the albumoses, but much more rapidly and severely. The animal becomes ill directly after the injection, gradually becomes more and more sluggish, and dies in coma, or, if a non-lethal dose be given, it recovers from the state of stupor gradually. After death enormous local subcutaneous œdema is found, with congestion and sometimes thrombosis of the small veins. Peritoneal effusion is occasionally present, and the spleen is usually enlarged, dark, and congested, or simply congested without being greatly enlarged. The fatal dose for a mouse weighing 22 grams is between 0·1 and 0·15 gram, death occurring in two to three hours.

The anthrax bacillus in digesting the alkali-albumin forms (1) proto-albumose, (2) deutero-albumose, (3) an alkaloid. The alkalinity of the albumoses may explain their toxic properties, being due to the fact that the alkaloid is in a "nascent" condition in the albumose molecule. The bacillus forms the alkaloid from the albumose, and it is possible that the living tissues have a similar action when the albumose is introduced into a living animal.

III. "On the Development of the Atrial Chamber of *Amphioxus*." By Arthur Willey, Student of University College, London. Communicated by Professor Ray Lankester, F.R.S. Received May 5, 1890.

Preface.

Last year, through the kindness of Professor Lankester, I had the opportunity of spending several months—May to August—in Sicily, collecting the embryos and larvæ of *Amphioxus*.

Since then I have been working continuously on the material I obtained in the laboratory of University College, under the direction of Professor Lankester. The period of the development, to which Professor Lankester determined first of all to give attention, was that before which Hatschek's well-known work stops short. He proposed that I should cut sections, so as to ascertain the mode in which the atrial chamber takes its origin and the subsequent history of the gill-slits, viz., as to how the slits on the left side of the pharynx originate. The relation of the larval to the adult mouth and the details of the curious process of movement of the mouth from a unilateral to a median position were included in the scope of our enquiries.

Professor Lankester received a grant from the Government Grant Committee in aid of the present investigation, and it is therefore necessary to state that he has constantly supervised my work, and allows

me to publish in my own name a summary of the results which I have obtained under his guidance.

Amphioxus occurs in great numbers in a comparatively small lake, or *pantano*, which is situated behind, and separated from the sea by, the village of Faro, near Messina. It is connected with the Straits of Messina by a narrow canal, some two or three hundred yards in length.

The bottom of the *pantano*, in contrast to that of the Straits, consists of foul mud; and it may be mentioned in this connexion, as I was informed by Professor Kleinenberg, that *Amphioxus* is only occasionally met with in the Straits, and is entirely absent from another larger *pantano* which lies behind the neighbouring village of Ganzirri, and is joined by a short canal to the one at Faro.

The embryos float on the surface, and are to be had by dredging on the surface at sunrise, but the readiest method of obtaining them in quantity is to take the adults in glasses and allow them to spawn there, if they will. Spawning takes place about an hour after sundown.

The ova, if fertilised, must be very carefully distributed among several glasses containing clean, but unfiltered, water from the *pantano*. If the water is filtered, or if sea water is employed, or if too many ova are placed in one glass, they will certainly either die or develop abnormally.

The first outward and visible sign of fertilisation is the separation from the egg-cell of the yolk-membrane (*Dottermembran*).

Most, if not all, of the ova that I obtained were discharged through the atriopore.

If Kowalewsky* had not seen them issuing from the mouth, it would not have been easy to understand how they could pass into the pharynx in opposition to the constant outflow of water between the gill-bars.

Segmentation always commences at dusk—between the hours of seven and eight—and goes on very rapidly through the night.

The early stages have been so fully described by Hatschek† that I will only refer to them in the briefest manner.

At 8 P.M. segmentation commences; at 11 P.M. invagination commences; at 1 A.M. the gastrula is complete; at 3 A.M. the gastrula begins to revolve by cilia within the yolk-membrane; and at 5 A.M. two pairs of myocœlomic pouches have been formed, and the embryo ruptures the egg-membrane and becomes free-swimming.

During the first day the embryo grows in length and adds several pairs of somites. By about eight o'clock on the second morning, that

* "Entwick. des Amph. lanc." ('Mém. Acad. Impér. des Sciences de St. Pétersbourg,' Series VII, vol. 11, 1867.)

† Claus's 'Arbeiten,' 1881.

is, thirty-six hours after the commencement of segmentation, the embryo has acquired a mouth on the left side of the body, and a gill-slit, which arises at first in the median ventral line, and subsequently comes to lie on the right side of the body.

The anus is formed soon after the appearance of the mouth and first gill-slit.

The embryonic period is now at an end, and the larval period begins. As Hatschek states, the only way of obtaining the larval stages is by pelagic fishing. This consists in dredging at depths varying from fifteen to twenty fathoms. At this depth the *Amphioxus* larvæ float in the midst of countless thousands of *Sagitta* larvæ.

A long, but not yet clearly ascertained interval (probably about a fortnight) elapses between the formation of the first and second gill-clefts.

In the period during which it is free-swimming the larva acquires from twelve to fifteen consecutive unpaired gill-slits, each one arising in the mid-ventral line, and then growing in such a manner as to lie on the right side of the body. This applies to the anterior two-thirds of the pharynx, but I am not quite clear yet as to whether the last two or three median slits ever move up to the right side. Meanwhile, longitudinal ridges which are subsequently concerned in the formation of the atrium have appeared (see fig. 6).

At the time of the completion of the atrium, which occurs at the close of the larval period, some remarkable changes in the relative position of parts of the body in the anterior region take place, by which the mouth becomes median, and the gill-slits are arranged in two series, a right and a left. The larva emerges from this critical phase in its development as a symmetrical animal, but the details of the process of symmetrisation—the strongly-marked character of which justifies the use of an otherwise undesirable term—are still rather obscure. The larva, now really a young *Amphioxus*, with atrium and paired gill-slits, ceases to lead a pelagic life, and takes to the sand, where it passes the rest of its life.

