**Zoology**. — "*On the Anatomy of the Larva of Amphioxus lanceolatus and the Explanation of its Asymmetry*". By Prof. J. W. VAN WIJHE.

(Communicated in the meeting of November 30, 1918).

For many years I have attempted to complete a part of the many lacunae in our knowledge of the morphology of Amphioxus, because this morphology is in many respects the basis for the comparative anatomy and embryology of the Vertebrates.

After the publication in 1914 in the Transactions of this Academy of my work on the changes of the larva of Amphioxus during the metamorphosis, during which growth ceases, I have managed to obtain sufficient material to investigate the larva during the growth-period. Now that the research and the necessary drawings have been completed — the text must still be written — I shall here discuss some results.

Many years ago (1893) I found that the mouth of Amphioxus was only apparently placed more or less symmetrically, that in fact however it lies exclusively on the left side as the nerves and muscles of the buccal cavity without exception belong to the left side of the body, which was later on confirmed by others. This fact is in accordance with the earlier discovery of KOWALEVSKY (1867) that the buccal opening in the larva of Amphioxus is situated, not mesially, but on the left side of the fore-end of the body.

Through its mouth, which is a mesial organ in all the other Metazoa, Amphioxus and undoubtedly also the related genus Asymmetron[1]) stand isolated as remarkable in the whole animal kingdom.

It has been repeatedly attempted to declare the mouth of Amphioxus for a mesial organ by accepting that it has removed to the left side. Its occurrence in the larva on that side had then to be taken up as an abbreviation of development. This removal would then be analogous to the phenomenon observed in the eye of the Pleuronectidae. The young larva of the flat-fishes has an eye on each side

[1]) TATTERSALL (Notes on the Classification and geographical Distribution of the Cephalochorda. Proceedings and Transactions of the Liverpool Biological Society, Vol. 17, 1903) has shown that in the Homomeria (Acrania) we can distinguish only these two genera: Amphioxus (Branchiostoma) and Asymmetron.

and swims like all other fishes with its back up, the side-planes turned to right and left.

Later on however the animal swims with one side-plane turned up and the other down. The eye of the white under-surface then removes to the pigmented upper-surface, where the other eye is already situated.

An analogous explanation of the situation of the mouth in Amphioxus is impossible, because it becomes clear through the nerves and muscles of the displaced eye in the Pleuronectidae that this belongs to the under- and not to the upper-surface, on which it has come to lie.

The nerves and muscles on the inside of the mouth of Amphioxus, which all come from the left side, prove on the contrary, that it cannot be considered as a mesial organ, but in fact belongs to the left side.

While the mouth is formed on the left side in the young larva, a glandular metamorphosed gill-pouch, known as the club-shaped gland, arises opposite to it on the right side.

Its discoverer, Hatschek (1881), knew the external opening of this gland, but not yet the intestinal opening. Its presence was established by later investigators.

It was now clear that the mouth of the larva of Amphioxus could be considered as a metamorphosed gill-pouch, and as an antimere of the club-shaped gland.

The question however arises with which organ of the higher animals the left-sided mouth of the Amphioxus larva is homologous. Is it the homologue of the left half of the mouth of the Craniota, that would then have to be considered as a product of fusion of the mouth of Amphioxus with the club-shaped gland; or is the mouth of the Amphioxus larva the homologue of the first left gill slit of the Craniota, which is known in the Selachii as the left spiracle?

Various grounds led to the conclusion that the latter must be the case. To my mind this was proved when it was found that the body-cavity of the lower jaw, the mandibular cavity, does not in Amphioxus lie *behind* the larval mouth, as is the case in higher animals, but *in front* of it.

Amphioxus, indeed has no jaws, upper- as little as lower jaw, but still it has a mandibular cavity that indicates the position of the lower jaw. It is self-evident that an opening, arising behind the place of the lower jaw, cannot be considered a homologue of a part of the mouth of the Craniota.

Such a primitive organ as the mesial buccal opening of the Vertebrates must however originally have been present in Amphioxus. In my opinion the opening of HATSCHEK's groove, which is a product separated off from the fore-end of the intestine, must be considered as the primitive mouth of Amphioxus.

However this may be, the original mouth of Amphioxus must have been, just as in the higher animals, a symmetrical organ situated in the mesial plane, rostrally to the spiracle.

Why has the original mouth in Amphioxus been lost and been replaced by the left spiracle?

