The Early Development of Amphioxus.

By

E. W. MacBride, M.A.,
Fellow of St. John's College, Cambridge; Professor of Zoology in the McGill University, Montreal.

With Plates 43—45.

The work which forms the subject of the present essay was carried out in the Cambridge Zoological Laboratory during the years 1895–7. The material on which my results are founded consisted of a collection of embryos and larvae which had been obtained by Mr. Sedgwick and Dr. Willey during their visits to Faro in 1890–91, and I have to express my warmest thanks to Mr. Sedgwick for placing this valuable material at my disposal.

It will, no doubt, be the opinion of many zoologists that a fresh paper on the early development of Amphioxus needs considerable justification, since it may appear superfluous to attempt to improve on the admirably clear account given by Hatschek (3 and 5) of this subject, an account which has become incorporated in all text-books, and forms part of the classical literature of zoology. That such an opinion, however, would not be well founded, and that on the contrary a large number of doubtful points of great theoretical importance remained to be cleared up, will be evident when we briefly examine the present state of our knowledge on this subject.

The foundation of our knowledge of the development of Amphioxus was laid by Kowalevsky, whose two papers (6 and
may be considered together. In these the segmentation of the egg and the formation of the blastula and the process of invagination are described. Kowalevsky states that the blastopore is at first posterior in position, but becomes later shifted on to the dorsal surface; he describes the formation of the central nervous system and its appendix, the neurenteric canal. He also describes the formation of the coelomic pouches (myotomes) as folds of the gut wall, and notes the fact that the first pair have thinner walls and a wider cavity than the rest, and communicate by a broader slit with the gut cavity. He likewise gives an account of the larval life of Amphioxus, and it is interesting to note that in his second paper (7) he anticipated the results which Lankester and Willey obtained later (9 and 13) as to the development of the gill-slits and the formation of the atrial cavity. The two longitudinal ridges, which by their union form the atrial cavity, were seen and figured by him, and the cavities they contained also described. His account of the gill-slits appeared, however, so extraordinary to his contemporaries, that it was supposed he was misled by pathological specimens, and it needed Dr. Willey's researches to convince zoologists of its accuracy.

A year or two after Kowalevsky's second paper was published Professor Hatschek undertook a re-investigation of the subject, and the account given of the development of Amphioxus in Korschelt and Heider's 'Lehrbuch der Vergleichenden Entwickelungsgeschichte' is taken directly from Hatschek's paper. Hatschek confirmed in his first paper (3) Kowalevsky's account of the segmentation of the egg; but with regard to the invagination he asserts that the blastopore is from the first in its definitive dorsal position, or, what comes to the same thing, that the closure of the blastopore is mainly effected by the backward growth of the dorsal (anterior) lip.

This statement has been seized on with avidity by certain workers in Vertebrate embryology as affording a possibility of twisting the developmental history of Amphioxus into accord-ance with theories which regarded the main developmental process in Vertebrates as a concrescence of two distinct halves
along the mid-dorsal line, or, as it was sometimes expressed, the meeting of the lips of a long slit-like blastopore.

Hatschek's account of the origin of the mesodermal structures is curious and interesting. Like Kowalevsky, he finds two longitudinal folds of the gut wall, but these he believes to terminate posteriorly in two large "pole" cells situated in the lip of the blastopore. By cross-folds the successive pouches are cut off from these longitudinal folds. Later two independent outgrowths from the alimentary canal form the "head cavities," of which the right retains longest its communication with the alimentary canal. The formation of a continuous ventral body-cavity by the fusion of the lower parts of the pouches (somites) is deduced from the fact that the divisions between the somites can no longer be traced in this part of the body. In a later paper (4) he describes in the larva what he terms "a genuine kidney," a tube lying in front of the mouth. This structure will be referred to throughout this paper as "Hatschek's nephridium." In his last paper (5) he gives an account of the derivation of the muscular and skeletal tissue from the ccelomic pouches, pointing out that everything in Amphioxus is essentially epithelial in nature, the connective tissue having no nuclei in it.

Lankester and Willey's paper on the development of the atrial chamber (9) confirms in most points Kowalevsky's statements, but the authors deny that the cavities in the metapleural folds are ccelomic in nature as Kowalevsky had imagined. The right atrial fold is described as extending much further forward than the left. They found also that Hatschek's nephridium opens into the gut; a result which Van Wijhe (11) announced as an independent discovery three years later.

Willey (13) in 1891 published an account of the development of the gill-slits and other organs appearing in the larval stage. He describes the internal and external openings of the club-shaped gland (an organ noticed already by Kowalevsky), which he regards as the fellow of the first gill-slit. He states that the oral hood which conceals the true mouth of Amphi-
oxus, and which was regarded by Lankester as a forward prolongation of the atrial folds, is a downgrowth of the upper margin of the præoral pit as far as its upper part is concerned, its lower part being formed beneath the mouth independently.

