be regarded as the most primitive members of the order Ascidiacea, and that *Botryllus* and the Styelinæ must take this position; for in the structure and development of the pharynx, as well as in other points, with which I shall fully deal elsewhere, the latter forms approach, more nearly than any other Ascidians, the ancestral type represented by *Pyrosoma*.

V. "Observations on the Post-Embryonic Development of *Ciona intestinalis* and *Clavelina lepadiformis*." By ARTHUR WILLEY, B.Sc. Lond. Communicated by Professor RAY LANKESTER, M.A., F.R.S. Received May 4, 1892.

The following is an account of some of the observations which were made by the author during an occupation of the British Association Table at the Zoological Station at Naples from October, 1891, to May, 1892.

In their admirable "Recherches sur la Morphologie des Tuniciers," ('Archives de Biologie,' vol. 6, 1887), Édouard van Beneden and Charles Julin came to a number of conclusions which, while they appeared to follow naturally from the facts observed, yet only added, if possible, to the perplexity surrounding any attempt to regard the Ascidians and *Amphioxus* from a common standpoint. Led away by the remarkable behaviour of the endostyle which I observed and described in the larva of *Amphioxus*, I easily induced myself to accept the views of the Belgian *savants*.

The observations on the post-embryonic development of *Ciona* described below oblige me, however, to reconsider the position which I took in my paper on "The Later Larval Development of *Amphioxus*" ('Quart. Journ. Micro. Sci.,' vol. 32, 1891), with regard to the mutual relations of the Ascidians and *Amphioxus*, and may, I hope, tend to the establishment of reasonable homologies between them.

It is necessary to recapitulate very briefly the views of van Beneden and Julin, in order to bring those which I am about to oppose to them in the most striking contrast.

The following table shows at a glance the homologies suggested by the above-named authors:—

(*a.*) The anterior intestinal diverticula of *Amphioxus*, the right one of which becomes the large head- or, better, proboscis-cavity, while the left becomes the præoral pit. = The primary branchial canals of Ascidians (*i.e.*, the first pair of gill-slits; see below).

(*b.*) The club-shaped gland of *Amphioxus.* } = The intestine of Ascidians.
(*c.*) Gill-slits of *Amphioxus*, *unrepresented* in Ascidians.
(*d.*) Atrial cavity of *Amphioxus*, *unrepresented* in Ascidians.

Of the above propositions, the last may be true; but I shall proceed to show that the others are untenable.

Fixation of the Larva of Ciona.

Just before the larva fixes itself, a narrow space can be discerned between the anterior end of the endoderm and the tract of ectoderm which bears the adhering papillæ; and in it lies a compact group of mesoderm cells.

Shortly after fixation this space swells up prodigiously, and then contains loose scattered mesoderm cells. I shall call this the *proboscis-cavity*. At its base (*i.e.*, where it joins on to the body) lies the endostyle dorso-ventrally. The primary position of the endostyle is extremely important. It behaves exactly as it does in the larva of *Amphioxus*, in that it occupies at first the most anterior region of the alimentary canal, and lies at right angles to the position which it assumes later.

In fact, the trunk of the young fixed Ascidian undergoes an actual rotation through an angle of 90° as the result of which the mouth, which was at first dorsal, becomes terminal, and the endostyle takes up its definite longitudinal position at the base of the branchial sac.

In comparing the accompanying figures (1 and 2) the attention should, first of all, be concentrated on the endostyle, and then the structures which precede and follow it in both cases should be taken into consideration. When that is done, I think the inadmissibility of van Beneden's and Julin's view of the homology of the first pair of gill-slits (primary branchial canals) of Ascidians with the proboscis-cavity and præoral pit of *Amphioxus* (anterior intestinal diverticula) will at once become evident.

In *Clavelina* the behaviour of the proboscis-cavity is essentially the same as in *Ciona*.

Origin of the Gill-slits in Ciona *and* Clavelina.