Spawning occurs at least from April to September inclusive. The best month, however, in which to obtain the embryos is June, while all the larval stages, up to the passage into the adult form, are to be found during July and August.

Previous View as to the Formation of the Atrium.

The hitherto accepted method of formation of the atrial chamber of *Amphioxus* is that described by Kowalewsky,* and more fully by Rolph.†

* 'Archiv für Mikrosk. Anat.,' vol. 13, 1877.

† 'Morphol. Jahrbuch,' vol. 2, 1876.

Kowalewsky says that after a certain number of gill-slits have been formed two longitudinal folds appear on opposite sides of the body, which grow round and meet, and finally fuse together in the median ventral line, leaving a wide aperture at one end—the atriopore. His figures, two of which are here reproduced (figs. 1 and 2),

FIG. 1.

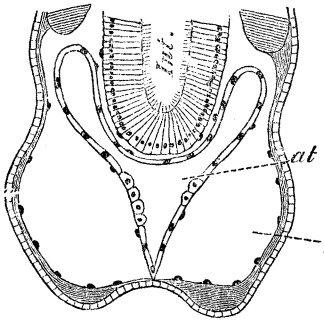
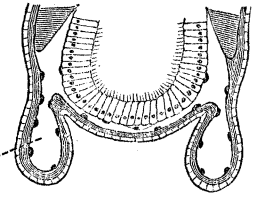


FIG. 2.

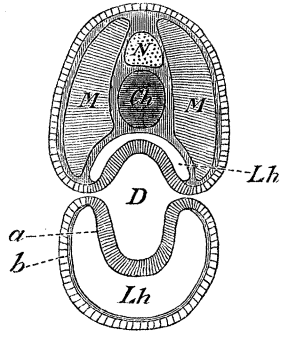


Copies of Kowalewsky's figures of transverse sections through a larva of *Amphioxus* with fully-formed atrium. Fig. 1 represents a section taken between pharynx and atriopore; and fig. 2, one taken just behind the atriopore of the same larva.

Int. Intestine. *at.* Atrium. *l.h.* Cœlom.

bear this description out, more or less, while Rolph's schematic figures bear it out entirely. The latter are reproduced in figs. 3, 4, and 5.

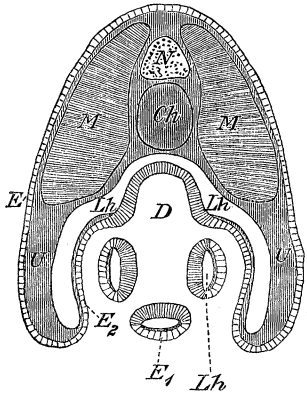
FIG. 3.



Copy of Rolph's theoretical section through the pharyngeal region of a larva, before the commencement of the so-called epipleural folds.

N. Nerve-cord. *Ch.* Notochord.
M. Muscles. *Lh.* Cœlom.
D. Intestine. *a.* Intestinal epithelium.
b. Epidermis.

FIG. 4.

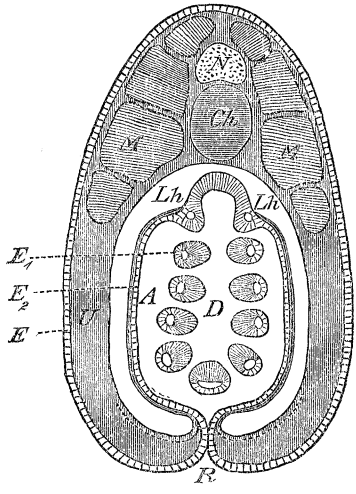


Copy from Rolph of a similar section through an older larva, showing the commencing longitudinal downgrowths.

E. Epidermis.
E_1. Inner epithelium of the (future) atrial cavity.
E_2. Outer epithelium of same.
U. Subcutaneous tissue.

Other letters as in fig. 3.

FIG. 5.



Copy from Rolph of a similar section showing the meeting together of the "*epipleura*" in the ventral middle line.

A. Atrium. *R*. Raphe.

Other letters as in figs. 3 and 4.

The most serious error in Kowalewsky's view lies in the fact that he makes the space in the lateral outgrowths continuous with the body-cavity, and consequently calls it "Leibeshöhle," or cœlom.

This space, as we shall see, does not belong to the true cœlom, and is not traceable as a space to the original myocœlomic pouches, but arises apparently as an inter-cellular space in the midst of the connective tissue—in fact, it would seem to belong to that category of spaces to which the term "pseudocœl" has been applied. In this respect it stands in contrast to the spaces in connexion with the dorsal and ventral fins, which have been shown by Hatschek to be derived directly from the myocœlomic pouches.

Rolph's figures (figs. 3, 4, 5) do not profess to be more than diagrams. They show the epipleur originating as a depending ridge on each side of the pharynx (fig. 4). Into this ridge the cœlom is extended. The epipleura meet finally in the middle line below the pharynx according to this theory (fig. 5). It is no doubt true that the scheme of growth thus sketched by Rolph, and based upon Kowalewsky's erroneous figures, would account satisfactorily for the condition of the atrial chamber and its epipleural walls as observed in the adult. It also gives a basis for the suggestion made by Kowalewsky that the epipleura are comparable to the opercula of Teleostean fish.

I now propose to show that this view is based on erroneous observation.

Formation of the Atrial Chamber as observed by me.

