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Anatomy. -- "*On the Metamorphosis of Amphioxus lanceolatus*".
By Prof. J. W. VAN WIJHE.

(Communicated in the meeting of April 25, 1913).

Amphioxus still continues to be one of the most interesting objects for the morphology of vertebrates, though the time is past in which he was almost considered as their ancestor. It is now pretty well generally admitted, that Amphioxus is not the grandfather of vertebrates. It has appeared that his organisation deviates so strongly from what must be regarded as the original type, that some morphologists do not take him for a genuine grand-father, but for a step-grand-father, who, in reality, does not belong at all to the family, and only confuses its relations.

There have been morphologists, and perhaps there are still some, whose theories appeared to be so much at variance with the organisation of Amphioxus, that they have proposed to strike him out from the group to which vertebrates belong, and if they had been able would willingly have brought him back to the group of snails, to which PALLAS in his time supposed him to belong, and for that reason gave him the name of *Limax lanceolatus*.

Though these investigators could not deny, that Amphioxus is affined to vertebrates, in order to save their theories, they were obliged to declare, that this relation is such a distant one, that it is certainly not necessary to make allowance for his organisation.

When however this organisation, both anatomically and embryologically¹⁾, became better known, it appeared more and more that Amphioxus shows indeed in many respects a very primitive organisation, which must be taken as point of issue for that of the higher vertebrates, whilst it presents, in other respects, such peculiar phenomena, that these must doubtlessly be regarded as deviations from types, that are represented among vertebrates.

I shall by-and-by discuss one of the most remarkable deviations. It is the placing of the mouth and the gill-slits in the larva before the metamorphosis.

¹⁾ How slowly our knowledge in this respect increases may appear from the fact, that the celebrated morphologist BALFOUR was in 1882 still of opinion that Amphioxus should possess no ventral nerve-roots, whilst, with regard to the dorsal nerve-roots, one is still searching where the cells lie, which, in vertebrates, form the spinal ganglia. I have discovered under the atrial-epithelium that covers the liver, the intestine and gut an enormously large number of splendid multipolar ganglion-cells, whose axis-cylinder runs along the dorsal nerve-roots to the spinal cord.

After the expiration of the embryonal period one distinguishes three stages in the development of *Amphioxus*: 1st the stage of the larval growth; 2nd the stage of the metamorphosis; 3rd the stage of the postlarval growth.

At the beginning of the first stage the larva is 1 m.m. long; at the end of it it has reached a length of between 4 and 5 m.m. At the beginning of this period only the first gill-slit is formed, behind it are gradually developed a second, a third etc. till a number of 14 to 16 is reached. All these gill-slits belong morphologically to the left side of the body; those of the right side appear only in the period of the metamorphosis. During the stage of the larval growth constantly new muscle-segments (myotomes) are added to the posterior part of the body, but at the beginning of the metamorphosis this number is already complete. The animal is then only 4 to 5 m.m. long, but it possesses already the complete number of 60 muscle-segments with the nerves appertaining to them, which are also found in the full-grown animal which is almost a finger long.

During the comparatively long time of the metamorphosis, which is divided by WILLEY into 8 subdivisions, astonishing changes take place, not so much in the nervous or muscular system (with the exception of the gill-muscles), but especially in the shape and the placing of the mouth and of the gill-slits.

The animal does not grow during the metamorphosis, for its length amounts, both at the beginning and at the end, to between 4 and 5 m.m.¹⁾ Differences in length do not indicate here a further development. A larva that is half a millimeter longer than another needs not be older than the latter, but is often younger. It has even appeared to me that, during the first half of the metamorphosis, the length rather decreases somewhat than increases, but the individual differences are too numerous to state this phenomenon as certain. I am of opinion, that the fact that the larva does not grow during the metamorphosis, must be attributed to its not taking food during this period. Presently I shall revert to the grounds of this.

Before the metamorphosis both the mouth and the gill-slits lie perfectly asymmetrically; the mouth does not lie medianly and ventrally as with all vertebrates, but on the left-side of the body, and of the gill-slits is only the row of the later left-side extant. They behave very curiously, for they do not originate on the left-side, but apparently in the median plane, whilst the foremost of the

¹⁾ Larvas from the neighbourhood of Messina are during the metamorphosis still smaller. According to the statements of the authors their length amounts to an average only to 3½ mm.

row soon remove temporarily to the right-side of the larva. They open freely outward and not in a gill-cavity or atrium, which is only formed during the metamorphosis by the fusion of a longitudinal fold, which has formed itself, during the larval growth period, on the left-side of the body over the gill-slits with a similar fold, which has developed itself on the right-side of the body.

