

**Zoology.** — *Observations on the adhesive apparatus and the function of the ilio-colonring in the living larva of Amphioxus in the growth-period.* By Prof. J. W. VAN WIJHE.

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### I. Adhesive apparatus.

In the November meeting of 1925 I reported on the occurrence of three papillae, partly observed also by others, at the anterior part of the larva of *Amphioxus lanceolatus* in its growth-period. Their presence has already been observed in the larva with a single gill-pouch. They get bigger during the larval growth, but perish as the metamorphosis advances. They secrete a mucous substance, which I suppose to be viscous.

The anterior papilla is unpaired, lies right in front of the mouth and is provided with glandular cells with flagellate threads, which carry its secretion product (together with that of the club-shaped gland) into the mouth. This will promote the formation of slimy strands in which the food-particles are captured.

The other two papillae are situated behind the mouth in the young larva. They form a pair: a right papilla and a left one.

During the larval growth-period the left papilla remains with its fore-part close behind the mouth, which ultimately extends caudally beyond the region of the 5<sup>th</sup> gill-slit. When the left pterygial fold appears just behind the papilla, the latter forms on this fold in caudal direction a long tape-like glandular stripe. This stripe does not reach the end of the pharynx, consequently it does not overlap that portion of the fold that in older larvae lies behind the pharynx.

In the course of the larval growth the right papilla also forms a long tape-like glandular stripe. This one is situated on the right pterygial fold. It extends about as far back as its antimere on the left fold. Its fore-part, however, does not shift caudally, but already in an early stage it grows rostrally as far as the basis of the ventral, rostral fin in the topographical median plane. This accounts for the fact that earlier authors maintained erroneously that the ventral rostral fin was continued caudally in the right side-fold.

Some authors suspected that the asymmetry of the larva is owing to the way in which it rests on the ground. They supposed the larva to lie on its side, just as the larva of the *Pleuronectidae*, of which the one eye, intended for the inferior side, migrates to the superior side, which contains the other eye. Analogously the left gill-slits of the larva of *Amphioxus* were supposed by these authors to migrate for a time to the right side.

Most adherents of this hypothesis assume a left lateral position of the larva. Of late years, however, the suggestion was made that the larva must lie on its right side.

But if my view be the right one, viz. that the two tape-like glandular stripes secrete a viscous matter by which the larva can affix itself for a time, then the larva does not lie on the bottom, but stands, as it were, on its pterygial folds. True, in that posture its sagittal axis is not vertical, but oblique, because ventrally the right and the left fold do not extend equally far. Whether the left- or the right side will incline towards the bearing surface, must depend on the fact whether the right or the left fold extends farthest ventral.

This changes with the age-period. In young larvae the left papilla lies in the topographical ventral median line, and the right papilla a little higher on the right side. Then the larva must incline the fore-part of its body dextrad. But later on, when the right pterygial fold reaches farther down than the left one, the front-end of the larva must be turned obliquely towards the left.

The presence of the adhesive apparatus in the larva of *Amphioxus* would not only afford another point of agreement with the larva of *Tunicata*, but would also clarify the origin of the peribranchial cavity in *Amphioxus*.

Now was the time to watch the living larva, and to ascertain whether it can affix itself or not.

The Direction of the "Biologische Anstalt" in Heligoland accorded to me the privilege of working in its beautiful, and well-appointed, newly-built aquarium. For 11 days in the first half of last August I studied there the living larva. When I arrived there, 11 larvae were available. They were from 4 to 5 mm. in length and had from 13 to 15 (left) gill-slits. The atrium had not made its appearance yet; every trace of right gill-slits was lacking.

They were lying on the bottom of the big glass-jar where they had been deposited. When the water was stirred they swam for a short while performing eel-like windings, but soon they sank to the bottom again.

By help of the pipette, I transferred some into a shallow glass, so that they could be watched with the binocular microscope. All of them immediately sank to the bottom, some lay on their left, others on their right side. Sometimes it happened that a larva, that had been lying on the one side, turned on the other for resting.

When shaking the glass a little I observed that some larvae shifted bodily to other places, which proved that they had been free to move about. Of others, however the fore-part appeared to stick to the glass, as the posterior (the largest) portion of their body was swinging to and fro.