The post-larval appearance of the gill-slits of the simple Ascidians has been studied to a certain extent only by P. J. van Beneden, Krohn, Kupffer, and Éd. van Beneden and Julin; and most thoroughly by the last two authors. The first three observed young Ascidians with two branchial apertures on each side. Van Beneden and Julin ("Rech. sur le Développement postembryonnaire d'une

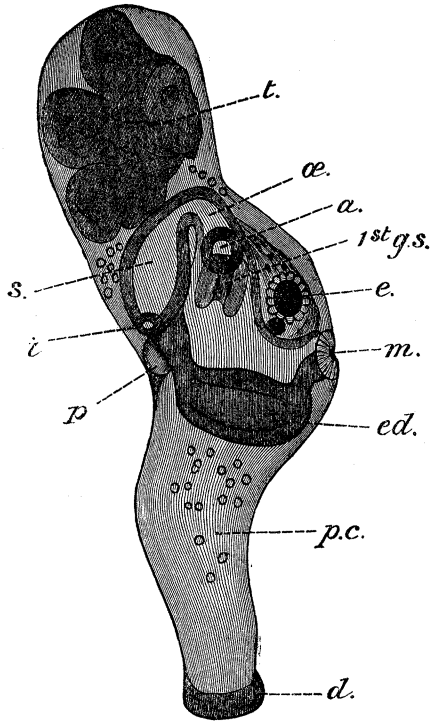

FIG. 1.—A young *Ciona*, shortly after fixation. From the right side. Drawn with cam. luc., Zeiss 3 C.

N.B.—The atrial aperture is merely the external aperture of the first gill-slit.

Explanation of Letters.—*t.*, remains of tail; *œ.*, œsophagus; *a.*, atrial aperture of this side; 1*st g. s.*, first gill-slit (in *Ciona*, double from the beginning); *e.*, eye; *m.*, mouth; *ed.*, endostyle; *p. c.*, proboscis cavity; *d.*, adhering disc; *p.*, pericardium; *i.*, exit of intestine from stomach; *s.*, stomach.

Phallusie," 'Arch. de Biologie,' vol. 5, 1884) commenced with individuals possessing four on each side. In all cases they were supposed to represent gill-slits which had developed by independent perforations.

Van Beneden and Julin, judging from the sizes of the slits, came to the conclusion that they formed in a very irregular manner, the first slit formed being the fourth of the series, and so on. In other words, the true first gill-slit of the Ascidians has been, up to the present, unknown; and it is owing to the ignorance which has hitherto prevailed with regard to this slit that the relations between the Ascidians and *Amphioxus* have been so little understood. Thus,

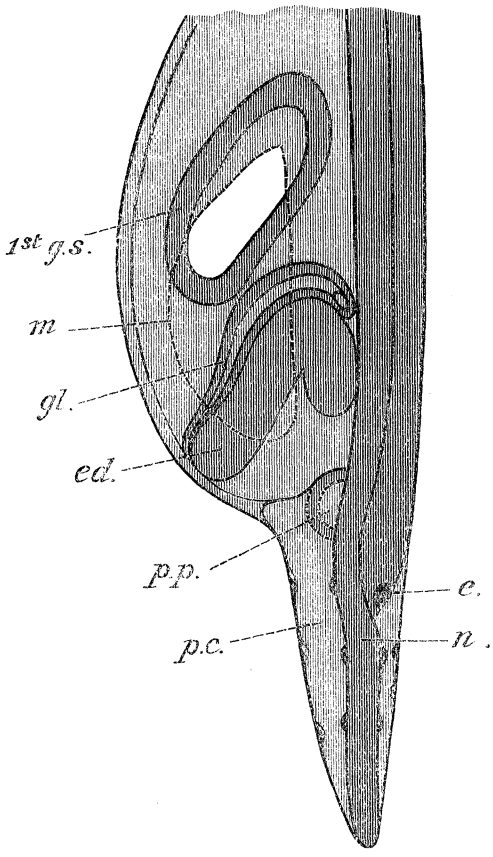

FIG. 2.—Anterior end of young larva of *Amphioxus*. From right side. Præoral pit (*p. p.*) and mouth are seen through.

gl., club-shaped gland; *n*, notochord. Other letters as in fig. 1.

up to the present time there has been no gill-slit described in Ascidians which possessed characters peculiar to itself, and which separated it from the rest. Such a gill-slit, however, exists, and I will now describe it.

Starting with the stage in which two oval apertures are present on each side (in *Ciona*), we find that, as time goes on, these elongate very considerably in the transverse direction, and eventually become twisted round in a curious way at their distal ends (that is, the ends towards the endostyle), and finally a small portion becomes constricted off from the distal end of each of the original slits (fig. 3).

