X. *On the Development of an Estherid Crustacean.*

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[Plates 18–24.]

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Introduction.

In the spring of 1922 the author obtained a sample of dried mud that contained eggs of Estherid Crustacea. It was collected in the neighbourhood of Baghdad by Capt. T. Buist, R.A.M.C. On placing it in water the Estherid nauplii hatched out within 24 hours. At the same time a large crop of protozoal and bacterial micro-organisms developed, and on these the nauplii fed. They thrived well on this diet and did not need any further food supply. They grew to the adult stage in about three weeks.

The adult specimens were identified by Mr. Robert Gurney as belonging to two genera. Out of about twenty adult specimens two were *Leptestheria* sp., while the remainder belonged to the genus *Estheria* (=Cyzicus or Eocyzicus). Since these two genera differ from each other only in minute details, and since the examination of the larvæ did not indicate two types of larvæ, they will be referred to simply as Estheria larvæ.

The following paper deals with some points in the post-naupliar development of these Estheria larvæ. The earlier development it was unfortunately found impossible to investigate. The eggs would only develop if they had been dried up in the mud, and from this it was not possible to isolate them owing to their minute size.

The thanks of the author are due to Capt. T. Buist, R.A.M.C., who supplied the mud containing the Estherid eggs, and to Dr. W. T. Calman, F.R.S., through whom the first supply of mud was obtained. To the latter, and also to Prof. E. W. MacBride, F.R.S., the author wishes to express his indebtedness for much advice and criticism.

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Method.

The larvæ were obtained at first solely from the mud that had been sent from Baghdad, but later a certain number of larvæ were reared from eggs that had been laid in the laboratory.

The adult females, after pairing, laid batches of about 100 eggs in their brood pouches. These were shed at the next ecdysis with the cast cuticle. They did not develop directly, but some did so after the mud into which they had been shed had been gradually dried, kept dry for several weeks, and then again placed in water.

When the mud first arrived, the number of larvæ that could be hatched from it was considerable, and a large proportion of these grew to maturity. After a few months, however, the yield of larvæ was much smaller and a far smaller proportion survived to maturity. There was certainly a diminution in the number that hatched, but the fact that so few survived must be attributed, partly at least, to the fact that the crop of micro-organisms hatching out with the nauplii became progressively smaller, and hence the food supply became insufficient. It was attempted to make up this deficiency with protozoal cultures reared in the laboratory, but without success. The micro-organisms at first consisted of various flagellates and ciliates as well as bacteria, but subsequently the bacteria only were evident and these in far fewer numbers.

The youngest stages were very easy to obtain, despite the fact that they were exceedingly minute, as they always collected at the surface of the water at the side of the vessel nearest the light. This may have indicated a positive phototropism in the larvæ, as has been recorded in the case of other Phyllopoda. However, it must be borne in mind that the larvæ may have gathered in the region where the food was most plentiful, the phototropism being exhibited by the food organisms rather than the larvæ.

Various fixatives were used. At first cold saturated sublimate in distilled water was used. Then it was found that strong Flemming gave quite good fixation. Finally Flemming without acetic was used, and this gave excellent results. Since this fixative is admittedly one of the best general cytological fixatives, it was used in all subsequent work. It was allowed to act for about $1\frac{1}{2}$ hours only. Longer fixation gave no better results.

The larvæ, which were about 180–200μ long on hatching, were each embedded separately in paraffin wax, xylol being used as the clearing agent. The larvæ were tinged with eosin in absolute alcohol to make them distinguishable in the paraffin bath. Each one was removed separately from this bath with a hot pipette and placed in a drop of melted wax in a watch-glass. They were then orientated under a $\times 16$ lens, using a hot needle for the purpose.

The larvæ, naturally, were not visible in the wax button when it had solidified. In order to mark their position in the wax, four marks were made on the solidifying button before this had cooled sufficiently to obscure the larvæ. Two of these marks were made on the line passing through the head and tail of the larva. The mark on the head side was coloured red by just touching it with a red " glass " pencil. The other two marks were on a line at right angles to this line, passing through the body of the larva. The

larva was thus situated at the junction of the lines joining the two pairs of marks, its head pointing towards the red mark. A strip of wax was then cut out along the line joining the red mark with the diametrically opposite mark if a series of transverse sections was needed, and along the line joining the other two points if a sagittal or frontal series were required. For sagittal sections the larva was embedded in the wax resting on its ventral surface, while for frontal sections it was supported on its side with the warm needle until the wax below it had solidified sufficiently to hold it in position. Although such small objects as the newly hatched larvæ could not be recognised as such in the paraffin bath, they could always be distinguished, if they had been coloured with eosin, from any particle of dust that happened to have found its way into the embedding bath. One of the main points of this method, however, is to use only freshly filtered wax in perfectly clean white porcelain basins for embedding.

The above details have been given because it was found possible by using this method to section any small object in any desired plane as long as that object was visible, at least, as the minutest speck that could be seen in the paraffin bath with a $\times 8$ lens. The drawbacks of the celloidin method were thus avoided and also a considerable amount of time was saved. One of the chief advantages of the celloidin method is that the thinnest membranes are supposed to be supported by the celloidin. However, with such small objects as these Estheria larvæ, the direct embedding was not found too drastic, the thinnest membranes apparently standing the process without breaking down.

A few specimens were cut at a thickness of 4μ while all the others were cut at 8μ. The sections of Flemming or F.W.A. material were usually stained with iron hæmatoxylin. In a few cases EHRLICH'S hæmatoxylin was used and in a few others MALLORY'S triple stain for connective tissue was used. The sublimate material was stained in MANN'S methyl-blue eosin.

Early Development.

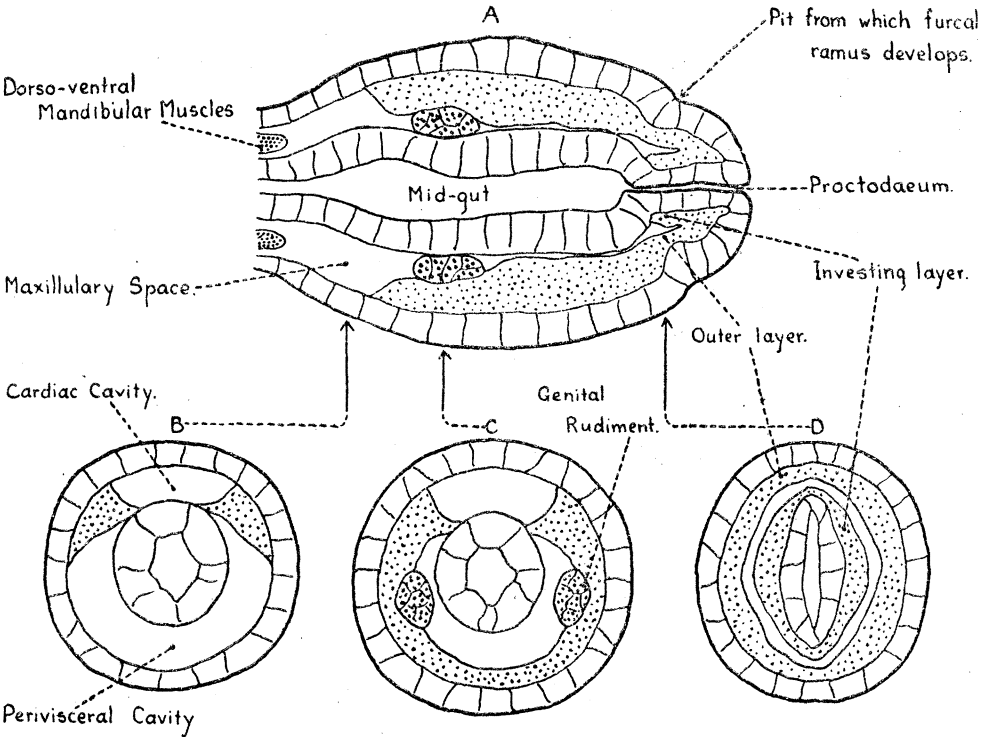
The larva of *Estheria* hatches from its egg as a true nauplius. Its three pairs of limbs show typical naupliar characters. The antennules are reduced to a pair of small prominences, each provided with a fine seta. The second antennæ and mandibles are both biramous swimming appendages. There is the usual masticatory seta on each of the second antennæ. The mandibles are provided with single minute processes on their basal joints. These are the rudiments from which the masticatory blades develop. They are probably functionless at this stage, as they do not point towards each other but project ventrally.

