viz. the continuity in the ventral median line of the transverse tracts of the Nemertea, is no doubt a consequence (*a*) of their origin, in a perfectly continuous plexus, (*b*) of the cylindrical arrangement of the muscular layers, which in most cases are uninterrupted both in the dorsal and in the ventral median line. It is all the more important to notice that, more especially in the primitive Carinellidæ, the tendency is very marked towards a scission of this muscular body-wall into a right and a left half.

This longitudinal scission is no doubt the first expression of

the phenomenon which shows us the musculature of the right and left half of the body, developing quite independently in the Chordata. It is easy intelligible how, as this phenomenon gradually becomes more and more marked, no more ventral connecting fibres across the non-muscular region were required for the innervation of the musculature of the right and left half of the body.

The process by which the transverse nerve-tract, with radial nerve-fibres leaving it at short intervals, both centripetally and centrifugally, gradually assumed the form of a nerve-stem with a dorsal and a ventral branch, such as we find in the spinal nerves, must have gone on pari passu with those numerous other changes which we cannot as yet fully trace, but which must have occurred when (1) the muscular metamery became gradually established, (2) the dorso-median medullary tract became so preponderant that an increase in mass, with economy of bulk, was only to be obtained by a process of folding-in already discussed above, and (3) the attachment of the spinal nerves (transverse tracts) to the medulla was modified in consequence of this process.

None of these phenomena, however, offer anything that is in any way inconsistent with, or opposed to, the general theory here developed.

We have now sufficiently insisted on the chief point of comparison here proposed, viz. that between the Nemertean medullary nerve and its metameric transverse nerve-cords and the Vertebrate cerebro-spinal axis and spinal nerves.

If Amphioxus were the only Vertebrate known, we should, recognising the phylogenetic importance of the plexiform arrangement still met with in the adult of that species, admit that, as far as we know at present, the primary lateral nerves with their anterior swellings of the Vermian ancestors had disappeared in the same measure as the dorso-median spinal cord had come more and more into the foreground.

But our consideration of other Vertebrates leads us to the conclusion that, when once the general homology between the two nervous systems is admitted, there may perhaps be secondary

points in regard to which the comparison can be further extended. And it must be recognised that, if we should also succeed in rendering more or less probable a comparison in secondary details, this might again be favorably interpreted for the primary and more important part of the hypothesis.

The search after these secondary points of agreement was instituted by me when the question above alluded to presented itself, viz. if any remnant could be traced of the central nervous system of Nemertea-like ancestors, i.e. of the brain-lobes and lateral stems, in those Vertebrate descendants in which the medio-dorsal tract had become so preponderant as to give rise to the unpaired medulla and brain.

It is clear that if it shall be possible to trace any such remnants, and to render their homology with the Nemertean central nervous system probable, they will have to be sought for—(*a*) in the head, as lateral more or less independent nerve-centra, innervating sense-organs of the integument, and passing posteriorly into parallel longitudinal stems; or (*b*) in the trunk, as longitudinal nerve-stems, in which the central character should be somewhat less marked than in the anterior swelling, but in which the original significance as parts of the central system should still be indicated either by histological or by embryological features.

To these latter conditions nothing can answer in the Vertebrate nervous system excepting the so-called ramus lateralis vagi. It is present in all Vertebrates above Amphioxus, long and important in the aquatic Ichthyopsida, gradually disappearing when the aquatic medium is exchanged for an air-breathing existence, and finally only retained in the higher Vertebrates as the inconspicuous ramus auricularis vagi.

Its course is indeed strictly lateral, and has always been a puzzle to anatomists. Stannius[1] characterises the existence and the course of this sensory nerve along the trunk down to the tail as "one of the most interesting facts of anatomy."

None the less startling is its development. Whilst Balfour attempted in this respect to bring it on one line with the other

[1] 'Das peripherische Nervensystem der Fische,' p. 108.

parts of the peripheral nervous system, the corresponding results of Semper, Goette, van Wijhe, and Hoffmann are all in the contrary direction. They have seen the nervus lateralis appear as an independent product of the epiblast, arising in loco along its whole length, its formation often even preceding that of the spinal nerves. These results have again been fully confirmed and definitely established by the latest investigator of the problem, Beard,[1] who also gives a detailed description and figures of the connection between the nervus lateralis and the vagus ganglion, both of them so much more massive and conspicuous in early embryonic stages than later on.