Lwoff's paper (10) deserves special attention not only because it is the first systematic study of the embryology of Amphioxus with modern methods, but also on account of the disagreement of the results he arrived at with those of Hatschek. Lwoff maintains that before invagination commences the cells constituting the blastula become sharply divided into two sorts, endoderm and ectoderm cells; that invagination first involves the endoderm cells, but that later the ectoderm bends round the dorsal lip of the blastopore, and displacing the endoderm forms the dorsal wall of the gut, and that the whole of this ectodermal stage is employed in the formation of the notochord, and in the production of mesoderm. Lwoff believes that the mesodermal "folds" are the mechanical result of the pressure of the nerve-tube and notochord on the upper wall of the gut; he asserts that the cavity of this fold disappears, and that the cavities of the somites appearing later have no connection with or relation to the enteric cavity; hence that Amphioxus is not an "enterocoelous animal." He finds that the pole-cells of Hatschek have no existence, therein confirming Wilson (14); and that the blastopore is closed not by the special growth of the dorsal lip, but from all sides.

It will thus be evident that the most interesting part of the development of Amphioxus, viz. the formation of the primitive germinal layers, was involved in great uncertainty, and it was with a view of clearing up the questions thus raised, and also from a feeling of dissatisfaction with the accounts given of the nature and origin of such structures as Hatschek's nephridium, and the cavities in the atrial (metapleural) folds, &c., that I was led to undertake a re-investigation of the whole subject. That the early development of Amphioxus is of great theoretical importance can, I think, be hardly denied; when we consider that in it we have the only instance of
Vertebrate development where, the yolk being evenly distributed, its disturbing influence is negligible; and when one recollects the weary controversies which have been waged round the meaning to be attached to the gastrulation and formation of the layers in the heavily yolked eggs of the higher Vertebrates, one must feel that a knowledge of the development of Amphioxus alone could bring these questions to a definite issue.

The results I have arrived at differ considerably from any obtained hitherto, and in claiming to have penetrated more deeply into the developmental processes than Hatschek or Kowalevsky I rely entirely on the higher grade of perfection which methods of dealing with small organisms have reached in the meantime.

All the embryos were embedded in the ordinary way in celloidin; but after hardening the celloidin with chloroform I adopted a plan of clearing on which really the whole success of the work depended. This was as follows:—The hardened celloidin was immersed for a minute or so in absolute alcohol, in order to remove any traces of moisture which might be present in the chloroform; it was then placed in cedar oil and left for a night in a warm place (the dish containing the cedar oil being placed on the top of the thermostat). In the morning the celloidin had become so transparent as to be almost invisible when looked at in the cedar oil. Little pieces of the block containing the embryos could then be cut out and examined with ease under a low power, and their exact orientation determined. They were then embedded in paraffin, and cut into series of sections from 4 to 5 μ thick, and stained on the slide.

The material at my disposal was preserved in a variety of ways, but except for the earliest stages my results are based only on specimens preserved in osmic acid. I wish to lay particular emphasis on this, as should any zoologist feel inclined to work over the same ground with a view of testing my results, and use such fixing reagents as corrosive sublimate or picro-sulphuric acid, he is foredoomed to failure. After such fluids the gut becomes swollen and the body-walls
collapsed, so that it is impossible to make out anything of the limits of the coelomic cavities. The greatest difficulty I have found in dealing with Amphioxus larvae is to stain the connective tissue; and I have, in fact, only found this possible with specimens preserved in osmic acid. Of course insufficient or careless preservation in this fluid is valueless, and leads as usual to maceration. For the stages up to the end of the gastrulation, however, before any real differentiation of tissues has taken place, almost any reagent gives fair results.

I do not intend in this paper to refer, except incidentally, to those features in the development of Amphioxus which have been satisfactorily worked out, and about which there is a general consensus of opinion. I may, however, remind the reader that the eggs are spawned in the evening about 7 o'clock; segmentation takes place rapidly, so that by 11 p.m. the blastula is complete and invagination has commenced; two hours later the gastrula stage is attained, and by 5 a.m. the mesoderm has appeared, and the embryo which has been for some time actually rotating within the egg-shell is hatched. On the morning of the next day about 8 a.m. the embryo has acquired the definite form of the larva, with a pointed snout, a swollen pharyngeal region, and a very attenuated body, and the mouth and first gill-slit are formed. No further development has been attained with embryos reared in aquaria, and in their natural habitat the rate of development is after this very slow, extending over several months. I shall divide my account of the subject into the following parts:—

1. The Gastrulation.

A specimen of the youngest embryos examined is represented in Pl. 43, fig. 1. It has the form of a regular sphere bounded by one layer of cells of approximately equal size, and is in fact a perfectly typical blastula. Although Hatschek figures a difference in size of the cells in the two poles of the
egg as being observable in the earlier stages of segmentation, I have been quite unable to find any trace of it in sections, and in this respect confirm Lwoff (10). In agreement with the latter author, I find when the first change ushering in the process of invagination takes place, namely, a flattening of one side of the blastula, that then for the first time a differentiation of the cells composing the embryo into two sorts become observable. Some of them, in fact, become taller and slightly narrower than the rest. Lwoff lays great emphasis on this phenomenon. He regards the more cylindrical cells as alone representing the endoderm of Invertebrates, any further portion of the blastula which may be involved in the process of invagination being denominated as ectoderm.