The post-mandibular region of the larva shows no signs of segmentation and appears to be one solid mass of cells. It is not provided with paired furcal processes, but at each side of the proctodæum there is a slight depression from which the caudal furca develops later. There is a very large upper lip, which is about the same length as the larva itself. It is a flat lobe projecting from the anterior end of the body, with its flattened plane at about 45° to the length of the body, and it contains three large and conspicuous labral glands.

It has been maintained by the author (5) that the function of the labral glands in the Daphnid *Simocephalus* is to produce a food-entangling substance. If this is the case generally in those Branchiopoda in which labral glands occur, the precocious development of the glands in the larva of Estheria finds a ready explanation. The labrum and the body together form a V-shaped structure which is forced through the water, apex forwards, by the oarlike action of the antennæ and mandibles. In the plane of the V the water will naturally be forced away from the nauplius, but in a plane at right angles to this the water will be sucked in between the two arms of the V, that is, into the region of the mouth opening. It is in this region that the duct cells of the labral glands open. Thus floating food particles would be sucked into the mouth region, where they would become entangled in the labral gland secretion. As the antennæ were flexed backwards in the actual swimming stroke, the masticatory setæ would push the entangled mass of food into the mouth. It is difficult otherwise to imagine how such a fine seta as this masticatory process of the antenna could push floating food particles into the mouth.

Sections of such a larva show that it is still in a very rudimentary condition. Its cells still contain a fair amount of yolk as yolk spheres. Even the muscles of the mandibles and antennæ contain yolk in their non-contractile portions.

The post-mandibular region is marked off from the more anterior part of the body by a pair of dorso-ventral mandibular muscles (text-fig. 1 and figs. 6 and 22). These



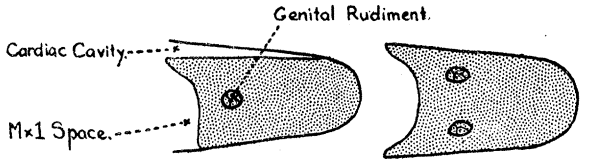
TEXT-FIG. 1.—A. Horizontal section through nauplius showing the earliest signs of a perivisceral cavity. B, C, D, are transverse sections through a larva a few hours older : B. Section through the Maxillulary space : C. Section through the Genital Rudiment : D. Section through the Proctodæum.

are attached in the mid-line ventrally, and extend in a slanting direction on each side of the gut to be attached to the dorsal ectoderm at the base of the laterally projecting mandibles. They are very distinct, and form a very useful guide in delimiting the mandibular region from the growing region behind. There is no body cavity in this region, except for a small space ventrally in the most anterior part (text-figs. 1 and 2). Here the mesoderm is absent. Elsewhere, together with the genital rudiment, it completely fills up the space between endoderm and ectoderm. The genital rudiment, which does not stain so deeply as the other tissues in the earliest stages, consists of a few large cells grouped together on each side between the mesoderm and ectoderm (text-fig. 1), and is placed at a level a little posterior to the region where the ventral mesoderm is absent. The mid-gut leads into a well-developed proctodæum (text-fig. 1), which extends into the post-mandibular region for about one-third of its length.

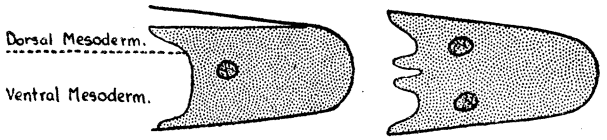
A few hours after hatching the whole body enlarges and a distinct body cavity appears in the post-mandibular region. Along the mid-dorsal line the mesoderm splits, and on each side of this split the mesoderm moves outwards, retaining at the same time its connection with both endoderm and ectoderm (text-fig. 1). In this way a cavity is formed dorsal to the gut (text-fig. 2), between the mandibular region and the proctodæum. Its roof is ectoderm, while its floor is endoderm medianly and mesoderm laterally. It is the rudiment of the cardiac cavity. It is continuous anteriorly with the space between the dorso-ventral muscles of the mandibles and hence with the general body cavity of the head region. Laterally and ventrally to the mid-gut the mesoderm plate, together with the genital rudiment, separates completely from the endoderm, and so forms a ventral cavity, distinct from the cardiac cavity, that becomes the perivisceral cavity in the adult (text-fig. 1).

The ectoderm forming the proctodæal invagination forms a tube flattened in the vertical plane. The mesoderm surrounding it very early differentiates into an investing layer and a layer lining the outer ectoderm. There is thus formed a continuous space around the terminal part of the gut, and through this space the cardiac cavity is in connection with the perivisceral cavity (text-fig. 1). Directly after the first ecdysis it can be seen that the investing layer of the proctodæum has differentiated into a layer of circular muscles and into dilator connectives stretching from the proctodæum to the outer ectoderm. At quite early stages these circular muscle cells send forwards thin fibrils at right angles to their length, which extend forwards over the outer surface of the mid-gut. They may branch and anastomose, but the direction of the fibrils is always more or less along the length of the gut. They are shown clearly in fig. 23. They have no special terminations and are presumably elastic fibrils. It is not until the larva has grown to a length of about 2 mm. that there are signs of circular muscles round the mid-gut (see later, p. 412).

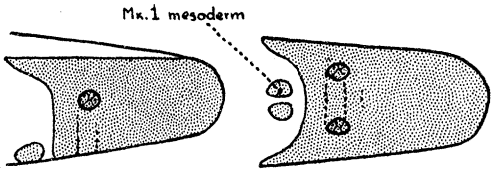
In the post-mandibular region, in front of the proctodæum, the mesoderm can be distinguished into two parts. Firstly, the dorsal mesoderm, consisting of the upper ridges



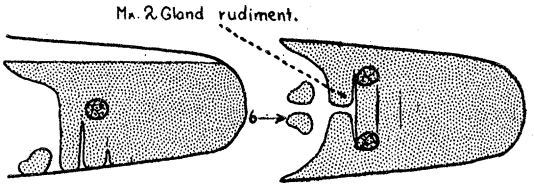
a. Cardiac Cavity just formed. Mesoderm lacking in Mx.1 space.



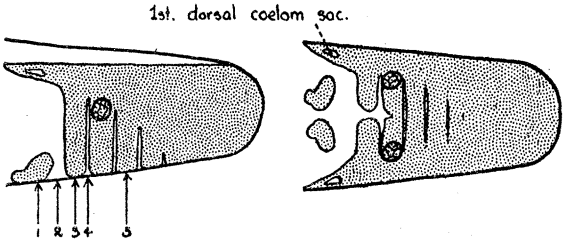
b. Tongues of mesoderm projecting across Mx.1 space.