In this way, therefore, we arrive at the stage with four branchial

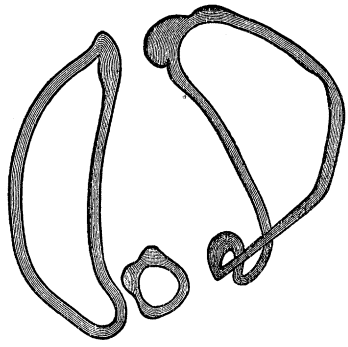

FIG. 3.—Primary branchial apertures of right side of *Ciona*, showing the way in which the stage with four slits on each side becomes established. Drawn from living object. Zeiss 4 B, cam. luc.

stigmata on each side. The slits which form after this (5th and 6th, &c.) arise by independent perforation.

The point is now to determine the origin of the first two slits, and this is by no means easy. By the study of great numbers of living specimens, and more especially of horizontal sections, I have convinced myself that the following is what takes place. It has been known for many years that the slits of *Amphioxus* become each divided into two halves by the formation of a tongue-bar. In the larva, however, the unpaired slits are simple, and remain simple till near the end of the period of metamorphosis. During this period the slits of the second row make their appearance, and very soon afterwards, the primary slits being still simple, tongue-bars begin to form in the secondary slits. Thus in this case the tongue-bars are considerably hastened in their development. If, now, they were hastened a little more, what we should see would be that the two halves of the slit would become independently perforated. *This is actually what occurs in* Ciona. I have very convincing evidence for this point of view, which I hope to produce in detail, accompanied by figures, later. The first four stigmata, therefore, are derived from, and represent, *one gill-slit.*

The primary branchial canals of van Beneden and Julin are simply the first pair of gill-slits. The atrial involutions are at first nothing but the ectodermic portions of a pair of gill-slits. They remain permanently in this condition in Appendicularia; but in the fixed Ascidians they become secondarily expanded to form a distinct chamber. The view of van Beneden and Julin, that the visceral wall of the atrium is endodermic, while the parietal wall is ectodermic, I consider to be unfounded.

The walls of the atrial chamber are apparently derived essentially from the ectoderm.

The first pair of gill-slits which becomes sub-divided in the remarkable way above described (totally different from the transverse sub-division which they subsequently undergo), is present in a simple undivided form in Appendicularia, and I consider it, both from its position and from its specialised character, to be undoubtedly homologous with the first pair of gill-slits in *Amphioxus*, which disappear at the close of the larval period. Both in *Ciona* and in *Amphioxus* this first pair of slits alone serves for the respiration of the larva (or, in the case of *Ciona*, young individual) for several weeks, during which the size of the animal is increased, but no new organs are added. In other words, there is a resting stage in the development of *Amphioxus* and *Ciona*, which is characterised in both cases by the presence of a single pair of gill-slits, namely, the first pair.

By the first pair of gill-slits of *Amphioxus*, I refer to the first gill-slit proper, and to the club-shaped gland. I have on a former occasion given cogent reasons for regarding these two structures as a pair of gill-slits.

In *Ciona*, at a later stage, the primary stigmata, whose origin has just been described, become divided in the usual way and give rise to the transverse rows of stigmata. *In* Clavelina *these transverse rows of stigmata form in the first instance, each aperture being an independent perforation.* Here, then, we have unequivocal evidence of considerable modification in the direction of a shortening of the development in the case of *Clavelina;* and we meet with similar evidence at every turn.

Origin of Pericardium and Heart in Ciona *and* Clavelina.

With regard to the origin of the pericardium, my observations appear to confirm, in the main, the account given by van Beneden and Julin as to its being endodermic; but, as I have never succeeded in finding karyokinetic figures in connexion with its development, a precise statement as to its mode of appearance is not at present possible.

In *Clavelina*, where it is comparatively easy to be persuaded of its endodermic origin, it arises at a much earlier stage in the development of the larva than it does in *Ciona*. In the former it arises before the formation of the body-cavity, while in the latter there is a wide body-cavity present at the time of its first appearance, containing loose mesoderm cells; and the failure to find nuclear spindles, combined with the extraordinarily small size of the object in transverse section, renders it extremely difficult to assign its origin to the endoderm

with certainty, although, from the appearances presented, and also from the analogy of *Clavelina*, it is probable that it arises in the same way in both cases.