And now that we are attempting to find out whether there is a possibility of comparing the lateral nerve-stems of lower worms with the nervus lateralis of Vertebrata, we are naturally led to consider, in the second place, the question whether the anterior swellings of these lateral stems (the paired brain-lobes of the worm) may have their morphological equivalents, their remnants, in the set of anterior nervous swellings that are found in the head on a level with the nervus lateralis, and longitudinally connected with it; viz. the variable set of ganglia of the cephalic nerves.

As to the origin of these ganglia of the cranial nerves I have no observations of my own, and must rely on the data of other observers.

It is suggestive to give the opinion of the three latest investigators of the development of these organs in different groups of Vertebrates in their own words.

Professor A. Froriep,[2] who studied Mammalian embryos, writes (loc. cit., p. 35):—"The ganglia (of facialis, glossopharyngeus, and vagus) enter into a peculiar, very intimate connection with the epiderm;" further (p. 40), "these ganglionic connections with the epiderm must probably be regarded

[1] "The System of Branchial Sense-Organs, &c., in Ichthyopsida," 'Quart. Journ. Micr. Sci.,' November, 1885, p. 95.

[2] "Ueber Anlagen von Sinnesorganen am Facialis, &c.," 'Archiv f. Anat. u. Phys.,' 1885, Anat. Abth.

as rudiments of organs which have phylogenetically disappeared, and which are only now retained in the ontogenetic development;" then (p. 43) "for the Gasserian ganglion there is no indication of a connection with the epiderm;" and, lastly (p. 52), "it appears to be hardly any longer possible to look upon these nerve-ganglia (Nervenknoten) as simply homologous with spinal ganglia."

Baldwin Spencer[1] writes (loc. cit., p. 129) concerning Rana temporaria: "Along certain lines the cells of the nervous layer proliferate, and it is by this proliferation that the rudiments of the cranial nerves are laid down;" further (p. 130), "the development of the ganglia at the level of the lateral line, and the fact of their long connection with the epiblast at this point, ... is of great interest in connection with certain points in the development of the Elasmobranch nerves."

Concerning the developmental phenomena of the trunk-region at this period, the spinal nerves are stated to be not yet visible, "though the nervous sheath is clearly developed and in this the lateral line."

The author next mentions observations made by him on Dr. Beard's sections of Elasmobranch embryos, and goes on to say (loc. cit., p. 131):

"The Gasserian ganglion is, at all events in part, formed directly from the epiblast . . . . . the same development takes place in the case of the ganglion of the third and seventh nerve—in that of the ciliary ganglion the development is particularly clear— . . . . . The ganglia arise along a level of the lateral line continued on the head."

He next says: "The curious origin of the ganglia of the cranial nerves points strongly to the conclusion that . . . . . their present condition and nature must . . . . . be regarded as a secondary and certainly not primitive condition.

"In passing, I may just notice that on this supposition an

[1] "Early Development of Rana temporaria," 'Quart. Journ. Micr. Sci.,' Suppl., 1885.

explanation is offered as to the origin and meaning of the two curious branches which unite respectively the ganglia of the fifth and seventh and fifth and third cranial nerves; they may be regarded as persistent parts of the lateral nerve . . . . in the head."

In the third place extracts will be given from Beard's more extensive paper.[1] He writes (p. 97) as an introductory statement: "At present we are acquainted with no Invertebrate nervous system which is built upon the same plan as that of Vertebrates," and then passes to the results of his investigations, chiefly carried out on embryos of Torpedo and a few other Elasmobranchs. I make the following selections (p. 101):

"At the point of fusion" (of the cephalic nerve with the epiblast) "a local thickening of epiblast has previously taken place. After the fusion has taken place a proliferation of some of the cells composing the thickening ensues. The proliferated cells form a mass of actively dividing elements still connected with the skin. . . . . This mass of cells is the rudiment of the ganglion of the dorsal root."