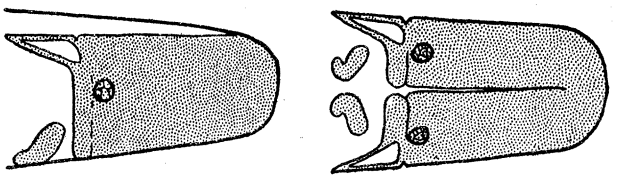
c. Mx.1 mesoderm separated off. Transverse grooves appear in ventral mesoderm.



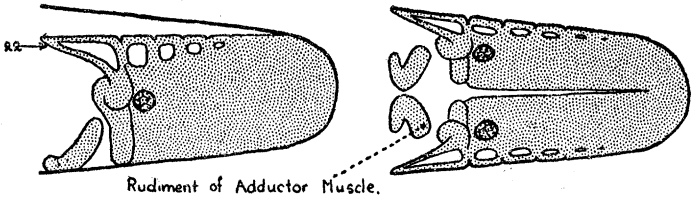
d. Ventral mesoderm segmenting. Mx. 2 Gland rudiments separate.



e. Ventral segmentation at maximum. Mid-ventral split extending backwards. 1st. coelom sac appears in dorsal mesoderm.



f. Ventral segmentation obliterated. Mx. 2 Gland rudiments form thickened pillars of mesoderm.



g. Further appearance of dorsal coelom sacs. Mx. 2 Gland rudiments form coiled rods of cells.

Lateral View. Dorsal View.

TEXT-FIG. 2.—A series of diagrams illustrating the segmentation of the ventral mesoderm and its subsequent disappearance and the appearance of the dorsal segmentation.

extending from endoderm to ectoderm that form the lateral floor of the cardiac cavity (fig. 5), and, secondly, the ventral mesoderm that lines the ectoderm ventrally and laterally, and is continuous at its upper limit with the dorsal mesoderm. The dorsal mesoderm extends forwards continuously on each side from the proctodæum to the inner side of the dorsal attachment of the dorso-ventral muscles of the mandibles. The ventral mesoderm extends forwards from the proctodæum, but stops short of the ventral attachment of the same muscles, thus leaving the small space anteriorly, which has been mentioned above, in which the mesoderm is absent. This space will be termed the maxillulary space.

After the first ecdysis, it is seen that the ventral mesoderm has sent forward on each side of the mid-ventral line a small tongue of five or six cells projecting across the floor of the maxillulary space and reaching as far as the dorso-ventral muscles of the mandibles (text-fig. 2). In this way there are formed two lateral bays, from which the mesoderm is absent. It is approximately from the margins of these bays that the shell folds originate. The mesoderm forming the posterior margins of these bays consists of a pair of thickened columns (fig. 3), and it is from these columns that the maxillary glands develop.

During the instar following the first ecdysis the perivisceral space enlarges considerably, and at the same time the cells of the ventral mesoderm become spread out into a layer one cell thick (fig. 5). They become flattened, and are by no means columnar. A further result of the enlargement of the perivisceral space is that the ridge of mesoderm that forms the connection between the main mass of dorsal mesoderm and the gut wall becomes drawn out into a thin membrane of flattened cells (figs. 1-5).

Signs of segmentation now appear in the mesoderm. The first indication is the separation of the maxillulary mesoderm tongues. They form a pair of symmetrically placed patches, each consisting of about ten cells (figs. 1 and 7), that reach as far forward as the mandibular dorso-ventral muscles, but which are separated from the more posterior mesoderm. Immediately behind the maxillulary region the mesoderm becomes segmented by the appearance of transverse slits (*c*, *d*, *e*, text-fig. 2). As many as four have been observed, but it is possible that more may appear. They can easily be overlooked in transverse sections. Each slit is only about 3–4μ from front to back, and hence occurs in the thickness of a section. They are seen clearly in a longitudinal vertical section a little to one side of the median plane (fig. 6). The first separates off the lateral anterior thickened edges of the mesoderm. The splits, which appear from before backwards, appear first as grooves and extend from the ventral side up the lateral parts towards the dorsal mesoderm, but they do not segment the latter. The segments so formed are taken as representing, for reasons that will appear later, the maxillary, the first, second, and third trunk segments. Behind this, no further segmentation of the ventral mesoderm was observed.

The apparent segmentation behind the maxillulary region is only transitory.

Proliferation of the mesoderm cells soon obliterates it (*f*, text-fig. 2). The maxillulary mesoderm, however, never again joins on to the more posterior mesoderm.

The cells forming the genital rudiment form a small roundish mass placed laterally in the first trunk segment resting against the ventral mesoderm (fig. 4, and text-fig. 1, *c*).

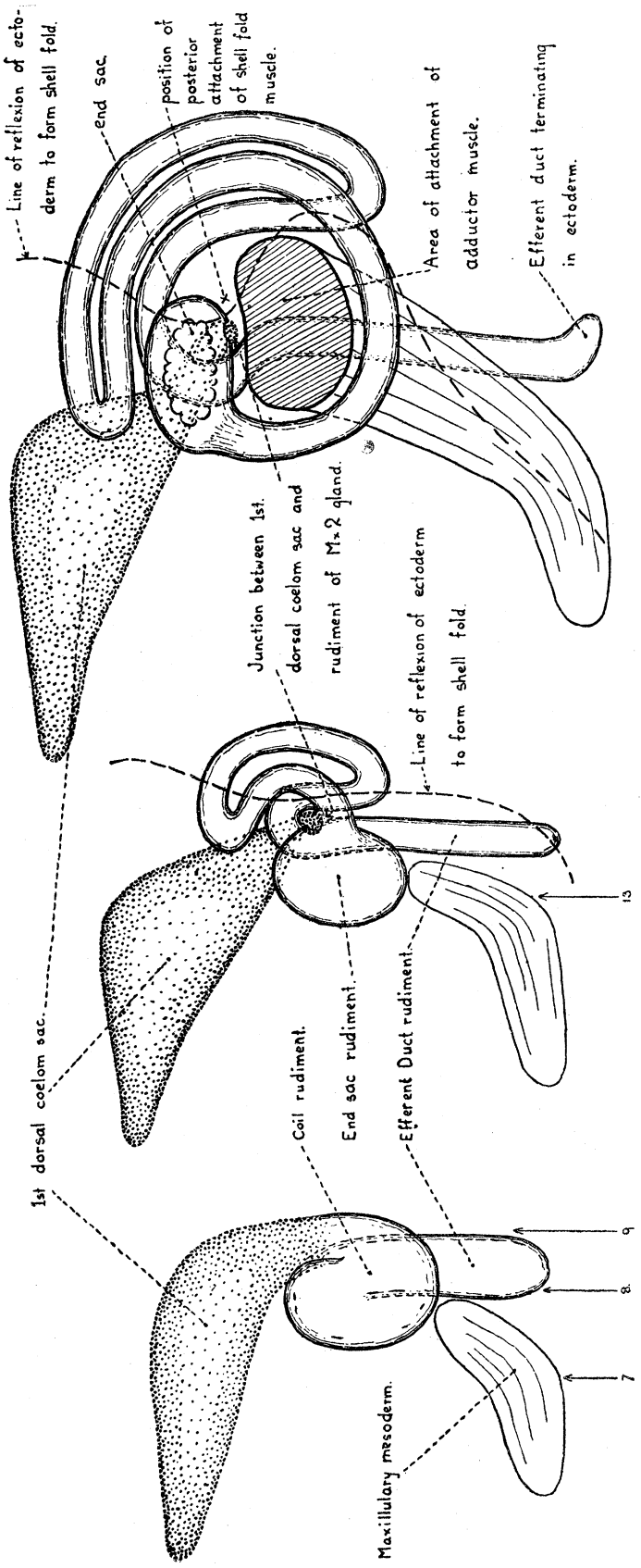
When the ventral segmentation is at its maximum, there appears in the mid-ventral line a split dividing the ventral mesoderm into right and left halves. It develops from before backwards and is a continuation backwards of the division between the pair of forwardly projecting tongues of mesoderm in the maxillulary space. About the same time there appears on each side a small cavity in the most anterior part of the dorsal mesoderm—that is, in the dorsal portion above the maxillulary region (figs. 1 and 7). Behind this another pair soon forms, but by this time the ventral segmentation has become obliterated. Ultimately there may appear as many as seven pairs of these cœlomic cavities in the dorsal mesoderm. They develop to a considerable size and become very conspicuous, especially in transverse sections (figs. 7, 8 and 19). A horizontal section that shows some of these pouches very clearly is shown in fig. 22.