The formation of the heart presents interesting differences in the two forms. In *Clavelina*, as already shown by van Beneden and Julin, the septum which, at first, divides the pericardium into two halves breaks down, and the heart forms as an involution of the dorsal wall of the pericardium. In *Ciona* the septum does not break down, and the heart forms by a splitting apart of the two layers which compose the septum.

Conclusions.

What has been said above is enough to show that the development of *Ciona* presents much more primitive features than that of *Clavelina*. It now remains to compare the conditions in *Ciona* with those that obtain in *Amphioxus*, and to seek to establish the true homologies between the various parts.

In *Amphioxus*, what I propose to call the proboscis-cavity is lined by a flat epithelium, and so is the rest of the body-cavity. In *Ciona*, the proboscis-cavity contains loose mesoderm cells in place of an epithelium, and so does the rest of the body-cavity. The distinction between mesoderm and mesenchym is no longer generally recognised as fundamental.

The presence of the præ-oral pit as a pair to the proboscis-cavity of *Amphioxus* seems, at first sight, to present a difficulty in the way of the comparison which I am making; but it is not so serious as might be supposed, and, for the rest, I need only refer here to what occurs in different species of *Balanoglossus*.

In instituting any comparison between the Ascidians and *Amphioxus*, the endostyle should be taken as the starting point, and the fact should be remembered that its primary axis is perpendicular to its definitive axis in both cases.

Making allowance for the secondary change of position which the mouth has undergone in the larva of *Amphioxus*, in correlation with the forward extension of the notochord, we find, therefore, that the relative position of the various organs from before backwards is precisely the same in *Ciona* and in *Amphioxus*, namely, (1) proboscis-cavity, (2) endostyle, (3) mouth, (4) first pair of gill-slits.

It should be remarked that the mesoderm which lies in the proboscis-cavity of *Ciona* has a bilateral origin, corresponding more or less closely to the pair of anterior intestinal diverticula of *Amphioxus*.

It is most important to establish the homology of the cavities of *Ciona* and *Amphioxus* on a sound basis. The endostyle admittedly occupies the same position primarily in both animals.

If, then, the question be asked, "What lies in front of the endostyle?" the immediate response is, "In both cases the proboscis-cavity."

I accordingly submit the following table of homologies:—

(*a*.)	Proboscis-cavity of Ascidians	= Proboscis-cavity and præoral pit of *Amphioxus*.
(*b*.)	Endostyle of Ascidians	= Endostyle of *Amphioxus*.
(*c*.)	Mouth of Ascidians	= Mouth of *Amphioxus*.
(*d*.)	First pair of gill-slits of Ascidians, in the improved sense of the term.	= First pair of gill-slits of *Amphioxus*.

The homology of the club-shaped gland of *Amphioxus* with the intestine of Ascidians, as suggested by van Beneden and Julin, would seem, therefore, to be quite out of the question.

It need hardly be pointed out that, if the homologies which I have advanced are really correct, then the relations between *Amphioxus* and the Ascidians become much less strained than they were on the views previously entertained.

I intend shortly to discuss the whole subject more elaborately in the pages of the 'Quarterly Journal of Microscopical Science.'

VI. "The Human Sacrum." By A. M. Paterson, M.D., Professor of Anatomy in University College, Dundee, St. Andrews University. Communicated by Professor D. J. Cunningham, D.Sc., F.R.S. Received April 18, 1892.

(Abstract.)

Owing to the now classical investigations of Gegenbaur and Frenkel, and the more recent researches of other observers, the several homologies of the vertebral column are distinctly understood. The specific or individual differences in the correlation of one region of the column to another can be adequately explained on the assumption of a suppression or excessive development of the potential costal element of the vertebral segment. This costal element may be metamorphosed in different ways to suit the needs of the animal economy, and the variations in individual cases affect the segments at the ends of a series where the vertebræ of one region possess characters resembling those of a neighbouring region. This hypothesis renders intelligible, not only the existence of cervical ribs, but also correlated variations of the thoracico-lumbar region, and abnormalities of the sacrum, differences in the number of bones, as well as asymmetry.