On p. 110 he adds: "Along with the separation of the (vagus) ganglion from the skin the sensory thickening begins to grow backwards along the lateral surface of the trunk. This thickening is the rudiment of the so-called lateral line. . . . . The so-called lateral nerve is formed from the deeper portion of the sensory thickening. . . . . That there is no actual growth backwards of the nerve is obvious enough."

Recapitulating, we must acknowledge that the mode of origin of the ganglia of the cephalic nerves, as described by these authors, is certainly a peculiar one—a mode of development sui generis. One of Beard's accompanying diagrammatic figures, reproduced in Wiedersheim's second edition (1886) of the 'Lehrbuch der Vergleichenden Anatomie' as woodcut No. 265, moreover, shows how the position of the cephalic ganglion, developing as an ectodermal proliferation, is in this

[1] "Branchial Sense-Organs in Ichthyopsida," 'Quart. Journ. Micr. Sci.,' November, 1885, No. CI.

early stage eminently lateral, a conclusion corroborated by the figures of his actual sections. This primitive position is, of course, gradually lost, and could never be predicted from a study of these ganglia and their position and significance in the adult animal. Yet it is not without significance when seen in the light of the suggestion here brought forward. And that the interpretation of the phenomena in question as given by these authors is not universally accepted, thus leaving room for new suggestions, is proved by the following citation from Ransom's and d'Arcy Thompson's latest article,[1] running as follows:—"Although the lamprey presents a well-marked lateralis nerve it has not also a regular lateral line, for the sense-organs of the skin are scattered and without segmental arrangement. The sense-organs do not, therefore, appear to be in direct relation with the spinal ganglia, and the view of the close connection between them (Spencer, Beard, Froriep) does not receive support. . . . . It seems more natural to consider the lateralis as a relic of the extensive and irregular commissure system connecting the posterior roots of Amphioxus."

Passing from a consideration of the embryonic ganglia to their connection in the adults, I must mention the connection of the ramus lateralis vagi with cephalic nerves anterior to the vagus. I will not here give a description of the numerous varieties presented by this nervous connection, but merely refer to the arrangement in Vertebrates so low as the lampreys. We there find, according to Johannes Müller, the ramus lateralis originating from the seventh as well as from the tenth pair of cephalic nerves, and if we compare the very satisfactory figure which was only lately[2] given by Ahlborn of this arrangement, we must recognise that this nervous connection is important, and has more the aspect of a direct forward continuation of the nervus lateralis than of a sensory

[1] "On the Spinal and Visceral Nerves of Cyclostomata," 'Zool. Anzeiger,' No. 227, July, 1886.

[2] 'Zeitschr. f. wiss. Zool.,' Bd. xl, pl. xviii.

branch from the facialis, establishing a connection between it and the vagus.

Ahlborn mentions the existence of a similar connecting stem reaching further forward still, and connecting the trigeminus and facialis. How these connections vary in the different adult Vertebrata will not be discussed here.

The different facts and speculations here brought forward in connection with the cephalic ganglia and the nervus lateralis vagi may suffice for the present. They may severally be brought to bear upon the question of the eventual homology of Vertebrate cephalic ganglia and nervus lateralis, on the one hand, and Vermian paired brain-lobes and lateral nerve-stems on the other. The parts here compared being indicated in figs. 1 and 2 of Pl. XLII, with corresponding letters (*Lg* and *ln*), a glance at these figures may further convey a notion of the purport of these speculations.

There is one fact, however, which is not indicated in these figures, which is nevertheless of very high importance for the views here considered, and which I must therefore develop more in detail.

It is the connection between the successive spinal nerves and the ramus lateralis vagi.

The existence of similar connections between the (eminently sensory and cutaneous) dorsal roots and the (similarly sensory and cutaneous) lateral nerve is for the first time mentioned by Ransom and d'Arcy Thompson for Petromyzon in the following passage (loc. cit., p. 422):

"The dorsal rami of the posterior roots pass up (over the lateralis nerve) to the skin of the back, but appear also to send fibres into the lateralis. (For this statement we at present rely only on sections, but we hope shortly to test it by dissections of the large Petromyzon marinus.)"

It hardly needs comment that if this observation should be confirmed the fact would be of the utmost importance for the hypothesis under discussion. We should then be permitted to consider these metameric connections between the dorsal roots and the nervus lateralis of Petromyzon, as the relics of an

earlier stage, still permanent in the Nemertea, where the metamerically consecutive transverse nerve-tracts similarly unite the medullary nerve and the lateral stems.