The further development can best be described in two parts—first, that dealing with the maxillary gland and the ventral mesoderm; and, secondly, that dealing with the heart and the dorsal mesoderm.

The Maxillary Gland.

At the time of the appearance of the first pair of dorsal cœlomic cavities the primordia of the maxillary glands are indicated merely by the thickened dorso-ventral ridges of mesoderm that form the anterior boundary of the post-maxillulary ventral mesoderm (fig. 3). Just before the second pair of cavities appears the dorsal parts of the thickenings have differentiated on each side into the solid rudiment of a coiled tube (figs. 8 and 9, and text-fig. 2, *g*). At the same time the ventral ectoderm on each side of the middle line has thickened to form the rudiments of the post-mandibular nerve cord, the thickening developing as usual from before backwards. The dorsal parts of the rudiments of the maxillary gland are continued downwards as a pair of straight solid rods of cells that end in the ectoderm immediately outside the rudiments of the nerve cords (fig. 8). They push in between the ectoderm cells but do not reach the exterior. The upper coiled part will be referred to as the coil of the gland, while the lower cord of cells will be spoken of as the efferent duct. Dorsally the coils are continuous with the posterior corners of the first pair of dorsal pouches (figs. 9 and 11).

At this stage the primordia of the adductor muscles which develop from the maxillulary mesoderm, and are described later, consist of paired rudiments passing outwards and backwards from their median point of attachment behind the dorso-ventral mandibular muscles, over the nerve-cord thickenings, to end against the ectoderm in contact with the lower edge of the coil rudiment (*a*, text-fig. 3).



TEXT-FIG. 3.—Three diagrams illustrating the development of the maxillary gland and of the adductor muscle.

At first there appears to be only one twist in the rudiment of the coil, and this is situated just below the dorsal mesoderm. A little later this dorsal coil has increased so that it forms a circular plate of cells lying flat against the ectoderm. In sections it is obvious that this is really a coiled rod, but it was not found possible to trace out these coils (fig. 12).

Just before this stage is reached the paired rudiments of the carapace have appeared. They appear first as lateral ectodermal folds (figs. 9 and 11). The line of reflexion of the ectoderm to form these folds commences ventro-laterally at the posterior edges of the adductor muscle rudiments and, in passing upwards, slopes backwards across the coil rudiment to a mid-dorsal point just behind the maxillary region where, at first, there is no reflexion of the ectoderm (*b*, text-fig. 3). These folds appear first dorso-laterally and extend ventralwards ; only later do they extend dorsally to meet each other. As the rudiments of the carapace develop the coils of the maxillary gland increase and project into the cavities of the shell folds. When the coil rudiment has become the circular mass of cells just mentioned, its ventro-posterior part is wedged firmly between the two layers of ectoderm of the shell fold. The efferent duct leaves this circular mass near its centre, and this point is situated in the angle formed by the lower edge of the first dorsal cœlom sac, which slopes downwards and backwards, and the reflected edge of the ectoderm forming the shell fold that slopes upwards and backwards (fig. 12).

Further development of the gland is not of much significance. It consists merely of the elaboration from the circular mass of cells of the end sac and the twisted duct leading from this sac to the efferent duct. The rod of cells forming the primordium of the efferent duct merely develops a lumen and remains as a straight tube leading from the coiled part of the gland to a papilla that develops on the lateral part of the maxilla. This duct is entirely mesodermal, there being no ectodermal invagination into it at its external opening. As the primordium of the coil of the gland develops, the ducts pass more and more into the cavity of the shell fold. The end sac, however, that terminates the coiled duct never really passes into the carapace. In the adult its outer surface rests against the outer ectoderm of the carapace, but most of its inner surface faces freely into the body cavity (fig. 15).

The connection between the first dorsal cœlom sac and the end sac that represents the junction of the dorsal and ventral mesoderm of the maxillary segment is retained until the adult stage is reached and may be retained throughout life (fig. 15) (see later, p. 411).

The development of the coils of the duct has been worked out in some detail, because in the adult the area of attachment of the adductor muscle to the carapace is completely surrounded by one of the coils of the maxillary gland, and it was not at first obvious how this arrangement could be brought about. The end sac first appears as the swollen end of the primordium of the coiled duct where the latter is continuous with the first dorsal cœlom sac (figs. 10 and 11). After this, the coil rudiment becomes the circular mass of cells already mentioned, in which the twisting of the coils cannot clearly be made out. Soon, however, these become clear, and the disposition of the coils can best be seen by

reference to text-fig. 3. There are three loops in this coil—one dorsal and two posterior. Resting against (and really continuous with) the upper side on the end sac is the lower corner of the first dorsal cœlom sac, while resting against the lower side is the upper end of the rudiment of the adductor muscle (fig. 13). The anterior of the two posterior loops now passes forwards ventrally to the end sac. In doing so it passes between the rudiment of the adductor muscle and the ectoderm, and finally, when it has reached the anterior side of the end sac, the outer end of the adductor muscle slips in between this loop and the lower surface of the end sac (text-fig. 3, and fig. 14). It is only at this stage that the adductor muscle becomes definitely attached to the ectoderm (fig. 15). The disposition of the coils is now that of the adult, and further development consists merely of the enlargement of these solid coils and the appearance of a lumen in them. The space between the lower surface of the end sac and the anterior loop, which, at first, is filled completely by the adductor muscle, enlarges so that there is a central area that is free from coils and end sac. The area of attachment of the adductor muscle spreads along the lower and anterior margins of this space. In this way the rudiments of the adductor muscle, originating from a single anterior median point, become spread out on each side into a fan-like band.

The Maxillulary Mesoderm.

It has already been mentioned that the ventral mesoderm of the maxillulary segment separates off from the more posterior mesoderm at a very early stage as two symmetrically placed groups of cells in the ventral part of the maxillulary space (text-fig. 2, *c*). As development proceeds these groups increase considerably in size, their growth being chiefly in a lateral direction. Later a division appears in the middle of the outer edge of each rudiment. This converts them into V-shaped masses (text-fig. 2, *e*, *f*, *g*). The apex of the V is at the ventral median point of attachment. The posterior arm of the V extends outwards, slightly backwards and slightly upwards, its end being in contact with the developing maxillary gland. It is from this that the rudiment of the adductor muscle develops on each side. The other arm of the V extends directly outwards and projects into a small prominence, which is the rudiment of the first maxilla (fig. 13). This arm gives rise to the lateral muscles that move the first maxilla (see later, p. 409). At the inner end of this posterior arm, between it and the nervous system, a group of two or three cells becomes separated off. This is the contribution of the maxillulary segment to the ventral longitudinal flexor muscle of the trunk.