During recent years this aspect of the subject and numerous

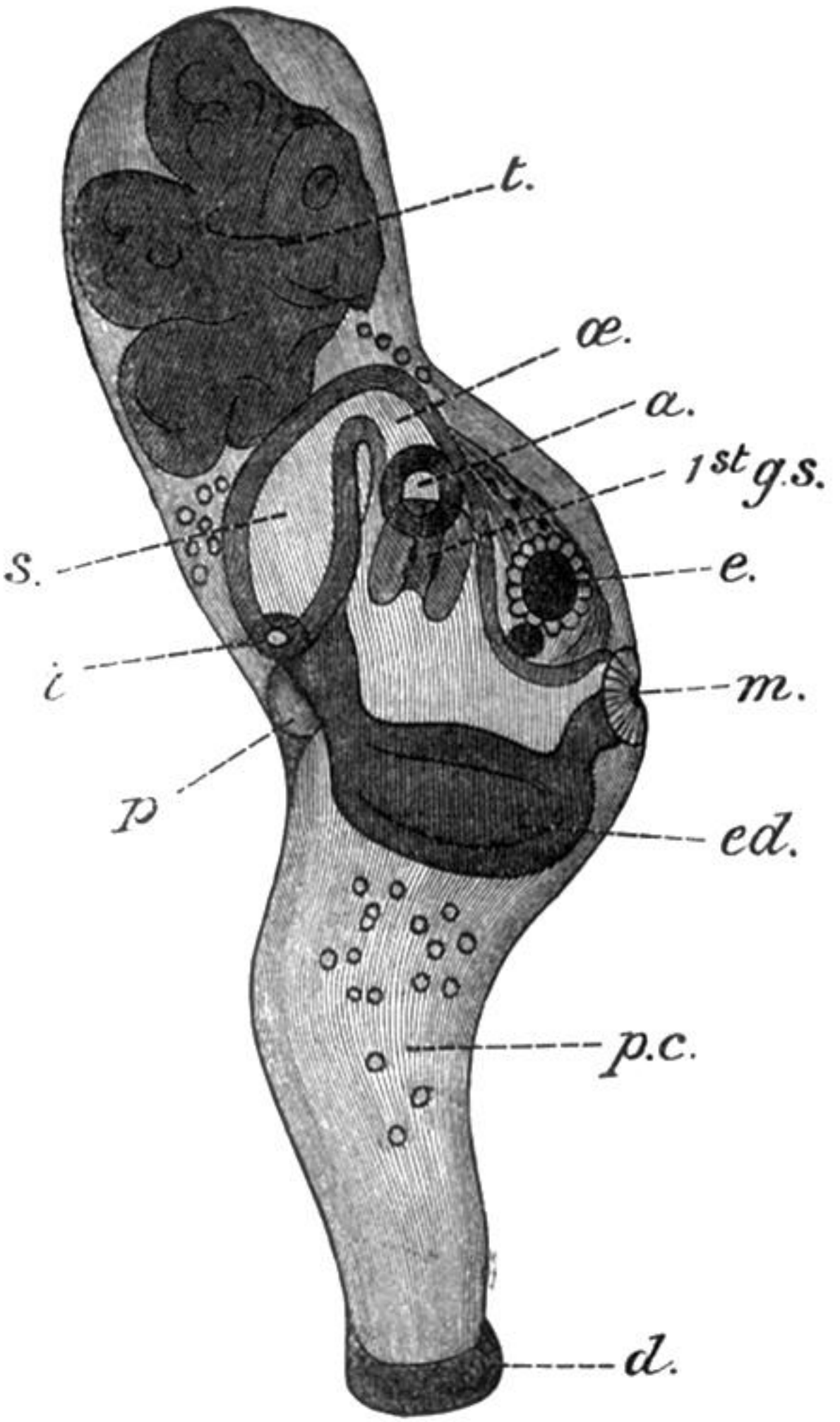

FIG. 1.—A young *Ciona*, shortly after fixation. From the right side. Drawn with cam. luc., Zeiss 3 C.

N.B.—The atrial aperture is merely the external aperture of the first gill-slit.

Explanation of Letters.—*t.*, remains of tail; *œ.*, œsophagus; *a.*, atrial aperture of this side; 1*st g. s.*, first gill-slit (in *Ciona*, double from the beginning); *e.*, eye; *m.*, mouth; *ed.*, endostyle; *p. c.*, proboscis cavity; *d.*, adhering disc; *p.*, pericardium; *i.*, exit of intestine from stomach; *s.*, stomach.