The first indication of the commencing formation of the atrial chamber is to be found in larvæ with nine or ten gill-slits on the right side. Behind the region of the pharynx we find that the mid-line of the body has become marked with a narrow groove, so that in section it is bifid (fig. 6). The short upstanding ridges which limit the groove are the metapleura of the adult. Though at first solid, the connective tissue within the ridge soon becomes hollowed, and forms a lymph space which never has any obvious connexion with the cœlom. These ridges can be traced from about the middle of the larva's body forward towards the pharyngeal region, where they diverge considerably from one another. That belonging to the animal's left side keeps a more or less median position, and can be traced (though but small in elevation) when twelve gill-slits are present as a ridge situated at the lower or ventral margin of the gill-slits and dying out in the anterior region of the pharynx. The right-hand ridge, or metapleur, takes a course to the right of the gill-slits (which, it will be remembered, are on the right side of the body), and overhangs the upper limit of the slits to a small extent. It dies out in front of the first gill-slit, where it bends towards the middle line.

FIG. 6. FIG. 7.

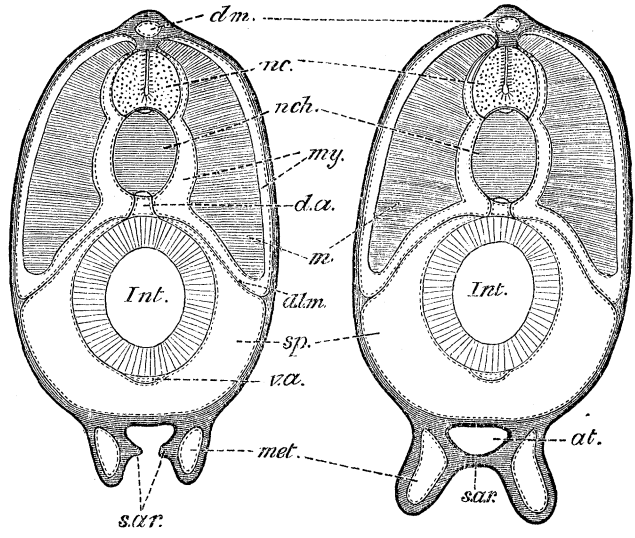


FIG. 6.—Transverse section through a larva with eleven or twelve unpaired gill-slits and with lateral mouth, showing the minute sub-atrial ridges.

d.m. Dorsal division of myocœl in which the fin-ray will lie when it is developed.
nc. Nerve-cord.
nch. Notochord.
m. Muscle-plate.
my. Cavity of myocœl.
d.a. Dorsal aorta.
Int. Intestine.
d.l.m. Double-layered membrane separating the myocœl from the splanchnocœl.
sp. Primitive splanchnocœl.
v.a. Ventral vessel.
met. Metapleur.
s.a.r. Sub-atrial ridges.

FIG. 7.—Transverse section through a slightly older larva. The sub-atrial ridges (*s.a.r.*) have fused for a short distance between atriopore and pharynx; but in the pharyngeal region the atrium is unclosed, and consequently the gill-slits still open directly to the exterior.

at. Atrium.

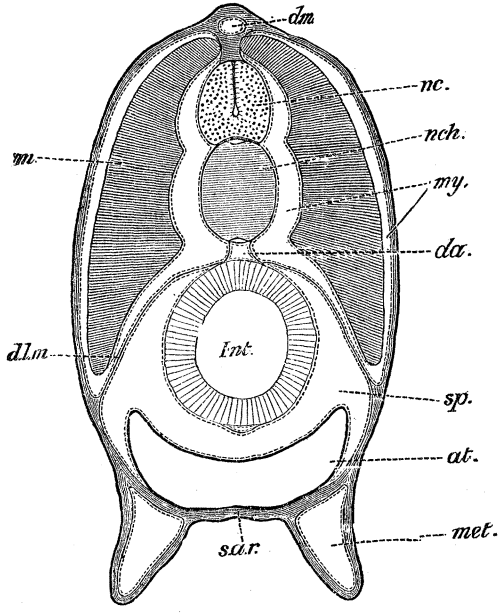
The atrium is formed by a small horizontal growth (*s.a.r.* in fig. 6), which starts from the inner face of each metapleur and floors in the deeper half of the groove or area between the two metapleura (fig. 7, *at.*).

These horizontal growths may be called the sub-atrial folds.

They are at first extremely small, and the atrial space floored in is a mere canal. Later the width of the atrial space increases greatly, and the sub-atrial folds consequently widen also, becoming that pleated expansible floor of the atrial chamber, with its transverse muscular layer, which all observers of *Amphioxus* know so well (fig. 9, *s.a.r.*).

The atrial groove becomes floored in first in the region of the atriopore. The growth of the sub-atrial folds extends gradually forwards, and the closure proceeds along one side (the right) of the pharynx. The whole atrium thus formed is a very small tube-like space. The closure by means of the small horizontal sub-atrial outgrowths in the region of the large gill-slits is somewhat difficult to explain. The small left metapleur actually moves in course of growth from the mid-line, and rises on to the right side somewhat. At the same time the much larger right metapleur is deepened, and overhangs the slits. Then the little horizontal junction is effected, and we get actually a nearly tubular atrium receiving the openings of successive gill slits. With subsequent growth the narrow atrial

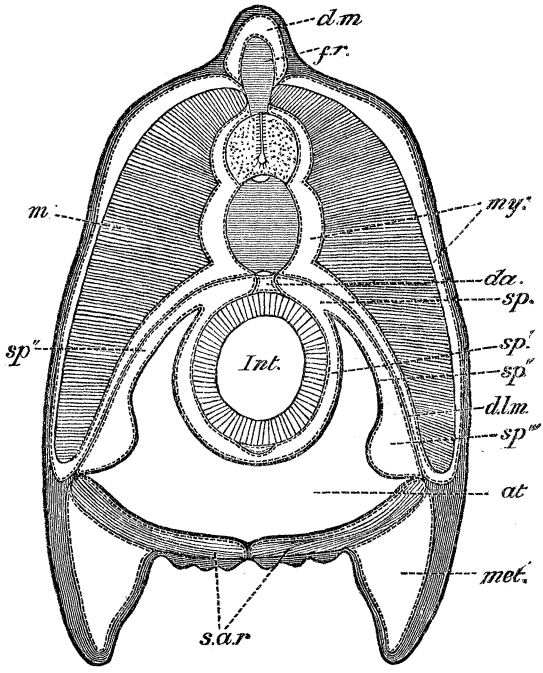
FIG. 8.