The manner in which the larvae that have just left the egg membranes move, can show us the way to come to an answer of this question. These larvae move forward spirally, by means of cilia, turning round the longitudinal axis from right to left. If we suppose that ancestors of Amphioxus possessed this form of motion permanently, then the left spiracle occurred in a more advantageous position to take in the sea-water necessary for nutrition and respiration, than did the mesially situated primitive buccal opening. This form of motion also makes the remarkable occurrence of the branchial apertures comprehensible. If we neglect the foremost pair, which is metamorphosed to mouth and club-shaped gland, the gill clefts do not occur in successive pairs as in all the Craniota. On the contrary, the apertures on the right side are altogether wanting in the larva during the period of growth; they do not appear until during the metamorphosis. During the period of larval growth the clefts on the left side alone appear. They make their appearance successively to a total of 14 or 15, apparently however not on the left side but in the *topographical* mesial plane of the pharynx. Shortly after appearing each of these clefts is removed to the topographical right side.

At first sight this phenomenon seems very extraordinary, but it becomes more comprehensible when we consider the location of the truncus arteriosus, which indicates in Amphioxus, as in the higher animals, the morphological mesial plane of the pharynx.

In the older larva of Amphioxus the truncus arteriosus does not run in the plane of symmetry under along the branchial intestine, but high dorsally along its right side.

The morphological right side has remained behind in development as a narrow dorsal strip in consequence of the excessive growth in breadth of the left side, which has hereby occupied the ventral territory of the right side.

Although the first 14 or 15 branchial apertures of the larva now lie on the *topographical* right side, they *morphologically* still belong to the left side. It is first in the following period, that of metamorphosis, that the right side of the pharynx also grows in breadth and gradually pushes the left side, with the gill-clefts back to the side where it belongs.

The right gill-clefts now arise for the first time, having earlier been hindered in their appearance by the crowding of the clefts of the left side.

With a screw-like motion turning from right to left such an occurrence of the gill-clefts is no longer odd. The secondary mouth of the larva takes up water that flows away through the gill-clefts by means of ciliary motion. The gill-clefts of the left side had now to remove[1]) to the topographical mesial line or better still to the topographical right side to prevent their taking in water.

In my paper of 1914 I mentioned already that the form of motion of the young larva can explain not only the origin of the mouth on the left side, but also the odd occurrence of the gill-slits. It was also mentioned that such a form of motion moreover makes it comprehensible that the organ of hearing (organ of equilibrium) could be lost, because the axis of a rotating object if the velocity be sufficient, is stable; while it cannot be expected in such a form of motion that the eye would be developed to a complicated organ, forming images.

At that time the criticism of ADAM SEDGWICK on my explanation of the asymmetric location of the mouth, in the third part of his text-book, published in 1909, was unknown to me. It is hidden in the chapter on the Echinodermata, in which the mouth originates symmetrically in the ventral median line, to remove later on to the left side.

Admitting that this case of secondary asymmetry differs from the phenomenon in Amphioxus, where the mouth is developed primarily asymmetrically SEDGWICK says that Amphioxus and the Echinodermata still have the left-sided location of the mouth in common. He continues (l.c. p. 162) "Here again we have a character which strikes us from its very rarity, for it is found in no other Coelomate nor so far as we know in any other member of the animal kingdom. It also strikes us by its strangeness and inexplicableness. In Amphioxus no serious attempt has been made to explain it".

My explanation was thus according to SEDGWICK *no serious attempt.*

---

[1]) The removal of a mesially situated organ to the left side is not without analogy; it occurs e.g. in the heart and stomach of man. As the nerves of these organs arise from both halves of the body it is also clear, without knowing their development, that they must have been situated mesially.

He also gives his reasons: "for no one, so far as we know, has ever attempted to bring the extraordinary features in the development of the gill clefts, of the endostyle, of the head-cavities, the asymmetric position of the anus and olfactory pit, into relation with the asymmetry of the mouth. The thing cannot be done. There is no sort of connexion between these various asymmetries. They seem to occur without rhyme or reason".

Of all these arguments there is only one that seems to be consistent viz. that concerning the anus. We shall consider them consecutively.

An explanation for the removal of the gill-clefts to the right side has just been discussed. The thyroid gland, indicated by SEDGWICK by the antiquated name of endostyle, is a mesial organ in all the Chordata. In Amphioxus it must also originate in the morphologically ventral median line of the gut. We saw that this line has been shifted to the right side in the branchial region of the larva. In the larva with three open gill-clefts (LANKESTER and WILLEY, 1890, fig. 1) it runs over the topographically dorsal (but morphologically ventral) edge of the gill-clefts. If we continue it slightly rostrally it cuts the angle in which the two portions (the later right and left halves) of the thyroid gland meet. In the larva the thyroid gland thus lies topographically asymmetrically; morphologically however symmetrically.