One day later, after the larvae had been set swimming in the big glass, I happened to see four times that one of them was hanging on the vertical glass-wall. It erected itself vertically with the rostrum turned upwards. On shaking the glass a little it appeared that, roughly estimated,  $\frac{1}{3}$  of

the body adhered to glass-wall, but the lower  $\frac{2}{3}$  was swinging freely to-and-fro.

The contact lasted, however, only a short time, about one minute, then the animal freed itself by some powerful to-and-fro strokes of the caudal part, to sink down motionless, or to swim to the surface and thence to subside without moving.

The next day I received four newly caught larvae. Prof. HAGMEIER gave me a high rectangular cuvette with parallel walls of cut glass and a binocular microscope with BRAUS' stand, so that the microscope could be mounted horizontally. But I was not so fortunate as to see a larva on the vertical wall. This failure I ascribed to the smooth inner wall of the cuvette, on which hardly any material from the seawater could have been deposited, so that I expected a better result the next day. Such was the case; then I was soon able to show a larva suspended in the way described just now. But before we could mount the microscope, we saw the larva set itself free and subside to the bottom.

The existence of an adhesive apparatus was now sufficiently demonstrated, so that next I directed my attention to other functions of the living animal. The day before my departure from Heligoland all the larvae but two had perished. This was perhaps owing to inadvertency, as on preceding days I had refreshed the water from the tap, instead of drawing it directly from the sea.

But also the two living larvae were more dead than alive. They were still transparent and not white as the others, but they did not move even when the water was stirred. I put both in the high cuvette where they went down to the bottom. Now by help of a pipette I brought one of them to the surface of the water close to the glass-wall. Without moving at all the animal sank horizontally to the bottom. A second time I brought it up, with the same negative result. But the third time the animal remained hanging on the glass-wall after having descended half way; then it assumed a vertical position, rostral part upwards; on moving the cuvette the caudal end swung to-and-fro. Now the binocular microscope was mounted horizontally and the left side of the animal appeared to be turned to the glass-wall. Owing to the low magnification — also with the strongest objective systems <sup>1)</sup> — we could not tell whether the left side of the larva was pressed against the glass-wall, or (as I suspected) whether the larva was only inclined to it at an acute angle. Several gentlemen working at the "Anstalt" could witness that the larva was hanging on the wall. When I looked at it again three hours later, it was of an opaque white and was dead. Infusoria were creeping with their cilia up-and-down the animal or were swimming round it. The next day it was still hanging there, but was hardly more than the skin. Its inside had been eaten up; of the intestines, even of the chorda nothing could be distinguished.

<sup>1)</sup> The thickness of the glass-wall prevented us from observing the larva under the strong objective-lenses of a monocular microscope.

I purposely dwelt on these observations at some length, because they tended to establish the fact that in its growth the larva of *Amphioxus* is provided at its front-end with an adhesive apparatus. Nor can it reasonably be doubted that this apparatus (cf. my paper referred to at the outset) is supplied by the right-, and the left papilla of the young larvae, which grow in the older larvae as far as the glandular stripe on the right and the left pterygial fold.

Now let me discuss the origin of the peribranchial cavity in *Amphioxus* and *Tunicata*, which many authors hold to be homologous.

#### *Origin of the peribranchial cavity in Amphioxus.*

Our knowledge of the origin of the peribranchial cavity in *Amphioxus* we owe in the first place to LANKESTER and WILLEY (1890).

For many years already I have been collecting and cutting material to observe the origin of the pterygial folds and of the atrium. In this paper I shall give a brief report of my experience, which I intend to illustrate copiously in a later work.

The first foundation of the pterygial fold appears as a small eminence on the wall of the pharyngeal region just behind the homolateral adhesive papilla. Initially its caudal growth is but slow up to the termination of the pharynx. After this its growth is quicker, so that with 18 gill-pouches shortly before the metamorphosis it covers a long distance beyond the pharynx.

The left pterygial fold originates considerably later than the right one. It does not yet exist in a larva with 7 gill-slits<sup>1)</sup>. As stated above it grows as far caudad as the right fold, but rostrally it does not grow so far, as it begins behind the mouth. The right fold on the contrary extends anteriorly up to the first gill-slit. Its apparent continuation up to the basis of the rostrum (in the topographical ventral median line) belongs to the right adhesive papilla.

Apparently the adhesive papillae have given the first impetus to the origin of the pterygial folds, which afterwards form the atrium. On this ground the papillae may be considered to be instrumental to the origin of the atrium.