It is difficult to find words to adequately characterise the artificiality and arbitrariness of such a view. The only circumstances under which it could be maintained would be if the supposed endoderm were sharply marked off from the ectoderm, and if further there were a pause in the process of gastrulation after the so-called endoderm had been invaginated, but before the invagination of the ectoderm had commenced. A glance at Pl. 43, figs. 2 and 3, will show that the taller cells on one side shade imperceptibly into the shorter and rounder, so that it is impossible to say where the one begins and the other ends. On the other side, it is true, there is an abrupt transition at the point marked a. This point I have found by careful comparison with one another of successive stages to correspond to the dorsal (anterior) lip of the blastopore, the very place where Lwoff supposes ectoderm to be invaginated. This spot is easily recognisable in the earlier stages of invagination (Pl. 43, figs. 4, 5, and 6), but becomes less recognisable in the later stages. As a tentative explanation of it I may suggest that here an active multiplication of cells takes place, and that those which are added to the invaginated portion of the blastula become laterally compressed and columnar, whereas those added to the ectoderm remain stretched by the internal turgidity of the fluid in the blastocoele or segmenta-
tion cavity. As invagination proceeds, the future dorsal surface of the embryo becomes recognisable by the close apposition of the layers of ectoderm and endoderm which subsists here, whilst on the other side the outer and inner layers of the gastrula diverge from one another (at the point marked \( o \) in fig. 7, for example). A little later we are able to perceive that the future dorsal surface has become definitely flattened, this being the first preparation for the formation of the neural plate, the rudiment of the central nervous system; and as the appearance of this structure enables us to determine the long axis of the future animal, we are able to say that the blastopore is at first posterior. At the same time the peculiar character of the dorsal lip at this stage, with the abrupt transition from ectoderm to endoderm, and the close parallelism of the two layers enables us with certainty to identify it with the corresponding part in earlier stages.

Thus I regard the gastrulation as a fairly uniform pushing in of the under or flattened surface of the blastula, accompanied by division and multiplication of the cells, such multiplication being at first most active in the dorsal (future anterior) lip of the blastopore. The blastopore, which is still wide, becomes rapidly narrowed by the upgrowth of the ventral lip (Pl. 43, figs. 8, 9, and 10): in contra-distinction to what Hatschek (3) asserts, the dorsal lip remains relatively stationary.

Coincidently with this increased activity of growth in the ventral lip, a sharp, abrupt transition becomes now observable in it from ectoderm to endoderm, a fact which supports the explanation given above of a similar phenomenon observed in the dorsal lip. In support of his view that there is an invagination of ectoderm round the dorsal lip of the blastopore, Lwoff speaks of a frequent accumulation of cells just inside the blastopore on the dorsal side, and figures two longitudinal sections of gastrulae in illustration of this point. I have to definitely state that no such appearances are ever seen in properly orientated sections, and that Lwoff has been misled by his inability to distinguish between oblique and sagittal
sections, though I do not admit that such an accumulation, did it exist, would prove his point.

If we examine a transverse section of a completed gastrula—such a one, for instance, as Pl. 43, fig. 11—we find no difference in character between the cells forming the dorsal wall of the alimentary canal and those forming the ventral wall, such as we should have the right to expect did Lwoff's hypothesis in any way correspond with the facts. Before leaving this subject, however, it is but just to notice a statement of Lwoff's, that had he been dealing with the development of Amphioxus alone, he should not have ventured to put forward the hypothesis of an ectodermal origin of the dorsal wall of the archenteron; but that as he found in other Vertebrates that this dorsal wall was entirely used up in the formation of the notochord and mesoderm, and did not take part in the definitive wall of the alimentary canal, and was in some cases apparently derived from ectoderm, he felt justified in reading this interpretation into the developmental processes of Amphioxus. Such an attitude of mind seems to me the entire converse of the proper one to be adopted under these circumstances. Quite apart from the superior value to be attached to the significance of the processes in Amphioxus owing to the primitive nature of the adult, it is one of the best known facts of embryology that the presence of large quantities of yolk clogs and utterly distorts the developmental processes, and that we have to interpret the cases where much yolk is present in the light of those where little yolk is present, and not vice versa. Moreover, a very simple and natural explanation can be suggested why in the Vertebrate embryo the yolk should be confined to the ventral wall of the archenteron. We know that many, if not most, developmental processes are ultimately reducible to processes of folding, such as would be rendered entirely impossible were the tissue in which they have to take place clogged with yolk. Hence in the higher Vertebrates the processes of invagination itself are profoundly modified; and, as explained in detail in the careful work of Will (12) (who in
this confirms the ideas of Balfour\(^1\), the bulky ventral wall of
the archenteron can no longer be folded in, and the persistent
invagination of the yolkless dorsal wall has the appearance of
an independent ingrowth of the ectoderm.