The symmetry is in so far imperfect in that the lower portion (the later left half) is longer and reaches considerably further rostrally than the upper portion (the later right half). We shall make use of this difference later on in refuting another argument of SEDGWICK.

That the morphological median line of the gut was shifted to the right side also anteriorly to the gill-clefts viz. in the neighbourhood of the mouth can easily be explained by the considerable enlargement of the mouth, which takes place not only in the longitudinal, but also in the dorso-ventral direction.

In the latter direction its lower border all but reaches the topographically ventral median line, which it even passes during the metamorphosis, so that this typically left-sided organ is partly found, not *temporally* like the first gill-clefts, but *permanently* on the right side of the animal.

The thyroid gland cannot take its permanent place at the ventral border of the gut until during the metamorphosis, after the gigantic larval mouth has been reduced, temporarily even nearly to nil. Then the difference in length between its left and right halves has also disappeared.

SEDGWICK's third argument are the extraordinary phenomena in the development of the "head-cavities" i e. of the portions of the coelom

66*

in the region of the head. The term "head-cavities" was introduced by BALFOUR, who discovered them in the development of the Selachii. In the branchial region of Amphioxus the coelom is divided by the gill-clefts into as many "head-cavities" as there are clefts, and the asymmetry of these cavities necessarily coincides with that of the gill-clefts. Here there is no new oddness to be explained.

But SEDGWICK (textbook part II, 1905, p. 34) wrongly understands under "head-cavities" only the foremost entoderm sacs of HATSCHEK, which are placed before the mouth, as it seems, as each others' antimeres. Soon however they take up a mesial position, whereby the right sac, a true head-cavity, is shifted before the left. In its further development the left sac has not a single character from which it could be deduced that it should be considered as a section of the coelom. Since 1893 — the last time more in extenso in 1914 (l.c. p. 63) — I have defended the opinion that the two sacs are only *apparent* antimeres, that they originally must have lain not opposite to, but before each other, and that the apparent antimeric occurrence must be the result of the asymmetry of the larva. In this opinion I however remained alone. The later investigators attached so much importance to the first appearance — certainly an argument of great weight — that they did not consider the important differences which soon arise. Undoubtedly the symmetrical situation of the foremost myotome (which must be considered as the second of the row; the first is included in the right entoderm sac) played a role in this. Yet in the rest — with exception of the third of the row — each myotome of the left side reaches, since its first appearance, half the length of a myotome more rostrally than its antimere of the opposite side.

As this wry symmetry is found only in the region behind the mouth, in front of it however not, it was apparently taken for granted that the true gut in the snout would indeed be properly symmetrical. This is however not the case.

When the first gill sacs of the right side make their appearance during the period of metamorphosis, the foremost of these does not lie opposite its antimere, but opposite the second gill cleft of the left side (cf. my paper 1914, fig. 3, 4, 5). In the fore-end of the branchial region the left side of the gut has been displaced rostrally over the whole length of a branchial metamere. [1])

---

[1]) In the adult animal, (and also in the more caudally situated clefts of the larva) this removal is not so large. The foremost half of a left cleft then, as is known, does not lie the whole, but half the length of a cleft rostrally to its antimere of the right side.

This removal also reaches to the part of the gut situated before the buccal aperture, as is evident from the above-mentioned (p. 1017) difference in length of the two portions of the thyroid gland, whose lower (later left) portion reaches much further forward than its upper (later right) portion (cf. my paper 1914, fig. 1, 2, 3 out of the metamorphosis and LANKESTER and WILLEY 1890, fig. 1 of a young larva with 3 open gill clefts).

As the entodermsacs of HATSCHEK now arise opposite each other just in front of the region of the thyroid gland the conclusion is obvious that their antimery is only apparent, even if we pay attention to their first appearance only, without considering their subsequent fate: The left sac is removed forwards and ought morphologically to lie behind the right sac, as is indeed the case later on.

The last arguments of SEDGWICK are the asymmetrical situation of the anus and the olfactory pit.

The removal of the olfactory pit, which develops out of the mesially situated neuroporus, to the left side is easily explained by the spiral motion with rotation of the axis from right to left: The olfactory pit had to catch up water and therefore to remove to the left side.