In the first stage of the metamorphosis the right and the left pterygial

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<sup>1)</sup> HATSCHKE (Zoologische Wandtafeln, herausgeg. von Dr. R. LEUCKART Taf. 72 Acrania) gives an instructive picture of a larva with 7 gill-slits, but in his explanation (l.c. Fig. 12) he confuses the right side of the animal and the left one. He says that the fold lies on the left (read: right) side, and the gill-slits lie on the left (read: right) side but that afterwards they shift to the right (read to the left).

It is remarkable that in his explanation of Fig. 14 he says that the anus of the larva in Fig. 12 lies to the right, which is correct (anyhow for the presence of 1 to 6 gill-slits), whereas in the larva with one gill-slit (l.c. Fig. 10) the anus on the picture is on the left side, which again is not correct.

folds coalesce behind the pharynx, but near their hinder extremity they leave a slit-shaped atrioporus open.

LANKESTER and WILLEY found that this intergrowth takes place postero-anteriorly; just the reverse direction is observed in the origin of the folds.

Towards the close of the first stage of the metamorphosis (after WILLEY 8 stages are distinguishable) the closing of the atrium is completed.

At first sight the direction of the formation of the atrium, and also of that of the pterygial muscle, is surprising, but a clue to it may be found, when the normal posture of the larva in rest is such that initially it is supported by its two adhesive papillae and afterwards by the glandular stripe of the pterygial folds. The folds form the side-walls of a longitudinal groove on the ventral side of the animal. The groove is closed by the object on which the animal rests so as to form a channel open behind. The row of the (left) gill-slits debouches into the groove, the bottom of which is turned upward.

The water that has served for respiration and feeding flows from the gill-slits into the channel, formed by the groove and the underlying object. It is transported further in caudal direction. In swimming there is no channel as the object is absent, and the groove is open over its whole length, unless its edges close up, which can often be seen on cross-sections. But this does not matter, as an apparatus for removing the used water from the vicinity of the mouth is not wanted, because the animal continually moves from one place to another.

In the resting larva the effect of this apparatus is increased when the metamorphosis has begun, because behind the pharynx the walls of the two pterygial folds, facing each other, get fused, thus forming the foundation of the atrium. Now in this region the pterygial muscle appears about simultaneously. Its contractions constrict the atrium, so that a sort of siphon arises, which will drive out the respiration water from the atriopore with greater force. The atrium now closes soon in anterior direction and the pterygial muscle grows in the same direction.

When the closure is accomplished the larva is provided with so powerful a respiratory organ (during swimming as well as during rest) that the gill-muscles will degenerate and ultimately disappear as the metamorphosis progresses. On the gill-slits of the right side, which do not appear before the closure of the atrium is completed <sup>1)</sup> muscles do not develop any more.

<sup>1)</sup> FRANZ (1925) (Kap. II p. 427 sqq.) believes that already before the formation of the atrium gill-slits of the right side are open to the outside directly. He pictures (l.c. Table 16 Fig. 5) a transverse section from a series, that appears to have been studied rather cursorily. The two openings *r, δ* and *l, δ* are not right and left gill-opening, as is asserted in the explanation of the picture, but the double section of a left gill-slit curved through contraction;

*end. c* is not the section of the "Endostylcoelom" for the collapsed truncus arteriosus can be seen in its proper place (on the nether lip of the right boundary-fold, left side of the figure) in the shape of four cohering cells; *end. c* is the cavity of a (left) gill-arch with some fibres of the gill-muscle cut longitudinally. All visceral muscle fibres represented

Now the adhesive apparatus has become superfluous and degenerates.

Besides being a respiratory organ the atrium has still to perform another function, viz. the protection of the pharynx and the mid-gut. Now the larva can modify its habitat, and hide in the sand, without any harm being done to its gills, its liver or mid-gut and without any chance of the gills being stopped up. The numerous larvae in the 2<sup>d</sup> to the 8<sup>th</sup> stage of the metamorphosis, that I received from Naples, had all been caught from the sand.

*Origin of the peribranchial cavity in Tunicata.  
A comparison with Amphioxus.*

I shall be brief about it for two reasons: 1<sup>o</sup> because I have but little experience of this origin in Tunicata, and 2<sup>o</sup> because in 1914 (pp. 71—74) I reported already on the comparison between Tunicata and the young larva of Amphioxus.