Transverse section through an advanced larva with fully-closed atrium. The latter has begun to encroach on the cœlom (*sp*).

Letters as in figs. 6 and 7.

FIG. 9.



Transverse section through an adult *Amphioxus*. The atrium has grown up so as to divide the primitive splanchnocœl into two portions—an inner or splanchnic, and an outer or parietal, portion (*sp'*. and *sp.''*).

sp. The portion of the primitive splanchnocœl which is not so affected by the atrium, and which persists as the dorsal cœlom.

sp''. The parietal part of the splanchnocœl.

sp'''. Its expansion as perigonadial cœlom.

f.r. Fin-ray.

Other letters as in figs. 6 and 7.

(The above figures 6—9 are all somewhat diagrammatic.)

tube widens and pushes itself right and left, so as to encroach on the space hitherto occupied by the cœlom, and finally it extends so far dorsalwards as nearly to surround the alimentary canal (see figs. 8 and 9).

The evidence of this history, in the form of careful drawings of consecutive sections from snout to atriopore, at various stages in the closure of the atrium, I purpose to publish shortly in the 'Quarterly Journal of Microscopical Science.' For the present I am anxious to point out, firstly, that this mode of formation of the atrium as a narrow groove, which closes and sinks (as it were) into the body of the *Amphioxus*,

is really different in important respects from the enclosure of a space by downgrowth of large folds, though ultimately no doubt the two contrasted modes of formation come to the same thing so far as the more obvious morphological relations are concerned. The mode of formation which really occurs in *Amphioxus* is readily harmonised with the existence of the post-atrioporal extension of the atrium which gradually tapers to a fine cæcal canal. It also gives us an essentially different view of the region called "epipleur" by Lankester, and generally so designated, from that which Rolph's theory necessitated. That portion of the epipleur into which the myotomes of the body-wall extend is seen now to be no downgrowth, no extension or fold. It is the original unchanged body-wall which bounds the sides of the animal's body in front of the atriopore, just as much as it does behind. The only *new* growth in the atrial region which takes part in the limitation of the surface is the sub-atrial growth formed by the two little horizontal folds which floor in the atrium when it is a mere canal. These in the adult are represented by the limited region of longitudinally pleated ventral wall between the two metapleura.

Lastly, the formation of the atrium as a narrow groove which closes, sinks into, and expands within the body of *Amphioxus*, is much more readily comparable to what is known of the formation of the atrial chamber in the Ascidians than is the Kowalewsky-Rolph scheme. In the Ascidian a pair of in-pushings are formed, each with a circular orifice of invagination; they expand within the body, fuse with one another to form one cavity, and one of the circular orifices disappears. In *Amphioxus* we have a single in-pushing with a longitudinal orifice of invagination, which closes as the invagination forms, excepting at its hindermost border, and then expands to a greatly increased volume.

The comparison of the so-called epipleura of *Amphioxus* with the opercula of Fishes has only a remote morphological basis, and probably no genetic relationship exists between these two structures.

IV. "On a Method of determining the Value of Rapid Variations of a Difference of Potential by means of the Capillary Electrometer." By GEORGE J. BURCH, B.A. Communicated by Professor BURDON SANDERSON, F.R.S. Received April 25, 1890.

In 1882 a paper by Professor Burdon Sanderson* appeared in the 'Biologisches Centralblatt,' in which an account was given of the

* Burdon Sanderson, "Die elektrischen Erscheinungen am Dionaeablatt," 'Biologisches Centralblatt,' 15 Oct., 1882.

FIG. 1.

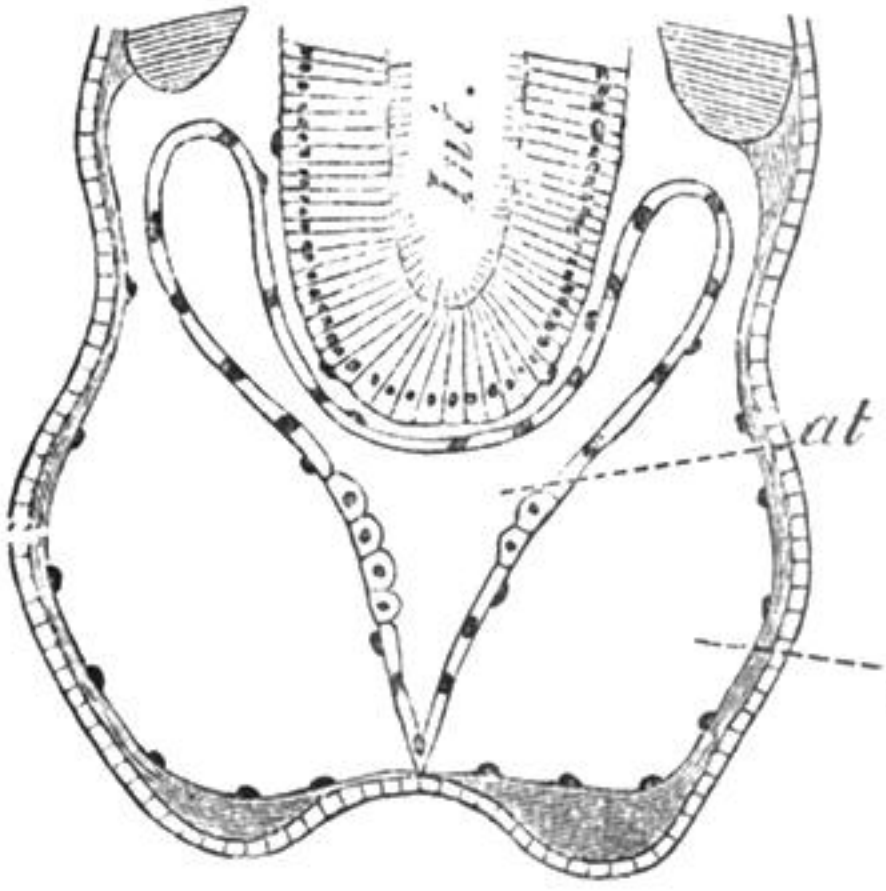
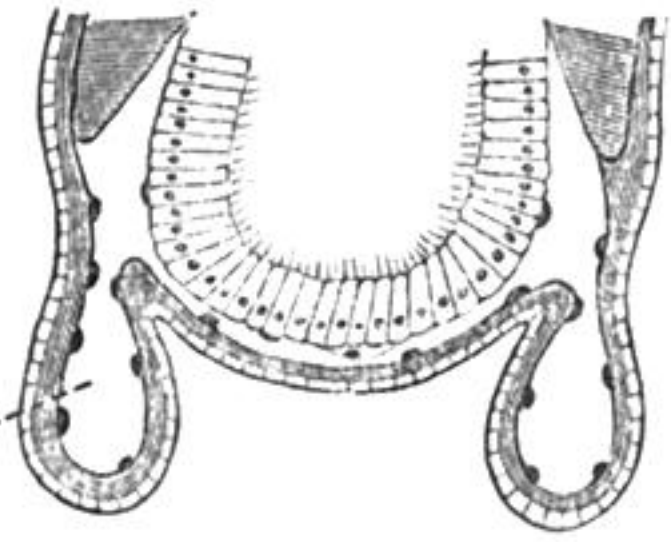