This connection is, as we know, also brought about in the Nemertea by the plexus, in those parts of it which spread out between the transverse tracts, and it may here be asked if relics of such a plexus between the successive precursors of the spinal nerves are perhaps retained, not only in Amphioxus (see above, p. 625, and Rohon, loc. cit., fig. 13), but also in Osseous Fishes, in the numerous superficial nerves described and figured by Stannius,[1] or whether we must rather look upon this multiplication of lateral nerves (one of which is called by Stannius the nervus lateralis trigemini, others, rami communicantes of the dorsal branches of spinal nerves, &c.) as derivatives from the nervus lateralis vagi.[2] This question can, of course, only be solved by careful anatomical and embryological investigations. That the nervus lateralis was often (Stannius) observed in the Petromyzontidæ only along a part of the length of the body (Schneider and Born, according to Ahlborn,[3] observed it as "bis an das Hinterende des Körpers") is not confirmed by modern investigators. Ahlborn's description (loc. cit., p. 304) of the variable situation of this nerve in Petromyzon is very suggestive in connection with the views here advocated. Ransom and d'Arcy Thompson consider that the regularity of the integumentary sensory apparatus is not yet established in Petromyzon, as may be concluded from the citation given above.

We have now considered the superficial ramifications of what I may call the lateral nerve-system, both in lower worms and in Vertebrates; we must now turn to the intestinal, to

[1] 'Das peripherische Nervensystem der Fische,' 1849, pls. ii—iv.

[2] It should be remembered that Beard is inclined (loc. cit., p. 139) to look upon the superficial longitudinal nerve-fibres, by which the successive epithelial modifications along the lateral line are often connected (Solger, Bodenstein), as such derivatives (by longitudinal fission in its very early stages) of the nervus lateralis.

[3] 'Zeitschr. f. wiss. Zool.,' Bd. xl. pp. 303 and 301.

the visceral branches of this same system, from which other and important data may be gathered for further elucidation of the hypothesis under consideration.

We have already seen that in Nemertea the typical innervation of the respiratory portion of the intestine is brought about—(*a*) by a pair of nerves directed backwards and springing from the anterior lateral swellings (the brain-lobes) of the lateral nerve-stems; (*b*) by numerous visceral branches starting from the plexus, directed inwards as branches that spread over the wall of blood-lacunæ and intestine.

In the Vertebrata, Amphioxus excepted, we also find that the innervation of the anterior respiratory portion of the intestine and of the circulatory apparatus is obtained from two sources, viz. (1) the cephalic nerves, amongst which the vagus nerve is in this respect the most important;[1] (2) the visceral branches of the spinal nerves, which are at the basis of what is afterwards more highly differentiated and separately recognised as the sympathetic nerve-system.

In Nemertea it is very difficult to determine in the anterior part of the intestinal wall which tracts belong to the so-called vagus nerve, which to this system of visceral nerve-branches.

So it is often in Vertebrata, and the blending together (in both divisions of the animal kingdom) of two systems, each of them again mutually comparable when separately considered, is an important point of agreement, and would, if no actual homology were at the base of it, be a very puzzling coincidence.

It is in this respect highly suggestive that Born notices, as early as 1827, what was afterwards confirmed by Ahlborn (loc. cit.) and others, that in Petromyzon, i.e. one of the lowest Vertebrates, the spinal nerves send out connecting branches towards the pneumogastric nerves. The existence

[1] Ventrally these nerves (e.g. the n. hypoglossus) are sometimes commissurally united with their representative of the opposite half of the body. It must remain an open question whether these commissures are in any way comparable either to the Nemertean vagus commissures (cf. p. 83), or to the general ventral commissural system of these worms.

of superficial metameric connections (Ransom and d'Arcy Thompson, vide supra) as well as of this set of deeper connections between the transverse and the latero-longitudinal nerve-stems (n. lateralis and n. pneumogastricus, of Petromyzon would thus be a remarkable repetition of the similar arrangement in the Nemertea, as it has been here for the first time demonstrated.