In Ascidians the origin of the peribranchial cavity is quite independent of the adhesive papillae, while the pterygial folds do not occur in Tunicata. Consequently their peribranchial cavity cannot be homologous with that of Amphioxus, which originates in quite another manner.

I agree with VAN BENEDEN and JULIN (1886, pp. 401—405) in assuming.

1<sup>o</sup>. that in Acopa this cavity must be derived from the pair of gill-channels of Copelata (Appendicularians);

2<sup>o</sup>. (l.c.p. 387) that the strand of entoderm-cells, which in the larva of (Appendicularians and) Ascidians is found in the "tail", is the rudiment of the gut in the trunk of the larva of Amphioxus, so that the post-branchial gut in Tunicata must be a totally different part of the body from that in Amphioxus.

For the rest the comparison made by the authors of the larva of Ascidians and that of Amphioxus is untenable, also in consequence of subsequent researches.

It induced me (1914) to draw the following conclusions:

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in Fig. 5 belong to the gill-muscles, that disappear during the metamorphosis. The foundation of the pterygial muscles is still cellulous, not fibrous, and FRANZ's assumption (l.c. p. 430) that in the larva of Fig. 5 they should already possess muscle fibres, is erroneous.

He presumes to have to correct my observations, but I cannot accept his view. On *transverse* sections of an adult Amphioxus it can easily be seen that the plates of the pterygial muscles look freely into the narrow "abdominal channels" and in the lateral part of the side-channel. True, the lower border of those plates is not free everywhere but here and there attached to the skin, by which the "abdominal channels" have originated. In the medial part of the side-channel the lower border of the plates is attached in the trunk region to an outgrowth of connective tissue, which, however, is lacking more laterally. So this outgrowth forms merely an incomplete partition wall. Laterally there is a free communication between the groove of the side-channel with spaces between the muscle plates. A *sagittal* section may involve errors here and has led FRANZ (l.c. Fig. 8) astray in supposing the pterygial muscle to be composed of little cases closed all round.

10. that morphologically the first pair of gill-slits, which in *Amphioxus* has been metamorphosed into mouth (tremostoma), and clubshaped gland, corresponds with the pair of gill-channels of the Appendicularians;

20. that the postbranchial intestinal loop of Tunicata has to be looked for morphologically in the second pair of gill-slits of *Amphioxus*, of which only the left antimere occurs as the first gill-slit in the larva, but disappears during the metamorphosis, while there is not so much as a rudiment of the right antimere.

It might be possible, though, that in some Tunicata this very antimere should form the intestinal loop. On this matter I have now a more settled opinion.

The intestinal loop of Copelata namely lies to the *right*<sup>1)</sup> of the median plane, which passes through the spinal cord and the gl. thyroidea (the so-called "endostyl"). In *Acopa* on the other hand it is morphologically located to the *left*<sup>2)</sup> of that plane.

The term "*Acopa*" is defective, as it is indicative of the absence of a character (absence of a tail). With sharply distinguished positive characteristics Tunicata may be divided according as the intestinal loop lies to the left or to the right, into *Laevicolica* (= *Acopa*) and *Dextricolica* (= Copelata).

It may now readily be suspected that in the large group of *Laevicolica* the intestinal loop corresponds with the foremost (left) gill-pouch of the larva of *Amphioxus*, in the *Dextricolica*, on the other hand, with the right antimere of this pouch, which is no longer developed in *Amphioxus*. This difference may account for a large differentiation of *Laevicolica*. They are distributed over some orders and many families, whereas the differentiation of *Dextricolica* is restricted to at most two families, if *Kowalevskia* is to be considered as a representative of a separate family.

## II. Swimming movements.

Many years ago I tried to interpret the remarkable asymmetry of the pharynx of the larva of *Amphioxus*, the disappearance of the primary mouth, its substitution by morphologically the first left gill-slit, and some more phenomena<sup>3)</sup> by advancing the hypothesis that the ancestors of *Amphioxus* had rotated "to the left" when they were swimming, round

<sup>1)</sup> In most Copelata also the anus lies on the right side. In *Megalocercus* and *Stegosoma* it lies in the median plane. If the suggestion expressed in the text be correct, it must originally have lain to the right in these two genera.