A good illustration of the influence of yolk is seen in the
development of Molluscs. There it has been customary to
regard the four macromeres as representing the endoderm.
These cells are, however, much too heavily loaded with yolk
to give rise to any definitive tissue; from them the smaller
micromeres which are to form the ectoderm are budded off,
and from them later, in continuity with these, the cells which
give rise to the epithelium of the intestine. (See Balfour’s
‘Text-book of Comparative Embryology,’ vol. i, Dev. of
Mollusca.) It is significant that assertions of the share of the
ectoderm in the formation of the alimentary canal should have
been made principally in such cases (eggs of insects, cephalo-
pods, &c.), where the accumulation of yolk is so great as to
preclude any possibility of the yolk-bearing cells being directly
converted into permanent tissue.

2. The Formation of the Mesoderm.

Shortly after the gastrulation is complete the outline of the
alimentary canal, as seen in transverse section, ceases to be
round. Its dorsal wall becomes flattened, and is then drawn
out into two lateral angles, and almost immediately afterwards
a median hollow ridge is formed, the first suggestion of the
future notochord. The lateral angles mentioned are the ex-
pression of two longitudinal hollow ridges or folds of the gut
wall. Lwoff regarded them as the mechanical effect of the
downward pressure of the nerve-tube as it became folded into
the interior of the body, and likewise of the notochord when it
was formed. The absurdity of this view is well shown by
Pl. 43, fig. 12; there these two lateral ridges are plainly seen,
whilst the nerve-cord is still a flat plate, and the notochord is
barely indicated. That these hollow outgrowths (which we

\(^1\) “A Comparison of the Early Stages in the Development of Verte-
may term the cœlomic grooves) are due to an independent process of folding, originating in the endoderm, is also shown by the fact that the endoderm is no longer in close contact with the ectoderm, a distinct slit-like blastocele being observable in several places, which certainly would not be the case if the endoderm merely passively followed the foldings of the ectoderm.

Shortly after the appearance of the cœlomic grooves a fresh pair of outgrowths from the alimentary canal make their appearance anteriorly. These, which will be denominated collar cavities, are shown in fig. 13. As will be seen, they are situated slightly nearer the middle line than the cœlomic grooves (which in the figure are seen external to them, separated by a very narrow fold of the gut wall), and their lumina are at first excessively narrow (at least in preserved specimens), but they soon enlarge, and their openings into the cavity of the alimentary canal become clear (fig. 14, c). About the same time the front part of the cœlomic grooves becomes constricted off from the alimentary canal, and thus a definite “somite” or myomere is formed (fig. 14, b). Behind, however, the cœlomic grooves still open into the alimentary canal, as shown in fig. 14, a. If we compare with such a series of sections through older embryos, such as those figured in fig. 17, d and e, we shall arrive at a clear comprehension as to how the cœlomic grooves are converted into a series of somites. We can always find at the hinder end of the embryo appearances (like those represented in figs. 14, a, and 14, c) of the gut wall being folded so as to produce a pair of cœlomic grooves, and we can follow the walls of the fold constituting the cœlomic groove into the walls of the last somite (compare fig. 17, d). The cavity of no other somite communicates with the gut; it is only the last somite, for the time being, whose cavity is in communication with the gut cavity through the cœlomic groove; and hence we see that as the embryo grows in length, and the cœlomic groove with it, this latter becomes progressively constricted from the gut and divided into somites at the same time, each new piece which is con-
stricted off becoming formed into a somite, and nipped off from the open part of the cœlomic groove, which then again grows in length, and the process is repeated. The formation of a somite, then, is essentially a process of obliterating the cavity of the cœlomic groove for a certain space, and the so-called last somite is really the undifferentiated hinder end of the cœlomic groove.

The entire independence of the collar cavities from the cœlomic grooves is emphasised by the fact that for some time after the latter are shut off from the gut the collar cavities still retain their openings into it. This is shown in the transverse section (fig. 14, c) and in the longitudinal section (fig. 15, a). These collar cavities are the "first protovertebræ" of Kowalevsky, and in his paper (7) he notes the fact that they communicate by a broader slit with the alimentary canal, and retain this communication longer than the rest. Later, it is true, the right collar cavity becomes completely shut off from the gut, but the left retains its communication, as is shown in Pl. 43, fig. 16.

Shortly after this period the embryo begins to diminish rapidly in diameter, owing to the consumption of the yolk in the endoderm cells, whilst at the same time it increases in length, and the cavities in its interior diminish in size, owing to the gradual shrinkage. Hence one requires now to have specimens preserved in such a way as to give firmness and resistance to the outer tissue, if one is to make out anything of the internal anatomy at all. The yolk, which is present not only in the ventral but also in the dorsal ectoderm and in the walls of the cœlomic folds, acts whilst it endures, somewhat like paraffin, in preventing too great shrinkage; after it has gone nothing but osmic acid will give any help.