During the metamorphosis appears likewise the right row of the gill-slits — 8, rarely 7 or 9, in number — which do not open directly outward but in the gill-cavity. The slits of the left side, which had temporarily removed to the right side, return now to the side to which they belong.

I can confirm WILLEY's observation, that the first left gill-slit aborts, and that also the 10th to the 16th disappear during the metamorphosis. At the end of the metamorphosis the young animal is then symmetrical with regard to the gill-slits, and there are 8 of them on the left-side corresponding with the eight on the right-side. It is of secondary importance that the symmetry is somewhat oblique; every left gill-slit does not lie exactly directly opposite the right one, but half the width of the slit more rostral. A similar oblique symmetry is likewise shown by the nerves and muscles of the body of the left-side compared with those of the right-side of the animal.

With the exception of the foremost slit, which remains undivided, as long as the animal lives, each slit is divided into two parts, in a longitudinal direction, by a clasp or "tongue" growing from the dorsal rim, till it reaches the ventral rim with which it fuses.

Directly after the metamorphosis the animal possesses thus, both on the right and on the left side, a row of 8 (rarely 7 or 9) gill-slits. During the rapid growth that follows now, this number regularly increases during the whole life-time of the animal, because constantly a new pair of slits develop themselves at the hindmost part of the gill-basket.

But after the metamorphosis the mouth¹⁾ seems to be a symmetrical organ; it is no longer situated distinctly on the left-side of the body as in the larval growth-period, but more ventrally and almost halved by the median plane as with all vertebrates.

The symmetrical placing of the *gill-slits* is real; since 1893 I have demonstrated however that the symmetrical placing of the *mouth* of *Amphioxus* is only so in appearance. In reality the mouth, also of the full-grown *Amphioxus*, is an organ of the left-side; for its inner parietes are exclusively provided for by nerves of the left-side, and

¹⁾ Not to be mistaken for the mouth of the larva, see the conclusion of this article.

its muscles belong all to those of the left-side. No nerve and no muscle of the right-side takes part in the provision of the mouth-cavity.

Here we are in the presence of a remarkable phenomenon: The mouth of *Amphioxus*, as organ of the left-side, cannot be homologous with the unpaired mouth of vertebrates always developing symmetrically, and we must surmise that on the right-side of the young larva a similar organ as the mouth, a counterpart of it, is found. This organ is, as I indicated a long time ago already, the so-called club-shaped gland and the mouth together with this gland form morphologically the first pair of gill-slits of the *Amphioxus*-larva.

Instead of gill-slits, it is more correct to speak of gill-pouches, for in all vertebrates, without any exception, a gill-slit is formed, because a pouch-shaped projecting part of the gut reaches the epidermis, fuses with it on that spot, and afterwards splits to the outside. With *Amphioxus* this is exactly the same; here also is every gill-slit formed as a pouch-shaped projecting part of the gut, and splits afterwards — before the metamorphosis — to the outside, after the beginning of the metamorphosis, towards the gill-cavity (the atrium). The epithelium of a gill-pouch can partly differentiate to glandular-epithelium, in this way e. g. in all vertebrates the thymus if formed, a glandulous organ, from the epithelium of some gill-pouches.

The club-shaped gland possesses all the essential distinctive properties of a gill-pouch; it is formed on the right-side of the body as a projecting part of the gut, which opens afterwards to the outside and possesses then two openings one inside in the pharynx, the other to the outside.

Though the greater part of its epithelium has differentiated into glandular epithelium, I found however laterally from it a ring-shaped strip of ciliated epithelium, corresponding entirely to that of the other gill-pouches.

The outside opening of the club-shaped gland is in the beginning placed near to or in the median plane; afterwards it removes in front of the mouth to the left-side of the body. This is again one of the remarkable phenomena of asymmetry in the larva of *Amphioxus*, the explanation of which I intend to give in my detailed paper ¹⁾, as it would lead me too far here. The club-shaped gland disappears in the course of the metamorphosis, and does not leave any vestige behind.