<sup>2)</sup> In *Corellidae* the intestinal loop lies to the right of the gill-basket, but DE SELYS LONGCHAMPS (1900) has found that in the larva the anus debouches into the foundation of the *left* atrium. The site of the intestinal loop on the right is, therefore, to be regarded as a secondary displacement.

<sup>3)</sup> Such as the absence of an organ of equilibration and of image-forming eyes. Afterwards also the initial location of the anal outlet on the right side of the body.



their long axis i.e. in the direction from right to left (along the ventral plane).

HATSCHEK (1881) observed this movement (which occurs also in the development of many Invertebrates) in the embryo, even for some time after it had disengaged itself from the egg-membrane.

Now, when FRANZ (1924 p. 6) had found in most cases a similar rotation in the adult animal when swimming, I also expected it in the larva, but this expectation was not realized.

I could not note any rotation in the eel-like windings, alluded to above — observed already by LEUCKART and PAGENSTECHER (1858).

It still remains to be seen whether the hypothesis is in any way substantiated by the mode of moving of younger larvae than those that I had at my disposal.

Other authors have endeavoured to ascribe the asymmetry to the condition of rest. This, however, seems impossible to me, especially after what has been said above about the behaviour of the larva during rest.

I have to assume that in its period of growth the larva lies attached to objects on the bottom of the sea, mostly temporarily, but sometimes also detached from them, now on its left, now on its right side. At intervals it swims higher up, and is then carried along by the ocean-currents. From the second stage of the metamorphosis upward — when the atrium has been fully developed, it also hides in the sand; at intervals it leaves its hiding-place. The vertical posture of larvae suspending freely in the water, as described by WILLEY (1891) applies probably only to stages of the metamorphosis. All the larvae, living or dead that I observed in Heligoland sank motionless in *horizontal* posture to the bottom of the vessel.

### III. Food transport.

The food is moved along the alimentary canal by the cilia, which, just as in the case of the full-grown animal, line the lumen of the intestinal canal over its whole length.

At the wheel-organ and the gill-slits, but also at the ilio-colonring the cilia have grown to long flagella, which form a living, brisk grating spectrum. Light causes in those two places two beautiful green spots, by which the larva of *Amphioxus* is recognizable<sup>1)</sup> among the many other larvae in the plankton. Muscular contractions were not observed at the intestine behind the pharynx. Indeed, muscle fibres are not known either in this part of the intestine of the larva.

In order to watch the progress of the food a *little* finely pulverized

<sup>1)</sup> LEUCKART and PAGENSTECHER already (1858 p. 559) called attention to these spots. They say that the larvae "im Pokale leicht erkannt [werden] an der zartgrünen Färbung, welche diejenigen Stellen des sonst durchsichtigen Körpers auszeichnet, an welcher die Kiemen und jene, an welcher die hintern Partien des Darmes liegen. Sie sinken, wenn sie sich nicht bewegen, im Wasser unter".



carmin was added to the water of the watchglass, in which a newly caught larva had been placed. It lay with the left side upward and had 15 gill-slits. The violent movement of the flagella of the wheel-organ caused the grains of carmin to be conveyed along the mandibular groove into the wide mouth-opening. The dorsal border of the groove had already been enlarged by the foundation <sup>1)</sup> of the left cheek that had just made its appearance, and which scarcely reached the front-end of the long, oval mouth-opening. A few grains flew up <sup>2)</sup> over the cheek-foundation; still, they got into the mouth-opening. While the organ set up a powerful current posteriorly, its violent undulatory movement was directed anteriorly.

The wheel-organ, seen in a side-view of the larva, presents the shape of a horse-shoe, with its open side turned caudally towards the mandibular groove. As the undulations of the threads in *either* limb <sup>3)</sup> of the horse shoe are directed anteriorly, the apparent wheel-like rotations of the apparatus are lacking, which JOHANNES MÜLLER (1841) has described at the extremities of the "finger-shaped figures" in the developed Amphioxus. At the top margin of such a figure he found the undulatory movement directed anteriorly, at the lower margin, on the other hand, it was directed posteriorly.

At the thick anterior border of the mouth-opening of our larva there was to be seen a bundle of long threads which quietly struck at the mouth at occasional short intervals. This bundle belongs to the unpaired mandibular papilla.