Just as the disappearance of the yolk is commencing, the third division of the mesoderm, the head cavities, make their appearance. The two head cavities really constitute the extreme anterior end of the alimentary canal, which grows out into two lateral horns. In Pl. 44, fig. 17, a, we see them still opening widely into the gut; but in the next section (17, b), taken
further back, we see them quite free from the gut, and so we can conclude that they are recurved. Hatschek (3) spoke of the two head cavities as distinct outgrowths from the gut, and further stated that the right one still communicated with the gut after the left had been cut off. This is not a correct account of what happens: the whole anterior part of the alimentary canal becomes shut off from the hinder part, and its two horns, which later become converted into the head cavities, still communicate with one another after separation from the gut has taken place (fig. 18, b).

We have thus seen that the mesoderm originates as five hollow outgrowths from the gut—an anterior median, viz. the head cavity rudiment, and two pairs of lateral ones, viz. the collar cavities and the cælomic grooves. Thus in the formation of the primary layers Amphioxus is in fundamental agreement with Balanoglossus, as described by Bateson (1). The main difference between the two types is that whereas in Balanoglossus the trunk cavity remains undivided, in Amphioxus it becomes broken up into a series of segments, a difference which we may plausibly correlate with the different modes of life pursued by the two animals. Pl. 45, figs. 25 and 26, are two diagrams intended to make these relations clearer.

3. The Fate of the Cælomic Cavities.

The two horns of the common head cavity rudiments rapidly become separated from one another; the right now shows itself as an irregular-shaped and thin-walled sac; the left, on the other hand, is composed of cylindrical cells, and remains small and round (fig. 18, a). The right soon after gets shifted ventrally, and forms the greater part of the cavity of the præoral snout during the whole of the larval period (compare fig. 21, a). I have not been able to identify it in the adult, and can only suppose that it becomes obliterated, the space corresponding to it being apparently occupied by connective tissue. The left, as is well known, acquires an opening to the exterior, and constitutes the præoral ciliated pit (fig. 21, a), which Hatschek first discovered. This præoral pit persists into
the adult condition as a ciliated area on the inner side of the praearal hood. By the time that the head cavities have commenced to appear, notochord and nerve-cord have become well advanced in their development. The notochordal fold has become completely shut off from the gut, and is quite solid in front, though still a groove behind, whilst the neural plate has passed through the stage of being covered by a flap growing from the adjacent ectoderm (figs. 13, 14, and 16), and has become converted into a tube (fig. 17, b, c, and d), still retaining an anterior opening, the neuropore (figs. 17, a, and 18, b). The collar cavities have now become large thin-walled sacs; the right extending by this time nearly to the mid-ventral line; the left does not extend so far, but it still retains its communication with the gut. This communication has by this time become drawn into an exceedingly narrow tube (fig. 17, c, neph.), and is in fact the rudiment of Hatschek's nephridium.

At the close of what we may term the embryonic development—that is to say, at the end of the second day of development—both collar cavities have undergone further changes. They have extended forward at the sides of the notochord, above the head cavities, to just beneath the neuropore (fig. 18, b); behind they have nearly reached the mid-ventral line, the left being more obliquely directed, as it has to pass over the area where the future mouth will be formed (fig. 19, b). When they have reached the ventral line they extend backwards to a considerable distance behind the first gill-slit, forming the ventrolateral angles of the body, and giving to the transverse section a squarish appearance ventrally, which contrasts strongly with the rounded appearance behind the point they extend to (compare Pl. 44, fig. 20, a and c). The inner walls of the dorsal portions of the collar cavities become like the corresponding parts of the somites converted into longitudinal muscles, and constitute in fact the first myotomes; but during early larval life, at any rate, the persistent cavity of this "first myotome" on the right side remains in open and obvious communication with the ventral part of the collar cavity.
THE EARLY DEVELOPMENT OF AMPHIOXUS.

(fig. 21, a). On the left side the front section of the ventral collar cavity seems to become solid, and from it are apparently derived the true oral tentacles, so well described by Lankester, and the muscles moving them. "Hatschek's nephridium" has now become a horizontally placed tube, the openings of which into the first myotome (collar cavity) on the left and into the gut are easily seen (fig. 21, b and c). It is curious that this internal opening completely escaped the observation of both Van Wyhe and Hatschek; it is perfectly easy to see in any good series of sections through a specimen preserved in osmic acid. Hatschek (4) states that this nephridium persists into the adult. I have, however, been able to find no trace of it after the metamorphosis.

The ventral extensions of the collar cavities appear in the larva to reach some distance behind the last gill-slit formed. In any series of sections in this region we get quite similar appearances to those represented in figs. 20, a, b, and c. The natural conclusion to be drawn from this is that behind the first gill-slit, as the gut grows and produces new gill-slits, the collar cavities grow pari passu.