The facts as they lie before us do not, however, admit of any very circumstantial comparison so far as the nerves in particular are concerned, and I purposely refrain from entering into any details. Yet it should be remarked:

(1) That the polymerous root of the Vertebrate vagus nerve is very readily explicable if we take the Nemertean arrangement as a starting-point (Pl. XLII, figs. 1, 2, *vag*), as is also the mixture of sensory and motor elements in this root.[1]

(2) That similarly, if the anterior cephalic nerves (e. g. the fifth) should prove to be polymerous, this would in no way be astonishing nor difficult to bring into harmony with that same starting-point.

(3) That the presence of superficial branches to the integument and to the musculature, and of deeper branches to the intestinal epithelium in those parts that will contribute to form the cephalic nerves, is similarly foreshadowed in the Nemertea.

(4) That the equivalent of the Nemertean vagus nerve will have to be sought for in such branches of the Vertebrate vagus as more especially innervate the intestinal epithelium,[2] whereas

[1] Rohon, "Ueber den Ursprung des Nervus vagus bei Selachiern," 'Arbeit. Zool. Inst. Wien,' vol. i, p. 159.

[2] I have good reasons, based upon actual observations made by my pupil, Mr. Dobberke, to believe that the ramus intestinalis vagi in adult Elasmobranchs may be traced centripetally from its region of innervation of the foremost portion of the intestinal wall, towards the brain, as a bundle of nerve-fibres running parallel to and combined with those for the branchial apparatus, but that, nevertheless, this bundle can be separately traced up to the vagus ganglion, without any further intimate relation to those branchial branches (cf. Beard, loc. cit., p. 110). If this should actually be the case, the possibility of a direct comparison between the Nemertean

the innervation of the Vertebrate gill-slits, which marks a later phylogenetic stage, in which these perforations of the anterior trunk region have appeared, may be as well put to the account of more superficial parts of the transverse tracts.

(5) That the common starting-point of the sensory, lateral, and the intestinal portion of the vagus has also attracted the attention of former observers. Ransom and d'Arcy Thompson write: "In the embryo dog-fish the second or ventral commissure, described by Balfour, &c., as uniting the roots of the vagus, ventral to the ganglia, is essentially a sympathetic commissure, whose (visceral) fibres pass on, as described by Balfour, to form the intestinal branch of the vagus. In that intestinal branch we have an outflow of visceral fibres, quite comparable to, e. g. a splanchnic branch of the dorsal sympathetic system. The connection between the origin of the lateralis and this ventral commissure connecting the vagus roots in the dog-fish, and similarly the relation of the lateralis to the loops uniting the ganglia of the fifth, seventh, and tenth nerves in Petromyzon, may probably be described as indicating a fusion in this region of the two great commissural systems which posteriorly are separate, viz. that of the sensory branches (lateralis) and the visceral or sympathetic.

"We agree with Gaskell that the term sympathetic should be suffered to fall into disuse, as tending to perpetuate the old conception of the primary importance of the longitudinal nerve-tract; whereas the leading fact is the metamerically recurring outflow of visceral fibres, which may or may not be united together by successive longitudinal commissures."

In the Nemertea this anterior "fusion of the great com-

vagus nerve and the Vertebrate ramus intestinalis vagi, of course, comes more closely within our reach. It need not be insisted upon that if these comparisons prove correct the separate intestinal nerve-systems (sympathetic nerve system) of other Invertebrates (Annelids, Arthropods, Molluscs) cannot be looked upon as homologous with the sympathetic nerve-system of the Vertebrates, but would rather be homologous with that portion of the intestinal innervation of the latter which comes to the account of their cephalic nerves, in so far as these represent derivatives of the Nemertean vagus, and are marked *v* in figs. 1 and 2 of Pl. XLII.

missural systems" is foreshadowed at the point where brain-lobe, lateral stem, and "vagus nerve" meet, or rather diverge. It has been attempted in figs. 1 and 2 to indicate the points here alluded to in a general way, special comparisons being, on the grounds that have been stated, purposely avoided.