The asymmetrical situation of the anus could however not be explained so easily. Not only does it lie on the left side in the adult animal, but according to HATSCHEK (1881) it is already situated on that side in the young larva with one gill cleft.

In the same way as the gill clefts serve for letting out the water taken up by the mouth of the animal, the anus serves for letting out the undigested food. The respiratory water as well as the faeces are propelled not so much by muscular contractions as indeed principally if not exclusively by ciliary motion, thus by weak forces.

To the same extent as it was profitable for the gill clefts to remove to the right side one would also expect this in the anus. It would have to be considered as a postulate of the theory that the anus either originated in the mesial plane, to remove later on, not to the left, but to the right side, or that it developed directly on the right side.

In the hope of being able to discover something in young larvae that would throw some light on the question, I found beyond expectation, that the anus in larvae with one gill cleft is situated not on the left but on the right side of the body.

This was clear beyond the slightest doubt in series of cross sections. In preparations in toto it was however impossible, even with the strongest dry lenses, to determine in such a young larva on which side of the body the extremely fine anal aperture was situated, because it could only be observed in profile, thus by focussing the microsope alternatively.

After however using strong oil-immersion systems (e.g. Zeiss obj. $^1/_{12}$ oc. 4) the situation of the anus on the right side of the body could clearly be seen also in these preparations.

When the anus has just made its appearance the tail-fin does not yet reach rostrally to it, but this is soon the case, even when there is only yet one gill cleft present. The anal aperture then lies on the right side of the tail-fin. Here it is also found in larvae with 2, 3, 4, 5 or 6 gill clefts.

Now however the remarkable phenomenon presents itself that it removes to the left side. In larvae with 7 gill clefts a lacuna arises in the tail-fin at the level of the anus, so that the tail-fin is interrupted at this place. The anus now enters this lacuna and thereby assumes a mesial position in relation to the body. This position is however of short duration. With the presence of 9 gill clefts the anus has already passed over to the left side, where it is further permanently found. The lacuna in the tail-fin vanishes and leaves no traces of its former existence.

I cannot give a reason for this removal. It must stand in connection to the manner of life, of which we know little or nothing. This does not however affect the principal question, the original situation on the right side, as it was postulated by the theory.

If the theory is not accepted then there is here especially occasion for speaking of a development "without rhyme or reason".

In the theory there is in any case a "reason" for the initial situation of the anus even if it cannot "rhyme" its permanent position with this fact.

The question whether a pancreatic gland occurs in Amphioxus does not stand in relation to the asymmetry discussed above.

This gland is known in all the Craniota, from the Cyclostomata to man, but it is the common opinion that it is not present in Amphioxus.

It arises in the Craniota as one or more bulbs protruded out of the gut epithelium in the immediate neighbourhood of the aperture of the ductus choledochus, which as a rule later on also forms an opening of the pancreas.

In the Lampreys, which in connection with Amphioxus must be considered in the first instance, the gland is not very voluminous. This must partly be ascribed to the fact that their pancreas, like the liver, officiates as an organ with internal secretion. The excretory ducts of these glands, as well as the gall-bladder, have disappeared after the metamorphosis, but in the Ammocoetes, the larva of the Lampreys, they are present.

The pancreatic gland of Ammocoetes lies hidden in the wall of the gut on the left side of the excretory duct of the liver.

At a quite analogous place I found the gland in Amphioxus from the first appearance of the liver in the period of the metamorphosis until the adult form. With good nuclear staining it can be seen, in preparations *in toto* of the newly metamorphosed larva, as a triangular stain on the left side of the wall of the gut immediately behind the place where the blind sac of the liver emerges. The rounded top of the triangle points forwards, the base is a transverse line, crossing the longitudinal axis of the animal at right angles. With a strong magnification it can be seen, more clearly still in cross section, that we have to do with a slight emergence of the wall of the gut.

In the sections we see that the gland possesses a strong ciliary epithelium, but it has moreover cells without ciliae which may be considered as the true glandular cells. In the adult animal the gland is stretched more longitudinally, and consequently the emergence of the gut wall is a longitudinal fold; its fore-end has been taken up in the hind-end of the blind liver sac. We find an analogous phenomenon in some fishes, where the pancreas is partly enveloped by the liver.

The reason why the numerous investigators of the anatomy of Amphioxus could not find a pancreas is easily given. The mid-gut epithelium presents in cross section so many folds [1]) that it cannot be expected that one could distinguish these from the pancreatic fold unless one's attention has been drawn to this in young animals.