The ccelomic cavities in the somites we saw at their time of formation to be exceedingly minute; in some cases it would be more correct to say that there was no cavity at all, but only a radiate arrangement of the cells round a virtual cavity. Such a state of things might in the minds of some suggest that there was something to be said for Lwoff's position that Amphioxus is not an enterocoelous animal, since its cælom could not be traced into continuity with the alimentary cavity. Such a position, however, appears to me to be quite untenable, even apart from the fact that the collar cavity is at one time in open and obvious communication with the enteric space. What determines whether an animal is to be regarded as enterocoelous or not is whether or not evidence is forthcoming to show that the walls of the body-cavity have been derived from those of the alimentary canal by a process of folding, for it is the walls only to which we can attribute an objective existence; the presence or absence of a cavity at any moment
of development is due to the relations of growth and pressure subsisting between them. No one who has seen well-preserved sections can doubt that it is by a folding process that the mesoderm is formed in Amphioxus.

Later the cavities of the somites enlarge, and the dorsal portions of their inner walls are like the corresponding parts of the collar cavities converted into longish muscles (Musc., fig. 20, a, b, and c), and form all the myotomes except the first. The ventral portions wedge themselves in between the gut and the posterior ventral extension of the collar cavities (V. Tr., figs. 19, c, 20, a, b, and c). Whether in the living condition these ventral portions are completely hollow, or whether, as is suggested by the examination of sections, they are partly represented by solid tongues of tissue, it is impossible to settle. Of course in the adult the ventral portions of the somites give rise to the dorsal ccelomic canals, and to the canals running in the primary and secondary gill bars (Lankester); the dorsal ccelomic canals are clearly represented in the larva, but I have found it impossible to be certain whether the rest of the ventral portions of the somites are open spaces in the larva or not. Just at the close of embryonic life the "myotome" becomes separated from the rest of the somite by a septum, and the ventral portions of the somites acquire communication with each other, about the region where the dorsal ccelomic canals afterwards appear. This ventral fusion of the somites was inferred by Hatschek from the fact that he could not trace the dividing lines between the somites to the mid-ventral line. The specimens of later larvae which were well enough preserved to rely on showed the trunk ccelom (derived from the ventral fusion of the somites) clearly only behind the gill-slits, where the collar cavities were dying out. Elsewhere the extreme difficulty of staining the connective tissue and peritoneal epithelium made it impossible to be certain whether a narrow slit-like cavity or only a wedge of tissue intervened between the gut wall and the collar cavity.
4. The Origin of the Atrial Folds.

Kowalevsky (7) was the first to discover that the atrial cavity was formed by the meeting in the mid-ventral line of two long ridges or folds. These, which were more exactly investigated by Lankester and Willey (9), are situated at the ventro-lateral angles of the body, and the atrial cavity is at first a small space situated in the middle line beneath the pharynx (fig. 23, b). Later the atrial cavity extends up at the sides of the pharynx, and the origin of the folds becomes consequently shifted up the body. This is the account of the origin of the atrial cavity given by Lankester and Willey; but it must be remembered that as the dorsal limits of the atrial cavity are from the beginning conterminous with those of the gill-slits, the process might be more correctly described as a great relative growth of the ventral region of the pharynx and surrounding structures. Lankester (8) terms the folds which actually wall in the atrial cavity "epipleural," and the projecting angles after these folds have united "metapleural." I shall use the term "atrial fold" to include the whole, of which both are parts.

It must already have struck the reader that the posterior ventral extensions of the collar cavities which I have described above occupied precisely the region where the atrial folds subsequently appeared; hence it will not be surprising when I state that the cavity of the atrial fold, termed by Lankester and Willey "pseudocellic," is nothing but the backward extension of the collar cavity. This I have succeeded in proving for the right collar cavity (comp. fig. 22, a, b, and c); and since the left collar cavity has precisely the course which Willey describes for the oral hood and left atrial fold, no one will doubt that this is the case for the left side also. From the walls of these two collar cavities the ventral muscles of Amphioxus are formed, and their lumen becomes occluded in the centre (fig. 24), but remains at the sides as the "metapleural lymph canal."

Lankester and Willey describe the atrial folds as first
appearing behind, and then growing forward; but the first recognisable trace of the future fold on the right side is an epithelial thickening (fig. 22, a) in the anterior region of the pharynx. This thickening, which later lines the outside of the fold, is recognisable even at the end of the embryonic period.

It will be remembered that the name “collar cavity” was given to the cœlomic pouches so denominated on account of their general resemblance in mode of formation to the collar cavities of Balanoglossus. The homology implied in this name is borne out by the subsequent history of the sacs in question; for (1) they remain distinct from the cavities derived from the cœlomic grooves or trunk cœlom, and (2) they swell out into ridges overhanging and protecting the gill-slits, just as the hinder edge of the collar region does in Balanoglossus, as Bateson (2) has pointed out,—only in that animal, of course, at most two gill-slits are protected.