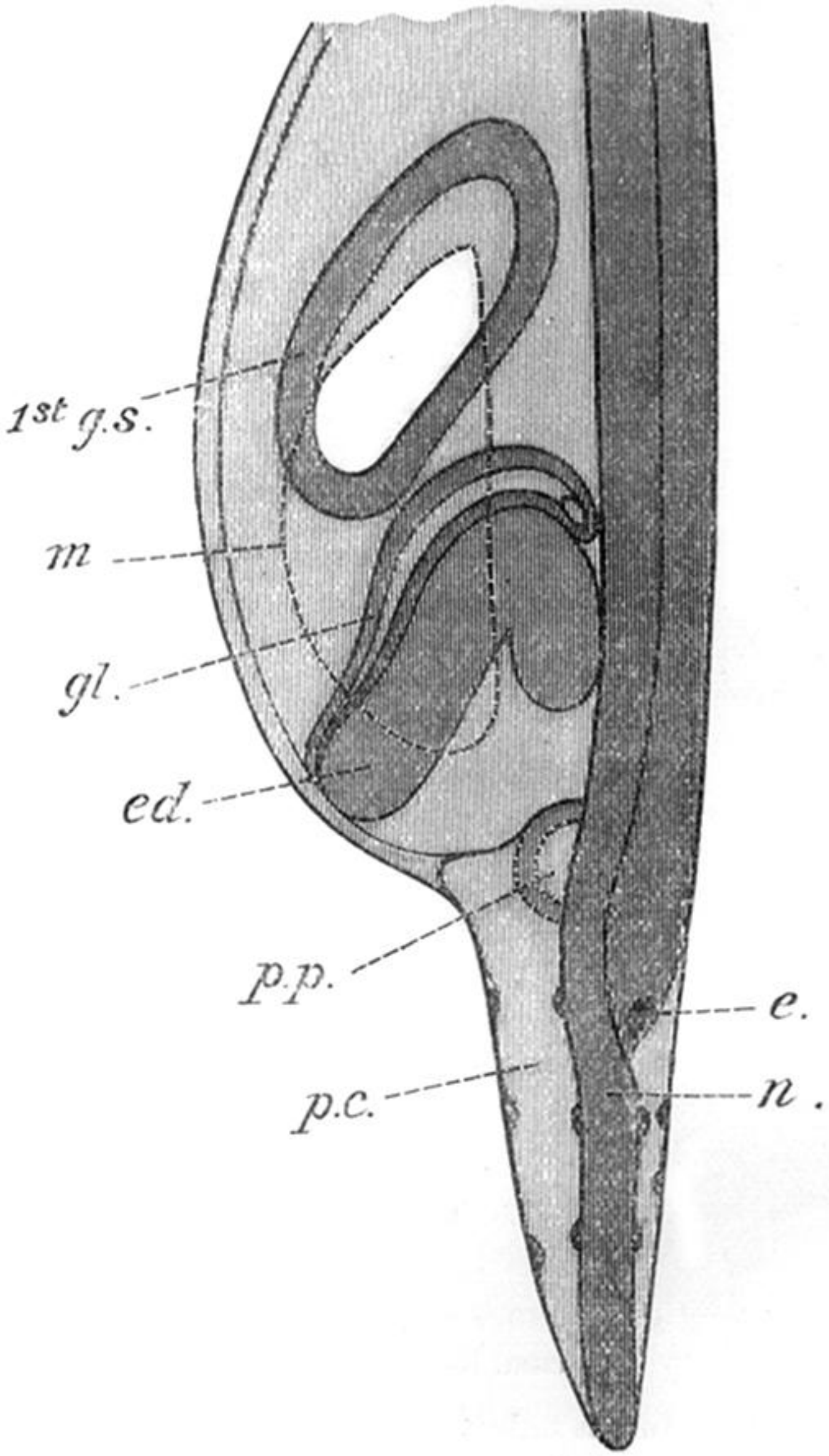

FIG. 2.—Anterior end of young larva of *Amphioxus*. From right side. Præoral pit (*p. p.*) and mouth are seen through.

gl., club-shaped gland; *n*, notochord. Other letters as in fig. 1.

FIG. 3.—Primary branchial apertures of right side of *Ciona*, showing the way in which the stage with four slits on each side becomes established. Drawn from living object. Zeiss 4 B, cam. luc.