If we now turn to Dohrn's and Semper's hypothesis we must recognise that no such satisfactory general comparisons are there possible. Even if we were inclined to accept the "turning over" of Geoffroy St. Hilaire, by which back and belly became exchanged, and to admit the brain-piercing œsophagus, regarding the Annelid supraœsophageal ganglion and the ventral nerve-cord as respectively homologous to cerebrum and medulla, it must still be conceded that we have not then in any way before us a nerve-system offering as many points of comparison with the Vertebrate system as does that of the Nemertea.

Concerning the Annelids we have no observations by which the cephalic ganglia and the cephalic nerves are so clearly foreshadowed, none which would throw light on the origin of the vagus, its connection with the nervus lateralis and with the anterior cephalic ganglia, none concerning the sympathetic system and its blending with the vagus system in the lowest Vertebrates, indications of which are even retained in the highest. Nor is the ventral nerve-cord of Annelids, with its undeniable double character and double origin a match, so far as comparison goes, for the Nemertean medullary nerve, with its transverse nerves preceding the spinal nerves of Amphioxus and the Cyclostomata.

And if we are then asked to consider the lens of the Vertebrate eye as a modified ectodermal branchial invagination, as the outer portion of what was once a functional gill-slit,[1] we feel that the ground under our feet is becoming rather uncomfortable, and that it is high time to reconsider whether all these ingenious speculations in which the most beautifully pliable hypothetical and unknown Annelids play a too conspicuous part should not be definitely abandoned, and a new

[1] Dohrn, 'Studien,' x, p. 459, 1885.

departure made by those who are interested in the phylogeny of the Chordata. In due time arduous and detailed morphological investigations on the Platyelminthes in general, and on the Nemertea in particular, may then lead us to more satisfactory conclusions than are the fata morgana that are so temptingly evoked before our eyes by the ingenious manipulations of the indefatigable founder of the first and foremost Zoological Station, when, following his lead, we find ourselves wandering in the barren deserts of that province of phylogeny in which he attempts to establish a close connection between Chordata and Annelida.

All these considerations have induced me to give this rapid outline sketch of the degree of comparison which I hold to exist between Chordate and Nemertean (more especially Palæonemertean and Schizonemertean) nervous systems, although I am perfectly aware that there is a growing tendency among those authors at present occupied with questions concerning the morphology of the Vertebrate nervous system (Froriep, Baldwin Spencer, Beard, Cunningham, Kleinenberg, and many others) to accept Semper's and Dohrn's views of the Annelidan descent of Vertebrates. Wiedersheim, in the new edition of his 'Vergleichende Anatomie' (1886), does not even hesitate to bring these results in their unripe phase before the more extensive public of students, and this generally in acquiescent terms. It is curious to see how, e.g. the question of the cephalic nerves and their comparison to spinal nerves, that of the nerve-roots, the cephalic ganglia and their respective connecting trunks, have given occasion to the most diverse twisting and retwisting of the facts in order to bring out a fixed scheme or diagram, which might then be compared to what obtained in Annelids, and in which the highest degree of similarity between the respective somites might be obtained, thus establishing a preconceived idea of the Vertebrate ancestor as a most rigorously segmented animal. The value of these speculations has been already tested above, and I may be allowed once more to express my conviction that our comparisons between the Chordata and their lower Invertebrate

predecessors may only be looked upon as in any way satisfactory so long as they remain on a very broad and general basis, and that any very special homology said to be demonstrated ought for that very reason to be more especially suspected.[1]

For my part I believe that, along the lines above indicated, a comparison between Vertebrate and Invertebrate nervous systems will in future prove to be more fruitful, but I wish to repeat that for the present we can only indicate general points of coincidence between the two, and must rigorously refrain from making comparisons in detail.

On the other hand, it is suggestive once more to consider what has been recorded in my 'Challenger Report' concerning the nervous system of Drepanophorus Lankesteri, when compared with that of certain Annelids; and we may, I believe, safely come to the conclusion which was formulated by me seven years ago, but which I now hold to be much more solidly established, that we have in the Nemertea an important group through which definite glimpses may be obtained at the sources from which both Chordata and Appendiculata (Ray Lankester) have respectively sprung. The proposition first formulated by Gegenbaur, about the phylogenetic origin of the ventral nerve-cord and œsophageal ring of the Annelida out of ancestors with lateral cords, has obtained new support from the arrangement which was met with in the species just mentioned. And just as we have before tentatively discussed the question, in how far remnants of the lateral cords were retained in those descendants in which the median one had been raised to the dignity of a medulla spinalis (the Verte-