Résumé and Conclusions.

The most important points established in this paper are as follow.

(1) The primitive gut or archenteron is formed in Amphioxus by a typical process of embolic invagination, the endoderm being at first not sharply marked off from the ectoderm. The blastopore is at first posterior, but subsequently becomes dorsal by the preponderant growth of the ventral lip of the blastopore.

(2) The mesoderm originates in Amphioxus as a series of true gut pouches, viz. one anterior unpaired pouch and two pairs of lateral pouches. Of these the first divides to form the two head cavities: the anterior pair give rise to the first pair of myotomes, and in addition to two long canals extending back ventrally: the posterior pair are gradually separated from the gut, and pari passu divided into a series of myotomes. The whole process of mesoderm formation is therefore referable to the type found in Balanoglossus, the main differ-
ence being that the pouch corresponding to the trunk coelom of Balanoglossus becomes segmented.

3. Hatschek's nephridium is the persistent connection of the left of the pair of collar-pouches with the gut.

4. The metapleural "lymph canals" found in the atrial folds are the persistent ventro-lateral extensions of the "collar-pouches."

The general conclusions which can, I think, be fairly deduced from the foregoing study are, in the first place, that all attempts to explain the formation of the nervous system of Vertebrates by the coalescence of the two halves of a nervous ring lying in the lips of a long slit-like blastopore must be given up, any appearances interpreted thus being due to secondary disturbances introduced by increasing food-yolk; for of any such process no trace is observable in the simple development of Amphioxus.

Secondly, that the theory of the descent of the Vertebrates from a form somewhat like Balanoglossus receives strong support from the early developmental history of Amphioxus. I say from a form somewhat like Balanoglossus advisedly, for it may not be superfluous to lay stress on this point, that we can no more suppose Vertebrates to be descended from Balanoglossus than from Amphioxus. The main stem of the Vertebrate phylum most probably continued throughout its whole history to lead an active predatory existence, and Balanoglossus and Amphioxus are to be regarded as degenerate offshoots from different levels of this stem. It is much more probable that the Tornaria larva of Balanoglossus gives the best idea of the remote ancestor of the Vertebrates, and in this respect the condition of the nervous system in the larval Amphioxus is of great interest. So far as we know central nervous systems are generally developed in close connection with prominent sense-organs. Now the Tornaria (in its later stages) has two main nervous centres—(a) at the apex of the praoral lobe, a sensory nervous plate with two eye-spots; (b) a short nervous tube in the collar region. Of these, the second has probably been developed in connection with a
series of sensory tentacles such as Cephalodiscus possesses in this region, and which probably correspond to the ambulacral tube-feet or tentacles of Echinoderms. The first is lost in Balanoglossus, owing no doubt to its burrowing life; but in the free-living Vertebrate ancestor this would not have occurred. As the præoral lobe became reduced in size (a process which may have been connected with the giving up of cilia as a means of progression and obtaining nutriment) the two nervous centres of the Tornaria-like ancestor would become approximated, and we should reach the condition which we actually find in the Amphioxus larva, viz. a sense-plate immediately followed by a nervous tube; for the part of nervous system under the neuropore becomes pigmented, and is sensitive to light. Figs. 27 and 28 are diagrammatic side views of Balanoglossus and an Amphioxus larva, and are intended to emphasise the immense diminution which the præoral lobe has undergone in the latter.

If these conclusions are well founded, Amphioxus would represent a more primitive offshoot from the Vertebrate stem than Ascidians, for the larvae of the latter possess a large vesicular brain, which only retains a small pore leading into the stomodæum. This deduction is, however, supported by the fact that whereas the Ascidian larva possesses a long post-anal muscular tail (a feature which has become more and more accentuated in fishes), in the Amphioxus larva the anus is as Hatschek pointed out, and as I can confirm, at the extreme posterior end of the body on a vertical neurenteric canal, and becomes only slowly and to a small extent shifted forwards during development.

Zoological Laboratory, Cambridge;
August, 1897.
THE EARLY DEVELOPMENT OF AMPHIOXUS.

LIST OF PAPERS REFERRED TO IN THIS PAPER.

EXPLANATION OF PLATES 43—45,

Illustrating Mr. E. W. MacBride's paper on "The Early Development of Amphioxus."

LIST OF ABBREVIATIONS EMPLOYED.


The last four figures are diagrams; the outlines of all the rest have been drawn with the camera lucida.

PLATE 43.

(All the figures drawn with magnification obtained by Zeiss C, oc. 2.)

Fig. 1.—Section of young blastula.

Fig. 2.—Sagittal section of blastula which is just commencing to flatten.

Fig. 3.—Sagittal section of blastula which has flattened on one side.

Fig. 4.—Sagittal section of gastrula in which invagination is just commencing.

Fig. 5.—Sagittal section of gastrula in which invagination is more advanced.