In the ontogenesis of the Craniota the liver (as an emergence of the gut) is first recognisable; somewhat later also the pancreatic gland. In Amphioxus this is just reversed. While the liver first makes its appearance as an emergence of the gut in the period of metamorphosis, the pancreatic gland can be followed back to the stage with two open gill clefts (in larvae with only one gill cleft it could not be seen). It is originally not limited to the left side, as is later the case, but envelops the gut like a ring. The ring is recognisable in that its nuclei are smaller and placed closer together

[1]) These folds are also found in the oesophagus, (whose hind-end corresponds morphologically to the stomach of the higher animals), and in the foremost portion of the end-gut. They serve for enlarging the resorbant surface. In young animals they are not present.

than is the case before or behind the gland. The ring however is not regular, but its left side is developed more strongly than the right. Soon however it disappears on the right side and through this the gland becomes asymmetrical.

As this asymmetry is also found in higher forms e.g. Ammocoetes, it must be independent of the above discussed asymmetry of the branchial gut.

Finally I should like to point out that another gut-ring the ilio-colon ring, in the larva with one gill cleft is of importance for the morphology of the alimentary canal in the Vertebrates. In Amphioxus this ring indicates the boundary between mid- and end-gut.

Physiologists have already long known that the nervus vagus in the higher animals and man helps to supply not only the fore- or "head"-gut, but also the whole mid-gut or "small intestine". Anatomists as a rule ascribed to the n. vagus the region of the fore-gut only, because the foremost of the two strands which form the continuation of the vagus plexus around the oesophagus, ends on the foremost wall of the stomach, while the hindmost strand is connected with the plexus solaris of the sympathetic nerve.

On account of this connection it was impossible to follow with certainty the ramifications of the vagus further distally than the stomach. Our countryman DONKER [1]) has however lately succeeded in doing this in apes. He could establish the fact that the ramification of the vagus reaches to the end of the mid-gut and that it does not extend to the "large intestine" (end-gut in a broad sense). In the section of his text-book which appeared this year MERKEL also lets the ramification of the vagus in man reach to the end of the mid-gut.

It is not surprising that the vagus, the tenth cranial nerve, supplies the fore-gut as it is a well known fact that the fore-gut is originally limited to the head region. The question now arises whether the mid-gut was perhaps originally situated in the head region also. To a certain extent the development of Amphioxus can give us an answer to this.

The ilio-colon ring, which forms the boundary between mid-and end-gut lies more rostrally the younger the larva is. The larva of LANKESTER and WILLEY with 14 gill clefts already has 61 myotomes, just as many as the adult animal. The ilio-colon ring lies under the 34$^{th}$, 35$^{th}$ and 36$^{th}$ myotomes. In their larva with 3 gill clefts and only 36 myotomes the ring lies under the 15$^{th}$ and 16$^{th}$ myotomes.

[1]) P. DONKER, Ueber die Beteiligung des N. vagus an der Innervation des Darmes. Anat. Anzeiger, Bd. 51, No. 8, 1918.

In the Selachii and, indeed, generally in the Craniota the first 9 myotomes belong to the head. It would now be interesting to know under which myotome the ilio-colon ring of Amphioxus is situated at its first appearance. This appearance takes place in the stage with only one gill cleft and an open anus.

Although in the course of time I have made hundreds of preparations of this stage, stained and imbedded in all sorts of ways, I have not been able to count the number of myotomes. Their boundaries, which are clearly discernible in earlier and later stages, were not visible in this stage, not even in series of sections [1]).

It is possible that these boundaries are to be seen in living larvae. In any case HATSCHEK (1881, fig. 64) indicates in a sketch of such a larva that there are 20 myotomes present; the ilio-colon ring has however escaped his notice. If now the place of this ring, which is very clear in my preparations, is compared with the sketch of HATSCHEK, one comes to the conclusion that it must be situated approximately under the 9th myotome, thus at the end of the head region.

In other words: Not only the fore-gut (prosenteron) but also the mid-gut (mesenteron) originally lies in the head region, and if this is also the case in the Craniota, as may be expected, then it is no wonder that also the mid-gut is supplied by a cranial nerve, the n. vagus. The conception "head-gut" ought then not, as is at present the case, to be synonymous to fore-gut, but must include the mid-gut also.

[1]) One could speak here of a cryptometameric stage, a characteristic — amongst others — that these larvae have in common with the Appendicularia and the larvae of the Ascidians, in which the metamery is at present still denied by various investigators.