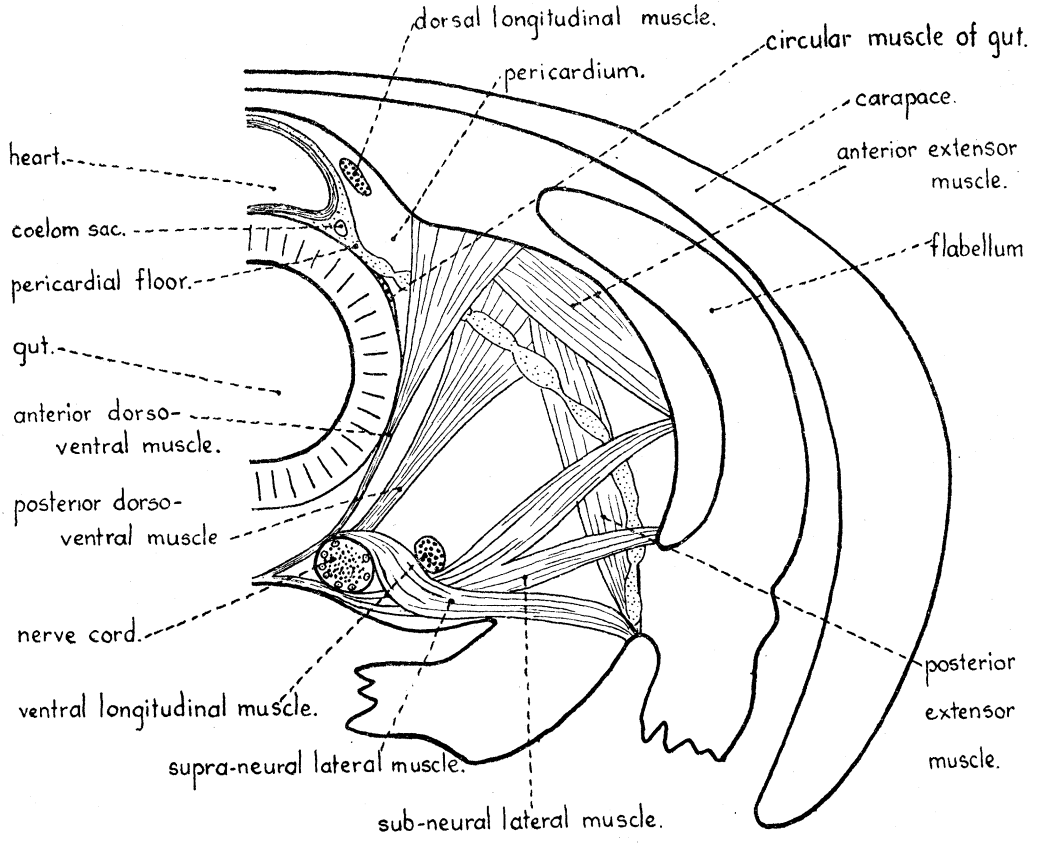
The development of the adductor muscle is associated with a marked change in the shape of the organism. At first the carapace is much broader than it is deep, and while this stage lasts the adductor muscle rudiments slope backwards from their median ventral points of attachment to their attachment at the maxillary glands. Later the carapace assumes the adult shape and becomes much deeper than it is wide. At this stage the two rudiments have fused together in the middle line, and the continuous muscle so formed passes directly outwards on either side without sloping backwards. The change

in the shape of the carapace is most probably brought about by the fusion and subsequent functioning of the two rudiments.

The union of the two rudiments is not muscular but consists of a tendinous band placed immediately behind the dorso-ventral muscles of the mandibles. It becomes raised up from the ectoderm to about the level of the top of the nerve cords, but remains in connection with the ectoderm through a group of strands—apparently prolongations inwards from the ectoderm cells.

The Adductor Muscle.

The main points of the development of the adductor muscle have been described above. It was attempted to discover, by the study of this development, the exact homologies of this muscle. In order to describe the conclusions arrived at it is necessary to give a description of the muscles of a typical trunk segment. In fully grown specimens it is difficult to make out with any certainty the arrangement of the muscles by the study of sections and, of course, the size of the organism makes dissection unpracticable. However, in young specimens showing six or seven rudimentary trunk limbs, all the larger muscles of the limb are already differentiated and can be made out without any difficulty in transverse sections. A diagrammatic view of a typical trunk segment of such a larva showing the chief muscles is figured in text-fig. 4 (compare fig. 18).



TEXT-FIG. 4.—Diagram of a typical trunk segment showing the chief muscles.

Running the whole length of the trunk are the longitudinal muscles. Dorsally are the extensor muscles. These are attached to the ectoderm in the mandibular region immediately on the inside of the dorsal attachment of the dorso-ventral muscles of the mandibles. Ventrally are the flexor muscles, and these are attached to the dorsal surface of the nerve cord in between the mandibular and maxillary segments.

Passing from the anterior face of the segment posteriorly the muscles figured are as follows :—

(1) *Anterior dorso-ventral muscle.*—This has a very thin tendinous attachment in the mid-ventral line. It passes upwards close against the nerve cord on its outer side and the gut on its inner side. It spreads out dorsally in a transverse plane and is attached to the dorso-lateral ectoderm.

(2) *Supra-neural lateral muscle.*—This arises from the dorsal surface of the nerve cord immediately behind the anterior dorso-ventral muscle. It is, also, apparently joined to the ectoderm in the mid-ventral line by a thin tendon. It passes laterally underneath the ventral longitudinal muscle, and is attached to the limb at the base of the flabellum.

(3) *Sub-neural lateral muscle.*—Close behind the supra-neural lateral muscle is the sub-neural lateral muscle. It is attached to the ventral ectoderm immediately underneath the nerve cord and passes to the lateral parts of the limb. It splits up in passing outwards, and is attached more dorsally than the other lateral muscle.

(4) *Posterior dorso-ventral muscle.*—This is attached ventrally to the dorsal side of the nerve cord by a thick tendinous attachment. In passing upwards it slopes posteriorly. It spreads out into a fan and is attached at the hinder limit of the segment over a dorso-lateral area that is in contact with the dorsal attachment of the anterior dorso-ventral muscle of the succeeding segment.

(5) *Anterior extensor muscle.*—This is attached dorsally immediately behind the dorsal attachment of the anterior dorso-ventral muscle and runs downwards to a lateral region just above the base of the flabellum.

(6) *Posterior extensor muscle.*—Between the dorsal attachment of the last muscle and that of the posterior dorso-ventral muscle is the dorsal attachment of the posterior extensor muscle. Ventrally it is attached to the lateral part of the limb immediately below the base of the flabellum.

The names given to these muscles have been given merely to facilitate reference to them and not to illustrate their topographical relations in the adult Estherid. It has been stated above that the text-fig. 4 is drawn from a young specimen showing only six or seven rudimentary trunk limbs. At this stage, as is obvious from the figure, the larva is broader than it is deep. It must be remembered that, when the shape of the organism alters,

this involves a shifting downwards of the limb relative to the nerve cord. In this process the limbs naturally carry with them the points of attachment of the limb muscles, and so the muscles that have just been described as lateral muscles now pass almost vertically downwards.