Fig. 6.—Sagittal section of gastrula in which invagination is still more advanced.

Fig. 7.—Sagittal section of gastrula in which invagination is well advanced; the first trace of the flattening which marks the dorsal side is visible.

Fig. 8.—Sagittal section of gastrula in which invagination is very advanced.

Fig. 9.—Sagittal section of gastrula in which blastopore is becoming narrowed.

Fig. 10.—Sagittal section of completed gastrula. The blastopore has been shifted to the dorsal side (in consequence of a slight obliquity a piece of the medullary fold is included in the section).

Fig. 11.—Transverse section of embryo about the age of that in Fig. 10 (7—8 hours from fertilisation).

Fig. 12.—Transverse section of embryo of about ten hours; first trace of neural plate and coelomic groove.
THE EARLY DEVELOPMENT OF AMPHIOXUS.

Fig. 13.—Transverse section of embryo of about ten hours; shows first trace of collar cavity distinct from coelomic groove.

Fig. 14, a, b, and c.—Three sections from a series through an embryo of from ten to twelve hours in age.
   a. Shows the coelomic groove.
   b. Shows the coelomic groove closed off in front so as to form the coelom.
   c. Shows the independent opening of the collar cavities.

Fig. 15, a and b.—Two sections from a series cut parallel to the sagittal longitudinal plane through an embryo of twelve to thirteen hours.
   a. Shows the coelomic groove and collar cavity opening into the archenteron.
   b. Shows neurenteric canal and division of trunk coelom into somites.

Fig. 16.—Transverse section of embryo of about thirteen hours, showing the left collar cavity only, opening into the archenteron.

PLATE 44.

(All the figures on this plate are drawn under the magnification of a Zeiss D, oc. 2.)

Fig. 17, a, b, c, d, and e.—Five sections from a series through an embryo of fourteen to fifteen hours.
   a. Shows head cavities opening into gut and anterior pore of the nervous system.
   b. Shows head cavities constricted off from gut.
   c. Shows downward extension of right collar cavity and persistent communication of the left with the gut (Neph.) (Hatschek's nephridium).
   d. Shows the last somite, which is still continuous with the coelomic groove.
   e. Shows the coelomic groove.

Fig. 18, a, b.—Two sections from a series through an embryo of about twenty hours.
   a. Shows the right and left head cavities becoming differentiated the one from the other, the collar cavities having shifted forward.
   b. Shows that further back the two head cavities still communicate with one another. The open neuropore is still seen.

Fig. 19, a, b, and c.—Three sections from a series through an embryo of about twenty-four hours.
   a. Shows complete separation of right and left head cavities and the ventral shift of the former.
   b. Shows inequality of the two collar cavities; the left retains a narrow communication with the gut (Neph.).
c. Shows backward continuation of the ventral part of the collar cavity 
\(V.\, Coll\). \(V.\, Tr\). A solid wedge of tissue which represents the trunk coelom formed by the fusion of the ventral ends of the somites.

FIG. 20, \(a\), \(b\), and \(c\).—Three sections from a series through an embryo of the same age as that figured in Fig. 19, showing the dying out of the ventral extension of the collar cavity \(V.\, Coll\).

FIG. 21, \(a\), \(b\), and \(c\).—Three sections from a series through a pelagic larva showing relations of the fully developed nephridium of Hatschek.

\(a\). Shows the opening into the cavity of the first muscular somite (left collar cavity).

\(b\). Shows appearance of a section across the middle of the structure.

\(c\). Shows the opening into the pharynx.

PLATE 45.

FIG. 22, \(a\), \(b\), and \(c\).—The ventral portions of three sections through an old pelagic larva, showing the formation of the atrial fold and the continuation of the ventral part of the collar cavity into it. \(gl\). Club-shaped gland.

FIG. 23, \(a\) and \(b\).—The ventral portions of two sections through a still older pelagic larva, showing a further stage in the development of the atrial folds.

FIG. 24.—Ventral portion of a section through a young adult Amphioxus, showing the cavities of the metapleural folds (the same as the collar cavities), and the schizocoelic artefacts which appear to the outer side of them.

FIG. 25.—Diagram showing the origin of the various portions of the coelom from the gut in Amphioxus.

FIG. 26.—Similar diagram to show the origin of the coelom in Balanoglossus.

FIG. 27.—Diagrammatic lateral view of an Amphioxus larva, showing mutual relationships of the head cavities, collar cavities, and trunk coelom.

FIG. 28.—Similar diagram of Balanoglossus.

(Fig. 22, \(a\), \(b\), and \(c\) are drawn with Zeiss F, oc. 2; Figs. 23 and 24 with Zeiss D, oc. 2).

N.B.—In Figs. 17 to 20 the hypoblast and mesoblast are coloured red, and the epiblast grey.

On the contrary, in Figs. 21 to 24 the epiblast is coloured red, the mesoblast blue, and the hypoblast, including the notochord, grey.