With a deeper focussing of the microscope HATSCHEK's groove revealed itself under the wheel-organ. On the bottom of the groove lay some small clusters of carmin-grains, that had got there accidentally. Here there seemed to be a perfect condition of rest, contrary to the violent movement at the border. Even after an hour the shape of the clusters had not altered appreciably.

At the gill-slits the undulation of the flagella could be seen beautifully. At the anterior border the direction of the undulation was dorsal, at the posterior border it was ventral. This is an opposite direction to that which JOHANNES MÜLLER has observed after the metamorphosis at the half-slits of the animal. He also observed that the wheel rotation is only a semblance, but that in reality the flagella expel the water in quite another direction viz. through the gill-slits.

The grains of carmin did not come out through the gill-slits — I had

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<sup>1)</sup> The foundation of the cheek grows later on rostrally over the pre-oral organ (wheel-organ + HATSCHEK's groove) as well as caudally over the mouth-opening.

<sup>2)</sup> In the description we have tried to give an impression of the velocity as it appeared magnified by the microscope.

<sup>3)</sup> The left limb is shorter than the right one. It follows the lower border of the left lateral muscle and terminates near the posterior border of HATSCHEK's groove. The right limb reaches farther backward and presents on cross sections a strongly bent course. This curve is not very well observable in a side-view.

used a small quantity of carmin on purpose — but they had accumulated in the shorter or longer mucus-strands, which entered the alimentary canal at the end of the pharynx. This mucus is secreted by the pre-oral organ, the mandibular papilla, and also in the pharynx itself. Directly when the red strands entered the gut at the end of the pharynx, they began to glide linearly with uniform velocity towards the ilio-colonring which they soon filled up. Here the transport of food was stopped for some time, while the strand was being revolved round its axis by the long flagella that line the inside of the ring.

After some time part of the strand entered the rectum and moved on slowly in a straight line, while the portion left behind in the ring continued revolving. When the ring was empty the strand had filled up the whole rectum as far as the anus. Soon the strand was thrown out slowly piecemeal. All the time the animal had been lying quietly and turned over only once from its right side on its left.

When the gut was emptied the ring was of a light pink, which manifested resorption. But also the clubshaped gland had in some degree assumed this colour, for which the blood must have been responsible.

The experiments with carmin were repeated several days, also for the purpose of determining the time required by a strand to pass through the various sections of the alimentary canal. This time, however, appeared to depend on all sorts of conditions.

It differed e.g. according to the length of the strand that issued from the pharynx; also according as a strand was or was not followed soon by others; and according as the flagella in the ring beat briskly or tardily (the latter occurred sometimes in animals that had been caught several days ago).

In the above-described experiment the strands had filled the ring in two minutes; after a rotation of 5 minutes the ring was empty, and the whole rectum was filled, and after another 4 minutes the whole intestinal canal was empty, so the whole time-interval in the post-pharyngeal part of the alimentary canal amounted to 11 minutes.

After the intestine had been emptied a very small lump of carmin grains glided from the pharynx in one minute up to the ring, remained there quietly for one minute; when entering the ring it revolved for one minute and ultimately also passed through the rectum in one minute.

The same distance covered by a large amount of food in 11 minutes, was gone over, a short time later, by a very small quantity through an empty gut in four minutes.

These time-data can only serve for examples, yet with other larvae c.p. I obtained time-values, that differed little from the above.

If a fair quantity of food glided from the pharynx after the ring was already quite full, it first stopped before the ring, but subsequently it also partook of the rotation, I suppose because it had been sufficiently agglutinated with the contents of the ring. This affords the clue for the

fact that the end of the mid-gut often exhibits a slight dilatation just in front of the ring.

During the comparatively slow linear movement in the rectum the food-strand was mostly broken to pieces, just as ANDREWS (1893) also observed in *Asymmetron*.

Time failed to experiment with other substances than carmin. Once I administered ammonium-carmin powder, which is easily soluble in distilled water, but appeared almost insoluble in sea-water. Contrary to my expectation its action was toxic. The ring was soon filled; the food began to revolve. This lasted about an hour! (55 minutes), then it entered the rectum in three several pieces, of which the hindmost appeared in the anus after 6 minutes. The front piece was not thrust out yet after 15 minutes. All that time the larva lay still, the right side uppermost. After a couple of hours the animal was markedly curved and dead; it still lay on its left side.