Comparison with the trunk musculature of Branchipus and Artemia as described by CLAUS (6) shows close agreement. The anterior and posterior extensor muscles are what CLAUS calls *a* and *b*, the anterior and posterior dorso-ventral muscles correspond to his γ and δ, while the supra- and sub-neural lateral muscles are apparently his *b'* and *a'*. He does not describe any other muscles except, of course, the dorsal and ventral longitudinal muscles, which pass through all the trunk segments, but he states that his muscle *a*—called here the anterior extensor, is augmented by an oblique strand from the segment in front, while his muscle *b*, called here the posterior extensor, is augmented by an oblique strand from the anterior extensor. This is certainly not the case in the young Estherid larva, but it is quite possible that in the change to the adult shape this alteration may take place.

Working from the posterior trunk segment forwards, it is possible to trace out all the muscles up to the anterior dorso-ventral muscles of the first trunk segment. Between these and the dorso-ventral muscles that mark the posterior limit of the mandibular segment, that is, in the maxillulary and maxillary segments, the muscles are naturally irregular. It has been attempted, however, to homologise the muscles that do occur with those in the typical trunk segments.

In the anterior part of this region there is the adductor muscle. Close behind this is a pair of dorso-ventral muscles attached ventrally to the nerve cord by a tendinous attachment. Wedged in between these two are the poorly developed supra- and sub-neural muscles of the first maxilla.

The adductor muscle and this dorso-ventral muscle correspond, then, in position with reference to the lateral muscles of the first maxilla to the anterior and posterior dorso-ventral muscles of the first maxilla, and it will be taken here that they represent these muscles. The ventral attachments of the paired rudiments of the adductor muscle, being situated in the most anterior part of the maxillulary segment, are in agreement with this. Further, while the dorsal attachments of these rudiments are peculiar, it is significant that the first rudiments of the carapace appear in that area to which the anterior dorso-ventral muscles would be attached were the muscles arranged as in an ordinary trunk segment.

The dorsal attachment of the posterior dorso-ventral muscles of the maxillulary segment is situated among the coils of the maxillary gland and is confluent with the attachment of the anterior dorso-ventral muscles of the first trunk limb along the posterior edge of the carapace fold. If the former attachment is taken to indicate the posterior limit of the ectoderm of the maxillulary segment, it would follow that the shell fold is confined to this segment, but also, it would imply that the dorso-lateral ectoderm of the maxillary segment is practically absent.

The musculature of the maxilla is very little developed. The maxilla bears on the outside the large papilla on which the efferent duct of the maxillary gland opens and on the inside a very small lobe beset with anteriorly directed spines that are probably used to assist food towards the mouth. A small group of cells in the gnathobasic portion probably represents lateral muscles, while a very fine tendinous strand immediately behind the posterior dorso-ventral muscle of the maxillulary segment running from the mid-ventral line to the efferent duct of the maxillary gland may represent one or both of the dorso-ventral muscles.

All the trunk muscles except the dorsal longitudinal muscle (see next page) are formed from the ventral mesoderm. This separates completely from the dorsal mesoderm except, as already stated, in the maxillary segment. The development of the separate muscles from the ventral mesoderm does not appear significant and has not been followed out in detail.

There is one muscle in the maxillulary segment that cannot be homologised with any separate trunk muscle. It develops very early in connection with the shell fold. It is called here for convenience the shell-fold muscle. It is attached anteriorly to the dorso-lateral ectoderm just posterior to the mandibular region. Posteriorly it is attached at the same level to the reflected ectoderm, forming the posterior edge of the shell fold, thus spanning antero-posteriorly the entrance to the shell-fold cavity. The cells from which it develops consist of a string of cells originally in contact with the ectoderm forming the lower edge of the outer face of the first dorsal cœlom sac (figs. 13, 16, 15). Since the dorsal mesoderm, apart from forming the heart tube, gives rise only to the dorsal longitudinal trunk muscles (see next page), and since it is the outer face of the cœlom sac that forms both the dorsal longitudinal muscle and the shell-fold muscle, it appears most probable that the latter is a portion of the former that has become separated off in connection with the development of the shell fold.

Development of the Heart.

In the part of this paper dealing with the earlier development of the mesoderm it has been stated that the ventral mesoderm becomes segmented before the dorsal mesoderm, and that as the segmentation of the ventral portion becomes obliterated through growth, the dorsal part shows the first signs of cœlomic pouches. Ultimately seven pairs of these cavities appear. They correspond, except the first pair, with the subsequent segmentation of the ventral mesoderm when it has differentiated into segmental muscles, one pair of pouches occurring in each segment. Whether the earlier transient ventral segmentation corresponds to the dorsal segmentation and hence to the later definitive ventral segmentation cannot be settled, since it becomes obliterated before the other segmentations have fully appeared. However, there does not seem to be any reason to assume otherwise than that all the segmentations agree and are all evidences of the same primitive segmentation.

Differentiation of the mesoderm of the dorsal pouches, after they have attained their maximum size, commences anteriorly, as does that of the ventral mesoderm, and extends posteriorly, and, as it proceeds, the cavities of the pouches become gradually smaller. Since, at the time that the most anterior pouch commences to differentiate, the posterior pouches have not yet grown to their full size, the early differentiation of the pouches is associated with a progressive diminution in size of the anterior pouches and a progressive increase in size of the more posterior ones (compare figs. 19 and 17).

The first five pairs of pouches give rise to the heart, and the development of these will be described first.

Each pouch is triangular in section (fig. 7). The three angles of the triangle may be referred to as the upper, lower, and inner angles. The upper and lower angles are in contact with the dorso-lateral ectoderm. The inner angle is, at first, in direct contact with the gut wall. Later the connection of this angle with the gut is drawn out into a thin membrane, as has already been mentioned (p. 401). The three sides of the triangle, which, of course, represent the three lateral faces of the pouch, can similarly be termed the outer, inner, and lower faces. The inner wall of one pouch faces the inner wall of the corresponding pouch on the other side of the body. The outer face is in contact with the dorso-lateral ectoderm, while the lower face faces downwards into the ventral body cavity.

It can be said at the outset that the inner faces give rise to the muscular portion of the heart tube, and from the outer faces develop the dorsal longitudinal extensor muscles of the body. After these have been formed, the remainder of the pouches forms the covering of the heart tube and the pericardial floor.

At the earliest appearance of the pouches their walls are only one cell thick, but they soon increase in thickness. The earliest sign of differentiation is the appearance of muscle fibrils, which form the rudiment of the dorsal longitudinal muscle, running antero-posteriorly in the outer cells of the outer face. At the same time the upper angle, keeping in contact with the ectoderm, commences to grow medianly. A little later the lower angle appears also to grow medianly, the thin, flattened membrane connecting it to the gut, at the same time shrinking, so that the inner angle comes again into direct contact with the gut wall. This can be seen clearly by comparing fig. 11 with fig. 16. As a result of the median growth of the upper and lower edges, the inner face becomes curved, the concavity facing medianly. The upper angle of one pouch soon meets the upper angle of the corresponding pouch of the other side of the body in the mid-dorsal line. In this way the cardiac cavity becomes enclosed dorsally as well as laterally by mesoderm, its floor remaining endoderm (figs. 14 and 20). The layer of mesoderm cells actually lining the cardiac cavity are now seen to be differentiated from the remainder. They are somewhat flattened and show very clearly muscle fibrils running in the transverse plane of the body (figs. 16 and 17). Their nuclei do not appear to differ markedly from the neighbouring undifferentiated mesoderm cells. In hæmatoxylin preparations these fibrils stain very deeply and are very conspicuous in transverse

sections of the embryos at this stage. This layer of muscle cells ultimately extends from the inner angles over the surface of the gut to the middle line (figs. 16, 17 and 19). In this way is formed in each segment a tube, the walls of which are constituted of circular muscles. These segmental tubes fuse up with each other dorsally, laterally, and, presumably, ventrally. The latter cannot be clearly made out owing to the muscles being in such close contact with the gut wall. The four pairs of ostia develop later, the anterior ones being formed before the more posterior ones. They appear as dorso-ventral slits that are inter-segmental in position in the lateral wall of the continuous heart tube (fig. 18). The first pair appears between the first and second dorsal pouches—that is, at the anterior boundary of the first trunk segment. The edges of these slits project inwards as thin flaps that are apparently non-muscular. They clearly form efficient valves, preventing efflux of blood from the heart through the ostia.