Has the mouth of the *Amphioxus*-larva originally also been a gill-slit? In my opinion there is no doubt about it. It is true that

¹⁾ This paper was offered last winter to be published in the transactions of the Academy.

it does not originate as a pouch-shaped projecting part, but this is impossible, because in the place where the mouth of the young larva will open, the pharynx lies already directly against the epidermis. Neither is a ciliated gill-epithelium formed in this place, but one has no right to expect it here, because the function of a mouth is so entirely different from that of a gill-slit. On the other hand the mouth possesses another lasting distinctive property, which is peculiar to every gill-slit during the period of growth, but disappears from these slits in the course of the metamorphosis. I found namely, that each gill-slit of the young larva is accompanied in front and behind by a strong gill-muscle, the fibres of which, for the greater part, run in a transversal direction with regard to the axis of the body. Some fibres however, surround the outside gill-opening and form a sphincter round it.

The mouth-opening is likewise enclosed by two such muscles. They degenerate also, but they are not lost without leaving a trace, as the gill-muscles proper, but produce the lip-muscles and the ring-shaped sphincter of the velum.

Mouth and club-shaped gland are counterparts, for they originate one under the second myotome of the left-side, the other under the second myotome of the right-side of the body.

In vertebrates the first pair of gill-slits originates nearly under the second myotome, of which in the head of Selachians nine are formed, as I demonstrated more than 30 years ago for the genera *Scyllium* and *Pristurius* ¹⁾. In Selachians the first gill-slit does not any longer function as such either; in rays it serves to admit instead of to let out the respiration-water, and in some sharks this slit, known by the name of spiracle, is shut by the fusion of its parietes. The mouth of *Amphioxus* is, according to what has just been seen, homologous with the left spiracle of Selachians, and serves, just as in rays, to ingest the respiration-water, but this water contains here the necessary nutriment for the animal.

If now the mouth of the *Amphioxus*-larva was originally the first gill-slit, then a primitive mouth, homologous with that of vertebrates, must have been extant before this secondary mouth.

This primitive mouth is, in my opinion, represented by the opening

¹⁾ BRAUS pretends that not 9 but at least 11 should be formed. A repeated investigation, which will be published afterwards, has taught me that my number 9 for *Scyllium* and *Pristurius* is correct, and may be admitted as the normal one for Selachians. In some genera however vertebrate elements fuse secondarily with the skull.

of the so-called praeoral pit, which is formed as a sejoined part of the pharynx and soon opens to the outside.

In accordance with this view is the place where the thyroid gland of *Amphioxus* originates. In all vertebrates this gland is formed as a median outgrowth of the epithelium of the pharynx immediately behind the mouth, between the first pair of gill-slits, if they are taken as fused with their ventral extremities. If now we see in the mouth of *Amphioxus* the homologue of that of vertebrates, then, in an incomprehensible way, the thyroid gland of *Amphioxus* would be formed in front of the mouth instead of behind it.

What explanation can now be given of the fact, that *Amphioxus* has lost its primitive mouth and has obtained secondarily the first gill-slit as mouth, whilst in the stage of the larval growth — now letting alone the club-shaped gland — not the gill-slits of the right-side, but only those of the left-side open to the exterior and moreover in the median plane, whilst they even partly remove temporarily to the right-side?

The key to this explanation is, in my opinion, to be found in the movement of the young embryo which has been observed by HATSCHEK. This embryo moves, turning on its longitudinal axis, helicoidally forward; the rotation takes place from right to left.

If now one admits that ancestors of *Amphioxus* have moved forward in this way, to which they may have been induced, because they missed an auditive or equilibrium-organ, the trace of which does not even appear in *Amphioxus*, then it is to be understood that the left first gill-slit must have had the predominance over the medianly placed primitive mouth as opening for the admittance of water, which must serve at the same time both for respiration and for nutrition. The following gill-slits had to evacuate the respiration-water, but this evacuation was for the slits on the left-side impeded by the way of moving of the animal. On account of the rotation from right to left, the following gill-slits on the left-side would be inclined to ingest water instead of evacuating it, and therefore they were obliged to remove from this side to the median plane, or still better to the right-side, where the evacuation of the respiration-water was exactly facilitated in consequence of the movement. By this removal, however, came the original gill-slits of the right-side in a tight place; they remained little, and this is the reason why in the *Amphioxus*-larva, they appear only in the period of the metamorphosis.