[1] Bateson (loc. cit., p. 562) seems to take a similar view of the efforts here alluded to. He says: "No doubt the cranial nerves may, by arbitrary divisions and combinations, be shaped into an arrangement which more or less simulates that which is supposed by some to have been present in the rest of the body, but little is gained by this exercise beyond the production of a false symmetry."—Dohrn himself, whose suggestions have so largely contributed to the accumulation of all this conflicting evidence, is now rather in the position of Goethe's Zauberlehrling, and writes ('Studien,' x, p. 468, 1885): "Auch auf diesem Gebiet (die Frage nach der Bedeutung der Hirnnerven) bildet die bisherige vergleichende Anatomie das Bild eines auf stürmischer See steuerlos herumgeschleuderten Schiffes."

brata), we might now consider whether any remnants of the median dorsal cord are retained in those descendants in which the lateral cords have differentiated into brain-lobes, œsophageal ring, and ventral cord (the Annelida). To this question I have no definite answer to offer, but I may call attention to the significant fact that the beautiful and exemplary investigations into the embryonic development of Lopadorhynchus, very recently published by Kleinenberg,[1] have demonstrated the existence in the larva of that Annelid of a nerve-stem answering to the conditions here required. It is dorso-medially situated, it is anteriorly connected with the brain, or rather with a transverse nerve-tract (Kleinenberg's prototrochal nerve-ring), which in its turn is connected with the brain,[2] it appears to be connected close to the anus with the ventral cord (the fused lateral stems), and though appearing in early larval life, and having only a temporary existence, it is regarded by Kleinenberg as having considerable physiological importance. If the light in which I am inclined to look at it is not deceptive, its morphological significance also can hardly be overrated.

In closing this chapter of general considerations we may once more bring before our minds the proposition with which it was opened. We have here and in the foregoing chapters adduced facts and arguments which appear to speak in its favour; we will once more rapidly enumerate the common characteristics of Nemertea and Cœlenterata, as well as those of Nemertea and Chordata.

The Cœlenterate characteristics that are also found in the Nemertea are the following:

a. The presence of nematocysts in the proboscidian epithelium.

b. The elaborate nerve-plexus in the integument, and its histological features.

c. The presence of epiblastic muscle-fibres separate from the general body-musculature.

[1] 'Zeitschr. f. wiss. Zool.,' Bd. xliv, Heft. i, ii, October, 1886, p. 107; pl. vii, fig. 27*a*.

[2] For comparison with the Nemertea, cf. Pl. XLII, fig. 1.

d. The presence and the chemical constitution of a sometimes very massive intermuscular jelly by which the other internal organs are at the same time surrounded.

e. The mode of development of the mesoblast (at least in Lineus obscurus), which is less specialised than in most other Invertebrates.

f. The absence of any distinct enterocœle.

The points of resemblance with the Chordata may be thus tabulated :

a. The general features of the nervous system.

b. The presence of a homologue of the hypophysis cerebri as a massive and important organ (the proboscis).

c. The presence of tissues which may have become converted into the notochord (viz. the material of which the proboscidian sheath is built up).

d. The respiratory significance of the anterior portion of the alimentary tract.

At the base of all the speculations contained in this chapter lies the conviction, so strongly insisted upon by Darwin, that new combinations or organs do not appear by the action of natural selection unless others have preceded, from which they are gradually derived by a slow change and differentiation.

That a notochord should develop out of the archenteric wall because a supporting axis would be beneficial to the animal may be a teleological assumption, but it is at the same time an evolutional heresy. It would never be fruitful to try to connect the different variations offered, e.g. by the nervous system, throughout the animal kingdom, if similar assumptions were admitted, for there would be then quite as much to say for a repeated and independent origin of central nervous systems out of indifferent epiblast just as required in each special case. These would be steps that might bring us back a good way towards the doctrine of independent creations. The remembrance of Darwin's, Huxley's, and Gegenbaur's classical foundations, and of Balfour's and Weismann's brilliant superstructures, ought to warn us away from these dangerous regions.

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PRINTED BY ADLARD AND SON, BARTHOLOMEW CLOSE.