FIG. 2.



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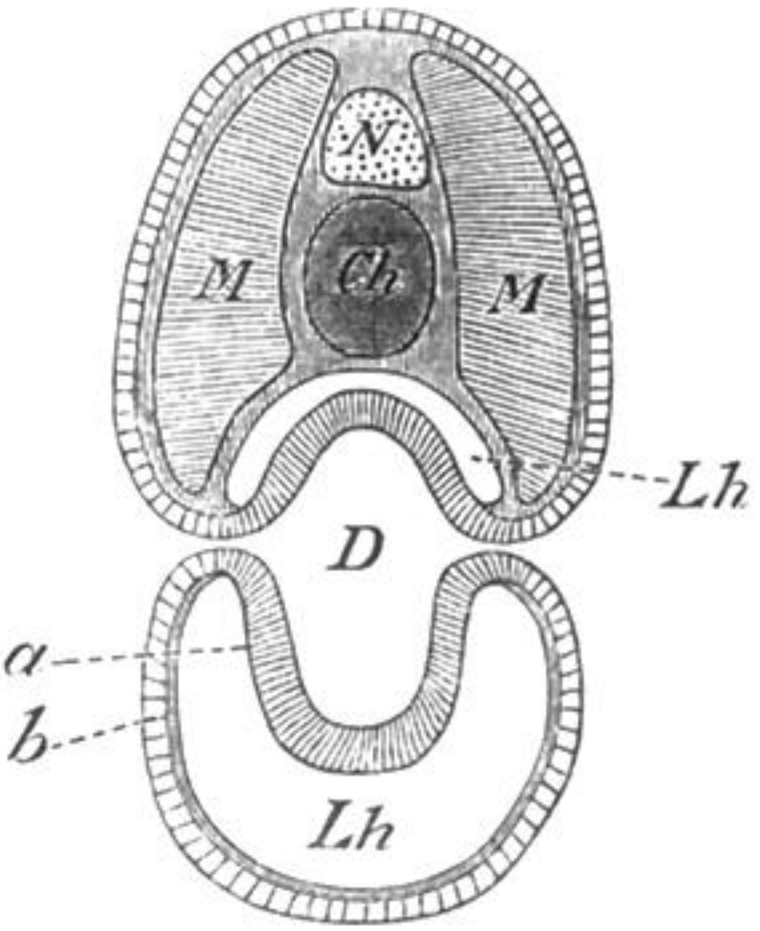
Int. Intestine. *at.* Atrium. *l.h.* Cœlom.

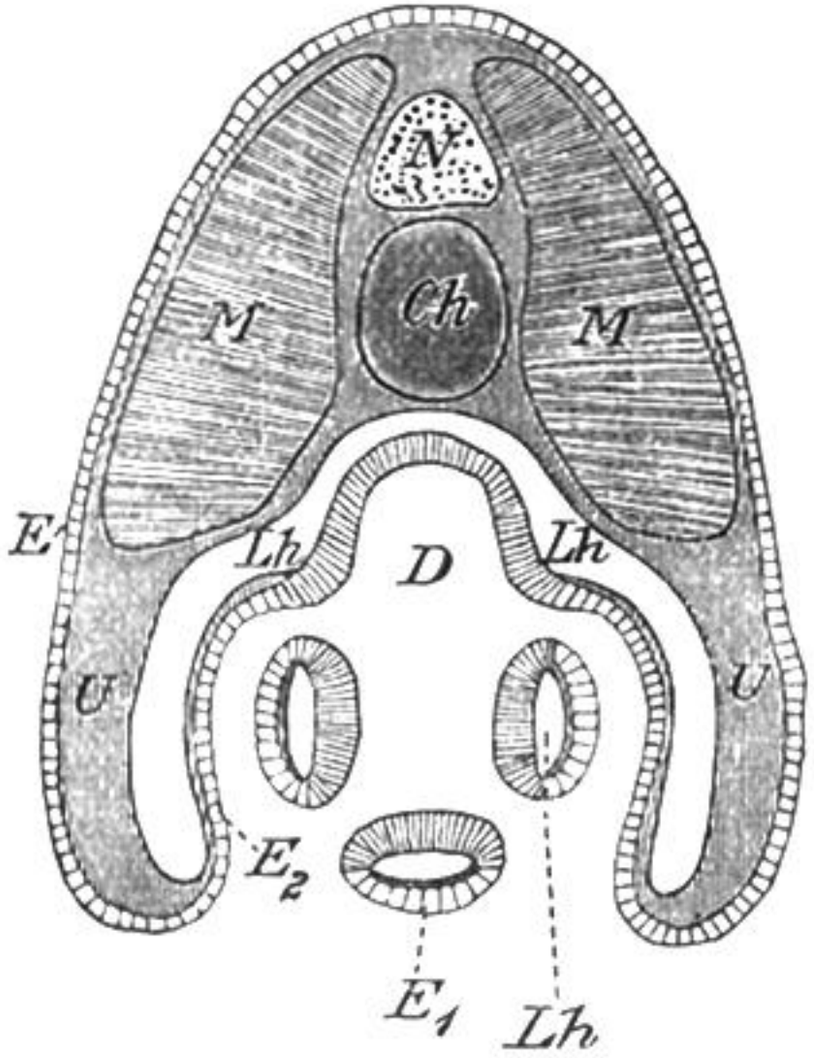
FIG. 3.