When later ancestors of *Amphioxus* gave up their swimming way of living and buried themselves into the sand, as he does still now,

to dash forward from it with the quickness of an arrow, when he is disturbed, and then to return immediately again into the sand, the reason for asymmetry did not exist any longer, the gill-basket became symmetrical again, and the mouth also tried to assume a symmetrical position, though it could only apparently succeed in it, as it is an organ of the left-side.

It is not to be wondered at, that there are investigators who oppose these views, because they cannot admit that such an ancestral organ, as the primitive mouth, should have had to give way to a secondary mouth. They admit, that ancestors of *Amphioxus*, which gave up the swimming way of living, have first passed through a period, in which they lay on the sand in the way of flat-fishes instead of burying themselves into it; that then the mouth has removed to the left side, just as, with flat-fishes, one eye, which otherwise would be directed downward to the bottom of the sea, removes to the upperside.

This theory is untenable especially for three reasons:

1^y. The mouth of the *Amphioxus*-larva does not originate medianly to remove afterwards to the left-side. It originates on the contrary on the left-side to take afterwards a pseudo-median position.

2^y. There is no reason why a median organ, when removing to the left-side should lose its nerves and muscles of the right-side. Not a vestige of such a phenomenon can e.g. be discovered in the heart and the stomach of man, which are for the greater part situated on the left-side.

3^y. One of the characteristic properties of the second myotome of Selachians is the fact, that its cavity remains, for a long time, in communication with the part of the body-cavity that is situated in the lower jaw and is known by the name of mandibular cavity. This communication continues to exist for a long time after the cavities of the following myotomes have sejoined from the body-cavity.

The same is the case with the larva of *Amphioxus*, and in order to make out, whether the mouth of this larva corresponds, either with that of vertebrates, or with their foremost left gill-slit, one need only state, whether the mandibular cavity of the *Amphioxus*-larva is situated before or behind the opening of the mouth.

On the base of investigations of GOLDSCHMIDT made on an affined larva, called by him *Amphioxides*, (and in the beginning supposed to be a developed form) I surmised at the time, that I could solve this dilemma in the sense that really the mouth of *Amphioxides* lies behind the mandibular cavity. In a later publication GOLDSCHMIDT

has not contradicted this conclusion, but MAC-BRIDE asserted in 1909 that in the *Amphioxus*-larva the mandibular cavity should lie behind the mouth. When I had the privilege of receiving a few years ago *Amphioxus*-larvas from the Zoological stations at Naples and in Helgoland, my attention was specially directed to this point, and I found in all the series of my sections of the larval growth-period, but also in the beginning of the metamorphosis, that the mandibular cavity does not run behind the mouth-opening, as MAC-BRIDE asserts, but before it. In my opinion it has hereby been definitely proved that the mouth-opening of the *Amphioxus*-larva is homologous with the left spiracle of Selachians.

In the course of the metamorphosis the mandibular cavity develops round the mouth, first in the shape of a horse-shoe and afterwards in the shape of a ring, because the extremities of the horse-shoe unite themselves with each other behind the mouth and form the ring-shaped cavity of the velum. As soon as this cavity has assumed the shape of a ring, one can of course no longer see, whether it was situated originally before or behind the mouth.

Finally I may be allowed to give a short communication of the remarkable variations which the mouth of the larva undergoes, of which we know already from HATSCHKEK that it invaginates to the inside and transits into the ring-shaped velum-fold, which, in the full-grown animal, separates the mouth-cavity from the throat (pharynx). This invagination is accompanied, during the metamorphosis, by the formation of a longitudinal fold of the skin, extending along the left side of the mouth of the larva and of the praeoral pit.

Hereby is formed an open cavity before the mouth of the larva: the mouth-cavity of the full-grown animal, in which likewise the praeoral pit is lodged, and which, by a longitudinal slit along which the cirri sprout forth, opens to the outside. This slit is known as the mouth-slit of the developed animal.