#### *Function of the ilio-colon ring.*

From the foregoing it follows that in the ring a complete mixture must take place of the ingested food and the digestive fluids. Moreover resorption takes place here for which there was no time <sup>1)</sup> in the mid-gut, because of the quick rate at which the foodstrands passed through it.

It can be said, therefore, that the ilio-colon ring in the larva of *Amphioxus* — presumably also in the full-grown animal — functions as a stomach, although morphologically it does not agree at all with the stomach of higher animals. For this stomach is a widened portion of the gut behind the oesophagus and before the embouchure of the efferent duct of the liver. In *Amphioxus* and its larva a stomach is absent in the corresponding place. But the ilio-colon ring lies on the border between mid-gut and rectum (end-gut) and corresponds morphologically in higher animals with part of the coecum, or with the appendix.

A coecum is found already in Selachians, but in Holocephali we find in its stead still a ringshaped glandulous piece of the gut.

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<sup>1)</sup> Time is available for it in some cases only in the hindmost part of the mid-gut. Also in the end-gut time is available.

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#### POSTSCRIPT.

Just when this paper had appeared in Dutch, FRANZ was so kind to send me his extensive works of this year on Amphioxus.

I cannot enter into them in detail and must confine myself to two points concerning the present paper.

10. I am happy that they drew my attention to the paper of J. H. ORTON: "On a Hermaphrodite Specimen of Amphioxus, with Notes on Experiments in Rearing Amphioxus", *Journal of the Marine Biological Association of the United Kingdom*, Vol. 10, 1913—'15.

In this paper ORTON (l.c. p. 511) already stated that larvae of Amphioxus, a fortnight old, when "only the first few gill-slits had appeared ..... stuck to the bottom of the vessel in the head region."

So ORTON was first in discovering the attachment of the larva to objects. But I cannot agree with his suggestion that this may occur by a secretion of the club-shaped gland, opening on the left side, and that "then the larva would be able to feed only from the right side of the body."

As we have seen, the feeding occurs just as well whether the animal is lying on the right or on the left side. Moreover, in my opinion, the very young attached larva will not lie on the left side, but seem to be resting on the right side, as noted above, p. 992.

20. FRANZ (*Morphologie der Akranier, Ergebnisse der Anatomie und Entwicklungsgeschichte*, Bd. 27, 1927, p. 593) figures a median section of a larva of Amphioxus with about 14 gill-pouches in the period of growth. The pre-oral organ is cut twice, showing two fossae, a foremost larger one, in contact with the notochord and behind this fossa, but more ventrally,

a smaller one. FRANZ says (l.c. p. 594) : "die grössere Grube wird zum Räderorgan, die kleinere zur HATSCHEK'schen Geisselgrube".

In a series of transverse sections, however, of a larva at this stage, one easily sees that the interpretation of FRANZ must be inverted : the anterior, larger fossa in contact with the notochord being the groove of HATSCHEK. The smaller posterior one, pushed away from the notochord is a part of the wheel-organ (Räderorgan). It is the deep curve of the longer, right limb of the horse-shoe mentioned above, p. 999 footnote.

The term "Geisselgrube" for the groove of HATSCHEK is a misnomer, as it shows no flagellation, at least not in the larva. The flagellate cells bordering the opening of the groove in front and at the sides<sup>1)</sup> belong to the wheel-organ.

The position of both fossae in a transverse section of a somewhat older larva (first stage of the metamorphosis) may be seen in LANKESTER and WILLEY (1890, fig. 13). Here (in the "praeoral pit") *s o* is the groove of HATSCHEK and *w o* the deep curve in the right or inferior limb of the horse-shoe noted above.

My sections show that this curve is straightened out during the period of metamorphosis. At the end of this period the "Flimmergrube" (which, 1901, I found in the adult, but is lacking in the larva) arises as an outgrowth from the posterior end of the groove of HATSCHEK. It is not a transformation of the "curve" as might erroneously be presumed and it does not arise from the wheel-organ.

Concerning its function in the adult I suggested (1901) that it would contribute in evacuating the mucus secreted by the groove of HATSCHEK. Probably the same function will be performed in the larva by the deep curve in the right arm of the wheel-organ (right limb of the horse-shoe).

*Groningen, December 1927.*

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<sup>1)</sup> Both arms of the wheel-organ (=limbs of the horse-shoe) uniting in front of the groove of HATSCHEK are prolonged rostrally (in the larva as well as in the adult) into an unpaired process.