Immediately outside the layer of muscle cells forming the heart tube are undifferentiated mesoderm cells. Dorsally these are continuous from side to side, but ventrally they are absent. In the most anterior part of the heart tube, that is, just posterior to the dorso-ventral muscles of the mandibles, the muscular layer of the heart tube comes away from the layer of mesoderm cells outside. This is shown clearly in fig. 21. The most anterior part of the heart tube thus constitutes a short aorta, consisting solely of muscle cells with no external epithelial investment. It extends as far forwards as the dorsal attachments of the dorso-ventral muscles of the mandibles.

Just previous to the appearance of the muscular fibrils in the walls of the heart tube the muscle cells, formed on the outer surfaces of the outer walls of the pouches, can be seen to have increased considerably. A gap now appears in between these muscle cells and the undifferentiated mesoderm (fig. 16) ; in other words, the actual outer walls of the pouches shrink away from the masses of muscle cells that they formed on their outer sides. This movement synchronises with the shifting of the inner angle medianly. The appearance of transverse sections at this stage suggests that the cœlom pouches are dragged away from the ectoderm and the longitudinal muscles that they have formed simply by the shrinkage of the thin membrane that connects the inner angle to the gut.

As the pouches pass inwards they diminish in size and become more and more wedged in the angle between the dorsal wall of the gut and the floor of the heart tube (figs. 15 and 17). The separate masses of muscle cells remain close against the ectoderm. However, before the cœlom sacs commence to shrink they have already joined up to form, on either side, one continuous longitudinal muscle. Later, this loses connection with the ectoderm and runs freely in the space left between the mesoderm and the ectoderm by the shrinkage of the outer faces of the pouches. This space is the pericardial cavity.

By the time that the pouches commence to shrink the ventral mesoderm can be seen to be differentiated in the post-maxillary region into the various trunk muscles. As the latter develop, the lower angles of the pouches, except the first, break connection

with the ventral mesoderm and grow out ventrally, each as a flat tongue of cells (fig. 19). At first these are separate and lie close against the inner surface of the anterior extensor muscles. Soon, however, they fuse up to form one continuous membrane. This membrane, on either side, forms the floor of the pericardial cavity. It extends from the angle between the gut and the heart, runs downwards parallel to the lateral body wall, and ends at the base of the limbs (fig. 20). It is naturally pierced by most of the trunk muscles—the anterior extensor muscles, however, are wholly external to it.

The cells forming the pericardial floor are not, at first, very much flattened. They are thick bi-convex cells that only later become much thinner. At first their cytoplasm is full of small granules which, by their staining reaction, are probably mitochondria, and in this feature they resemble the blood cells.

About the time that the lower angles of the cœlom pouches commence to grow downwards to form the pericardial floor, circular muscle cells can be seen on the outside of the gut wall in the angle between this and the lower faces of the cœlom sacs (figs. 17–20). Since they appear always first in this region they are without doubt mesoderm cells that are budded off from the lower faces of the cœlom pouches, and that later grow ventrally and completely surround the gut. Up to this stage, however, the gut is naked on the outside except for the fibrils from the proctodæal region (fig. 23) that have already been described (p. 399). As far as the author can see, this late development of gut musculature has not been described elsewhere, nor have the longitudinal fibrils been described. It is possible, therefore, that Estheria is peculiar in this respect. Claus (6), in his description of the muscles of Branchipus, states that longitudinal gut muscles are absent, as in the case described here, but he figures circular muscles round the gut in his figures of the earliest nauplius.

The cavities of the cœlom pouches finally disappear, AND THIS DISAPPEARANCE RESULTS ENTIRELY FROM THE SHRINKAGE INWARDS OF THEIR WALLS AND IS NOT DUE TO THOSE CAVITIES LOSING THEIR IDENTITY BY OPENING INTO THE GENERAL BODY CAVITY. The collapsed walls of the pouches are finally represented by the pericardial floor and by the layer of cells on the outside of the heart muscles. Neither of these layers of cells apparently extends underneath the heart tube, so that the heart is attached directly to the dorsal wall of the gut.

The development of the sixth and seventh pairs of pouches differs from that of the more anterior ones only in that the inner faces of these pouches do not form a heart tube. Their outer faces give rise to the longitudinal muscles, and then the cavities dwindle and the remainder of the pouches go to form the pericardial floor.

The development of these two pouches forms a transition between the development of the anterior pouches and that of the posterior mesoderm, where no pouches are formed. Here the dorsal mesoderm is distinguished from the ventral mesoderm in that it connects the gut wall with the ectoderm, whereas the ventral portion merely lines the ventrolateral ectoderm. It forms the dorsal longitudinal muscles in the usual way and then grows downwards to form the pericardial floor.

The Genital Rudiment.

The genital rudiment retains its original position until very late in the development of the larva. As stated previously, it is situated just posterior to the developing maxillary gland—that is, just ventral to the second dorsal cœlom pouch. As the lower angle of this pouch grows ventrally to form its contribution to the pericardial floor, it surrounds the genital rudiment (figs. 17 and 18). The latter thus comes to be represented at this stage by a small mass of cells situated in the pericardial floor, lying in between the anterior and posterior dorso-ventral muscles of the first trunk limb.

The genital rudiment shows no change until after the larva has grown to about 2 mm. in length. The small mass of cells on either side then grows backwards as a solid string of cells in the pericardial floor at about the level of the dorsal side of the gut. This rudiment projects as a ridge into the perivisceral space. A lumen appears later in this solid chain of cells, which becomes the cavity of the gonad. The formation of the oviduct has not been followed, but from the position of the oviduct in the adult it is undoubtedly formed from or in the pericardial floor.

The cœlom sac that grows downwards to surround the genital rudiment is earlier in connection with the ventral mesoderm, from which develop the muscles of the first pair of trunk limbs. The second cœlom pouch must clearly belong to the first trunk segment, and hence, since there is only one pair of pouches between this and the mandibular muscles, either the maxillulary or the maxillary pouches are absent or else they are fused together. That the first dorsal pouch represents at least the maxillary pouch is clearly established by the fact that the maxillary gland develops in connection with it. Whether it also represents the maxillulary pouch cannot be stated for certain. It is a very long pouch, being prolonged to end just inside the dorsal attachments of the mandibular dorso-ventral muscles. In one series of horizontal sections that showed the dorsal pouches most clearly, this anterior pair seemed to be slightly constricted, but this cannot be taken as sufficient evidence on which to state that the first pair of pouches really belong to two segments.