Copy of Rolph's theoretical section through the pharyngeal region of a larva, before the commencement of the so-called epipleural folds.

N. Nerve-cord.
M. Muscles.
D. Intestine.
b. Epidermis.
Ch. Notochord.
Lh. Cœlom.
a. Intestinal epithelium.

FIG. 4.



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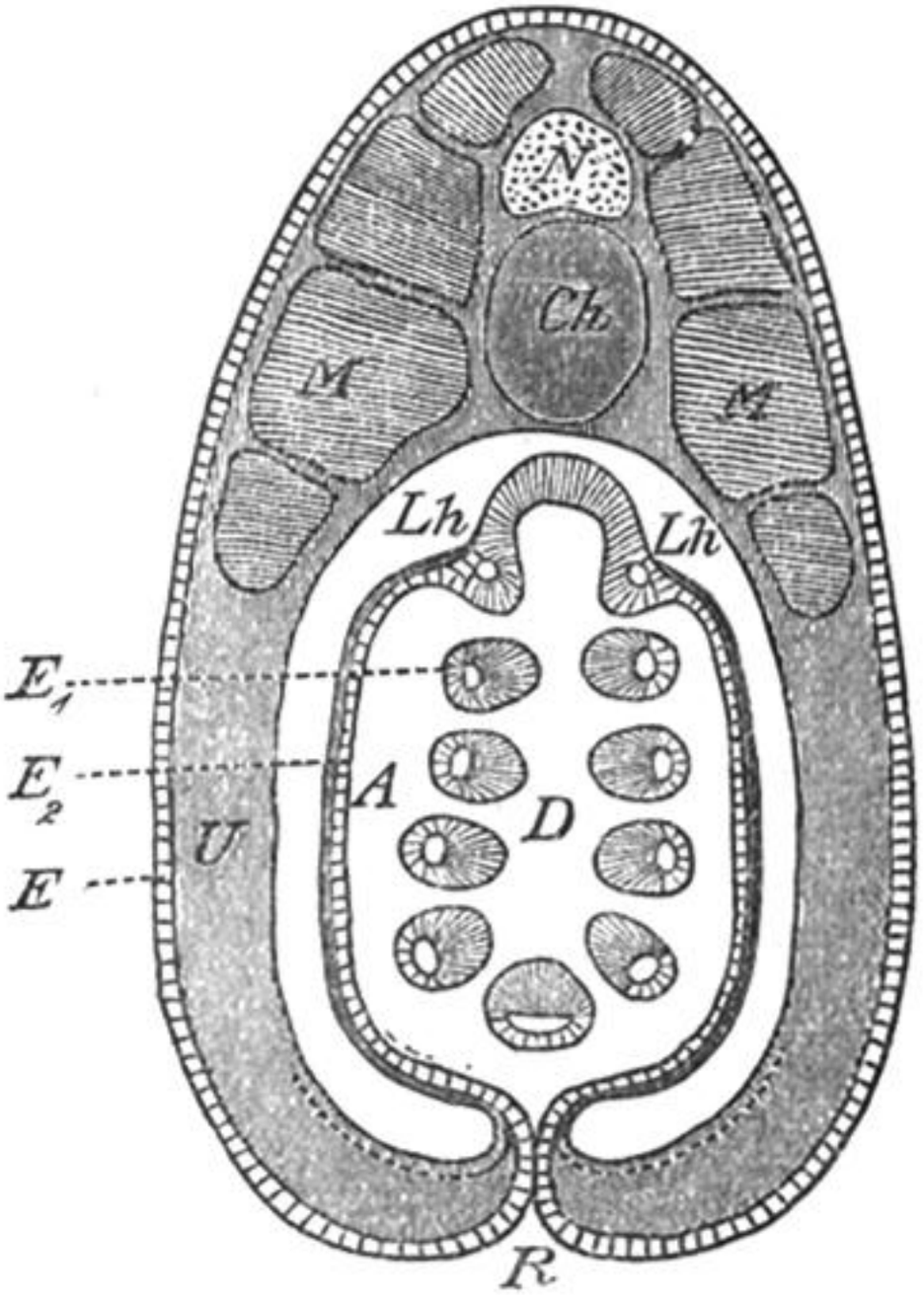
Other letters as in fig. 3.

FIG. 5.



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A. Atrium. *R*. Raphe.

Other letters as in figs. 3 and 4.