At the end of the embryonal period, when the larva is only 1 m.m. long, and the first gill-slit is on the point of opening to the exterior, the mouth is a little almost round opening on the left-side of the body under the second myotome. It lies then opposite the club-shaped gland, which is found under the second myotome of the right-side.

With the growth of the larva the mouth-opening, which is now oval and becomes afterwards slit-shaped, increases giganticly in length. When three gill-slits are extant, the mouth reaches as far to the back as the back-rim of the first slit, and at the end of the larval growth-period it reaches even the back-rim of the fourth or fifth

gill-slit. This gigantic enlargement¹⁾ of the mouth indicates that the larva, during its growth, must be a very gluttonous animal, if the words gigantic and gluttonous may be applied to an animal that is not even 5 m.m. long. The gluttony is also of a very inoffensive nature and consists in swallowing water, for only minimal remnants of food are found in the gut.

During the metamorphosis remarkable modifications occur at the mouth of the larva.

One modification regards its size. In the first half of the period of metamorphosis the gigantic mouth becomes constantly smaller, till, in the middle of this period, it is an extremely little round hole. But for a little sickle-shaped slit this hole is moreover closed by the formation, at the rostral rim, of the first tentacle in the shape of a little tongue.

Now the falling asunder and the resorption of the gill-muscles is in full swing. The fibres of these muscles let loose from their insertions and have partly been broken into pieces. Just like the loosened cells of the club-shaped gland these pieces float in the fluid that is found in the body-cavity.

From this phenomenon, from the cessation of the growth and from the minimal size of the mouth I deduce, that the animal ceases to take food from outside, and continues to live at the expense of part of its own texture: the gill-muscles and the cells of the club-shaped gland.

Obviously the gill-muscles have become superfluous, in consequence of the formation of the atrium, which now regulates for the greater part the movements of respiration. No trace of gill-muscles appears at the slits of the right-side of the body, which never open directly to exterior, but only indirectly by means of the atrium.

In the second half of the metamorphosis the mouth enlarges again gradually, and becomes the opening of the "velum", round which three more tentacles develop, completing the number of four, which this opening is provided with.

The diminution of the mouth has already partly been observed by LÉGROS, but vehemently contested by WILLEY, who supposes this diminution to be only apparent, an optical effect, caused by the rotation of the mouth on a sagittal axis.

According to WILLEY, who does not base his views on the study of sections, but only on that of the larva in *toto*, this rotation should commence already at the beginning of the metamorphosis. I found

¹⁾ This enlargement of the mouth contributes to the temporary removal of the foremost gill-slits of the left-side to the right-side.

however that this rotation, which is incomprehensibly denied by LEGROS does not set in before the mouth has reached its minimal size, consequently in the middle of the period of the metamorphosis. In consequence of this rotation the rostral rim of the mouth of the larva becomes right-rim, whilst at the same time the posterior rim becomes left-rim.

The mouth-opening having become velaropening lies now symmetrically with regard to the median plane, but the nerves, that surround it, indicate that it continues to be an organ of the leftside.

In the higher animals the middle-ear originates from the first gill-pouch, whilst amphioxus lacks the auditive organ entirely. If we wish to express ourselves in a popular way, we may say, as I did already on a former opportunity: Amphioxus cannot hear; he eats however with the left ear, and has consequently lost the mouth.

Mathematics. — “Applications of SONINE’s extension of ABEL’s integralequation.” By Dr. J. G. RUTGERS. (Communicated by Prof. W. KAPTEYN).

(Communicated in the meeting of September 27, 1913).

SONINE¹⁾ has given to ABEL’s integralequation an extension which comes to the following.

The unknown function u in the equation

$$f(x) = \int_a^x \varphi(x-\xi) u(\xi) d\xi \quad (1a)$$

is determined by

$$u(x) = \int_a^x \sigma(x-\xi) f'(\xi) d\xi \quad (2a)$$

where we suppose $f(x)$ to be finite and continuous, $f'(x)$ finite, $a \leq x \leq b$, and $f(a) = 0$. Moreover σ and φ are connected in the following way:

Suppose

$$\varphi(y) = \sum_0^{\infty} c_n y^n, \quad \frac{1}{\varphi(y)} = \sum_0^{\infty} d_n y^n,$$

¹⁾ Acta Matém. 4; 1884.