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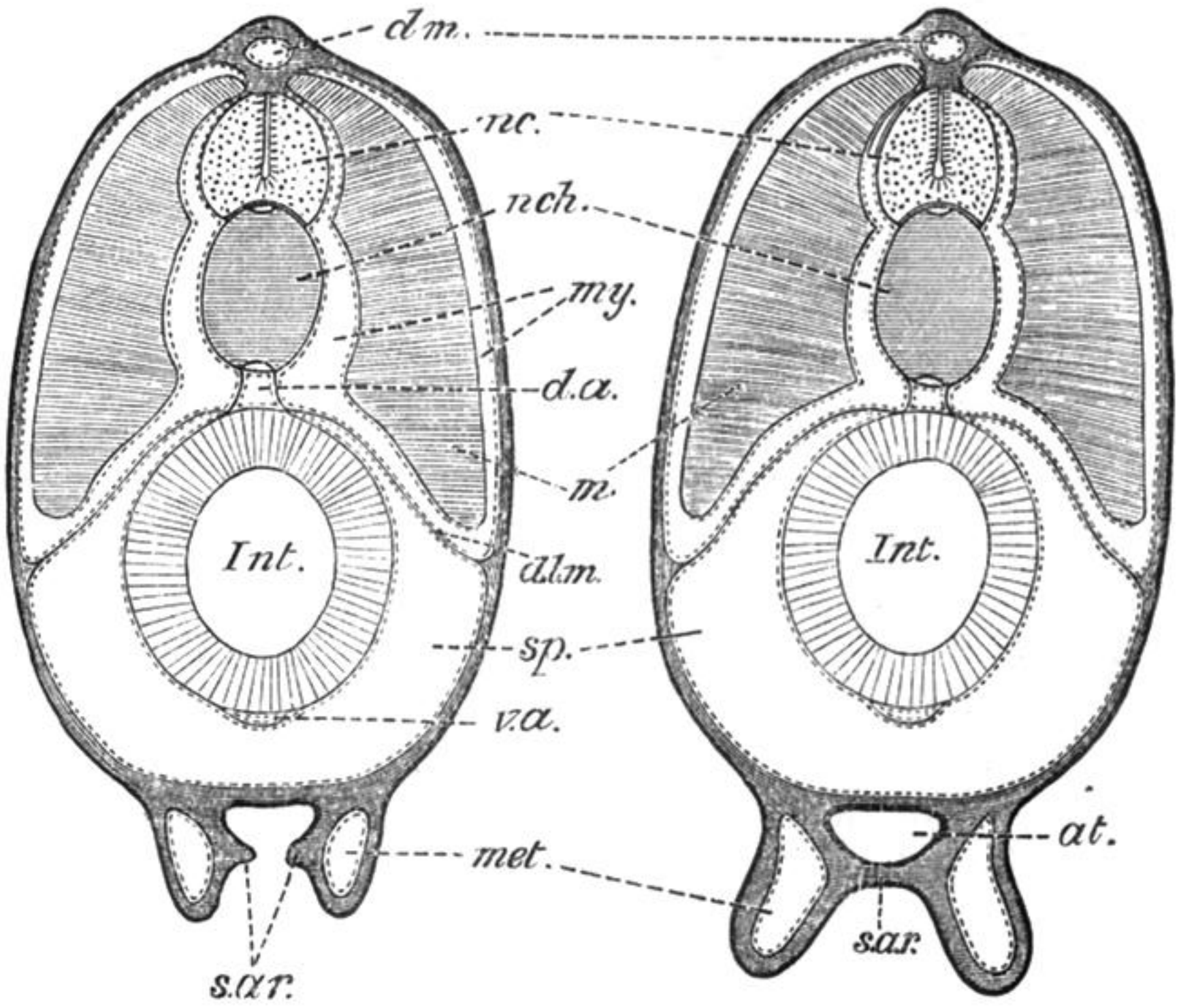

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my. Cavity of myocœl.
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Int. Intestine.
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sp. Primitive splanchnocœl.
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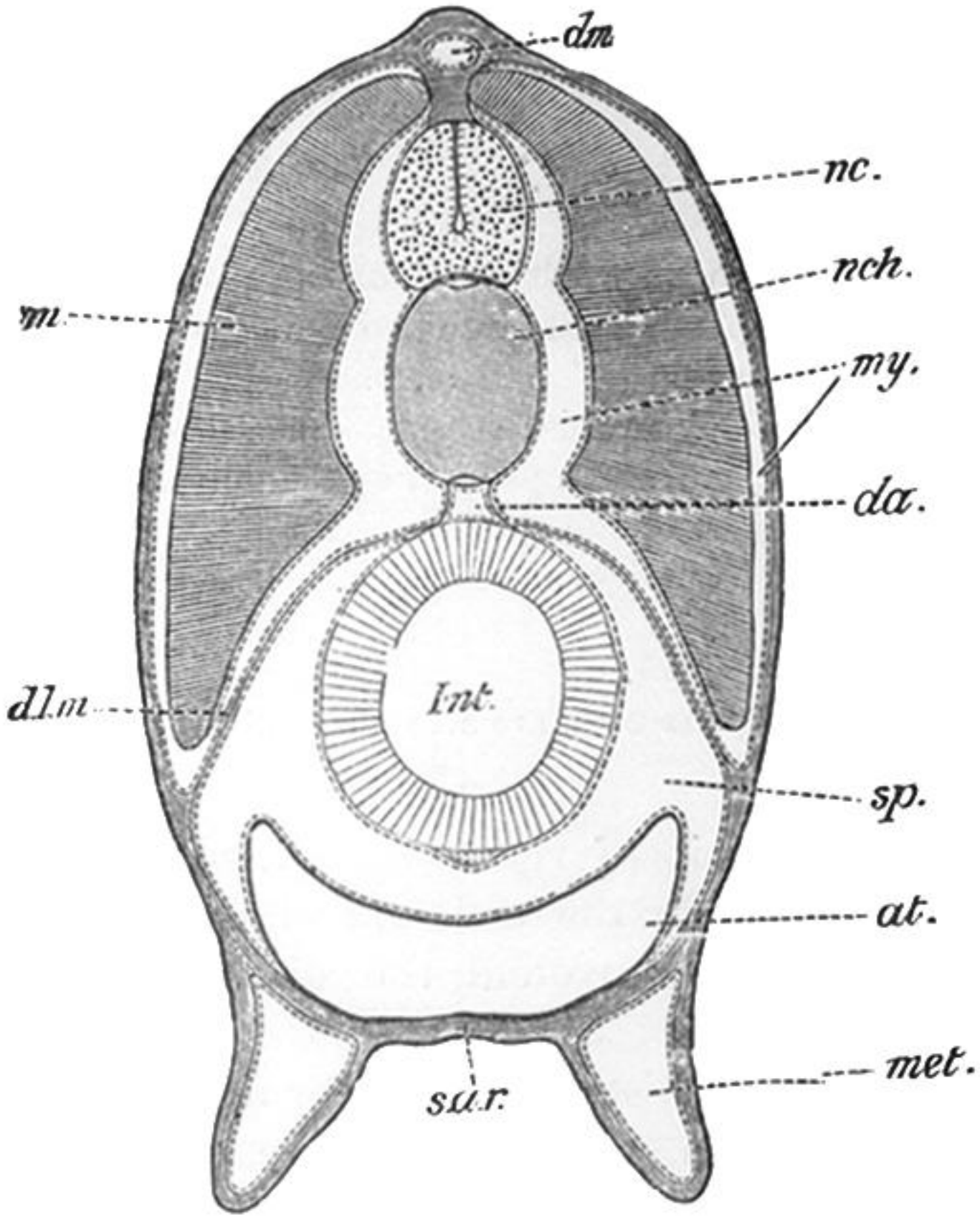
FIG. 7.—Transverse section through a slightly older larva. The sub-atrial ridges (*s.a.r.*) have fused for a short distance between atriopore and pharynx; but in the pharyngeal region the atrium is unclosed, and consequently the gill-slits still open directly to the exterior.

at. Atrium.

FIG. 8.



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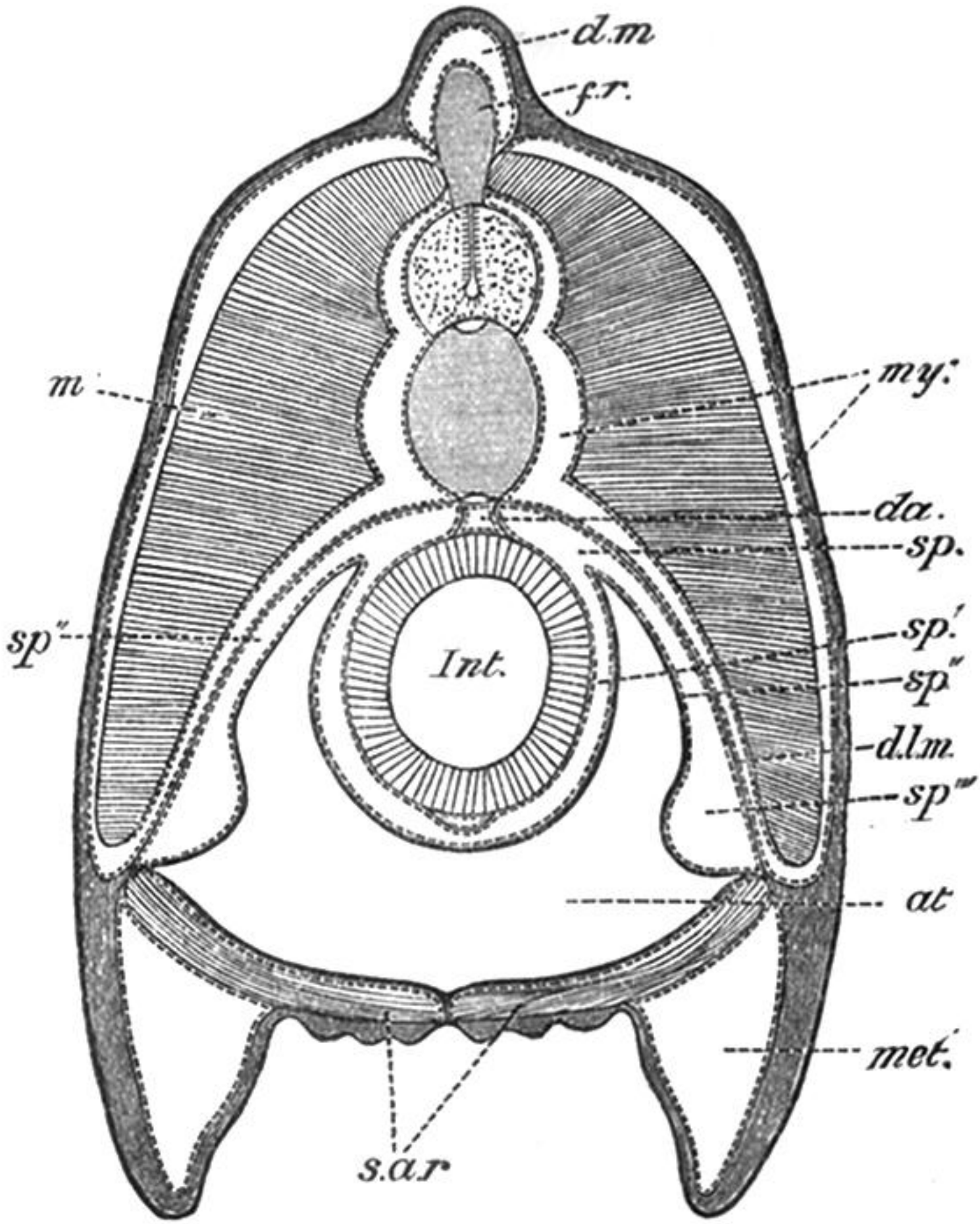

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