The first pouch obviously differs from the others in that its lower angle does not grow ventrally to form the pericardial floor in the usual way. However, at an early stage, before the dorsal and ventral parts of the more posterior mesoderm separate, the lower face of this pouch forms a non-cellular membrane (fig. 16) that later becomes continuous with the more posterior pericardial floor. Ventrally this membrane appears to end against the end sac on the maxillary gland (fig. 15).

It has been previously stated (p. 409) that the lower edge of the first cœlom sac gives rise to a small muscular strand—called here the shell-fold muscle—that spans the entrance to the shell-fold cavity. The connection between the first dorsal sac and the maxillary gland is, at first, underneath the rudiment of this muscle. Later, however, the cells forming the hinder limit of the lower edge of the cœlom sac completely surround this muscle, so that there is a connection both above and below it (fig. 16). Ultimately the

lower connection is lost, so that the connecting tongue of cells passes entirely dorsal to the shell-fold muscle (fig. 15). This detail may appear unimportant, but it has been given here as otherwise the figures might appear misleading.

Discussion.

In all the classes of Arthropoda other than Crustacea, so far as observations have been made, the occurrence of metameric cœlomic cavities is firmly established and is of regular occurrence. In the Crustacea, however, their occurrence is much less certain. The accounts given are few, but are conflicting.

Fric (7), working on various species of Cyclops, Diaptomus, and other Copepoda, states that in the later nauplius the mesoderm forms two long streaks of cells in which can be seen paired cavities. He considers that these correspond to mesoblastic somites. In the young stages they are large and pressed one against the other. During later development they separate from each other, dwindling in size during the process, and come to rest on the nerve cords. Here they finally disappear. Urbanowicz (22), also working on various Cyclopidæ, gives quite a different account. He agrees with Fric in stating that the mesoderm of the nauplius appears first as paired mesoderm bands, and that in these bands paired cavities appear. As the larva grows, however, it is stated that the mesoderm bands also enlarge, and new pairs of cavities are formed behind the first pair, each pair corresponding to a segment and one pair of appendages. The dissepiments between the somites do not persist for any length of time, but the mesenteries persist throughout life. The dorsal mesentery forms, by the separation of its two halves, a dorsal sinus that plays an important part in the circulation in the absence of a true heart. According to this observer, then, the primary body cavity persists in the posterior part of the body only as the dorsal sinus. The perivisceral cavity, being formed by the fusion of the somite cavities, is thus a secondary body cavity. It is obvious that the account given by Urbanowicz differs entirely from the generally accepted view as to the relations of the body cavity of the Crustacea. The careful researches of Grobben (8) on Cetochilus do not lend support to the findings of Urbanowicz. He states that in the metanauplius of Cetochilus there are transverse plates of connective-tissue cells that divide the body cavity more or less into compartments. These plates are placed inter-segmentally, and from their position might be regarded as dissepiments. He points out, however, that their incompleteness and the fact that they are made up of very few cells does not support this view.

Among the Phyllopoda there is a paper in Russian by Nassonow (18), in which there is a figure of a horizontal section of the nauplius of Artemia showing a paired series of cavities in the posterior segments of the body, and which, according to Korschelt and Heider, are taken to represent a temporary development of paired cœlomic vesicles. In the nearly related genus Branchipus, Claus (6) did not find this state of affairs. Possibly the cavities figured by Nassonow merely represent splits in the mesoderm which

is in the process of differentiating into muscles. This is also suggested by one of NASSONOW'S figures, in which one of the supposed cœlomic cavities is not separated from the ectoderm by mesoderm on the outer side. Further, horizontal sections of late nauplii of Estheria usually show cavities in the limb rudiments, but these cavities are simply due to the separation of the mesoderm preparatory to forming the various muscles of the limbs, and, in transverse sections, are usually found to be continuous with the general body cavity.

Among the higher Crustacea there is a statement by GROSGLIK (10) to the effect that WASILJEFF had observed somite formation in Oniscus, but had not published an account of it. In Astacus, REICHENBACH (20) states that, at the stage where the rudiments of the abdominal feet appear, the mesoderm, which was originally scattered, becomes grouped into paired segmental masses. In the centres of these masses there appear cavities which can be considered, according to REICHENBACH, as true cœlom. The position of these sacs supports this contention, but their late appearance suggests again the possibility that they indicate merely a grouping of mesoderm preparatory to its differentiation into segmentally arranged structures. Also, until this work has been repeated, using less crude technique than did REICHENBACH, this description cannot be taken as definitely established.

In Estheria, paired cavities appear in the post-mandibular mesoderm. That these are strictly homologous with cœlomic cavities occurring in other classes of Arthropoda is evidenced by the spatial relation of those cavities to the rest of the body, but more especially by their relation to the formation of the heart.

The descriptions of the method of heart formation in Arthropoda are essentially the same in all classes except the Myriapoda Diplopoda. It would serve no purpose to give here a detailed comparative account of them. The account given by SEDGWICK (21) for Peripatus may be taken as typical. A cavity, which must be regarded as the remains of the blastocœle, is formed by the shrinkage of the endoderm from the ectoderm. The medio-dorsal part of this is the cardiac cavity. It becomes wedged in between the dorsal apices of the somites, and, finally, the walls of the heart are formed surrounding this space from the adjacent walls of the somites. The formation of the ostia is not known for certain in Peripatus, but in Agalena, where, according to KAUTSCH (15), the development of the heart is typical, the contributions of the separate somites to the muscular walls of the heart tube fuse together dorsally and ventrally to the cardiac cavity, but not laterally. The lateral gaps thus left are the ostia.

In Peripatus the somites divide into an upper part that forms the heart and a lower part that forms, in the jaw segment, the salivary gland, and in the other segments the excretory organs.

In Scolopendra, according to HEYMONS (13), while the method of heart formation is the same as in Peripatus, there is the difference that the somites divide into three portions, a dorsal portion concerned as before in heart formation, a lateral portion that gives rise to muscles in all except the intercalary segments, where it gives rise to two

masses at the sides of the œsophagus considered by HEYMONS to be the homologues of the nephridia, and hence of the salivary glands of Peripatus, and a ventral portion that extends ventrally to meet its fellow. The latter portions are unrepresented in Peripatus, and from their walls is formed the ventral vessel.

In Julus, according to HEATHCOTE (12), the somites are divided into a " somatic " portion that remains in the body and a " crural " portion that passes into the limbs and develops the musculature. The heart is said to develop by the grouping together of certain cells that are left scattered throughout the yolk at the close of blastoderm formation. In the segment of the gnathochilarium, the somatic portions of the somites give rise to the salivary glands.

If the salivary glands of Julus are regarded as homologous with the ectodermal salivary glands of insects, the account given by HEATHCOTE cannot be accepted. If, however, as seems more probable, the salivary glands are considered to be the homologues of the œsophageal masses in Scolopendra, and of the salivary glands and nephridia of Peripatus, then their mesodermal origin described by HEATHCOTE would appear correct. Accepting this homology, it appears that the " somatic " portion of the somite of Julus is comparable to the lateral portion of the somite of Scolopendra and to the lower portion of the somite of Peripatus. The upper portion of the somite of Peripatus and the dorsal portion of that of Scolopendra would then be represented in Julus by the cells scattered through the yolk which form the heart. These, according to HEATHCOTE (11), " are the direct descendants of the first segmentation masses." It is not clear how these cells were definitely traced from the segmentation stages to the later stages of organogeny. Apparently they had no conspicuous cytological characteristic—they are stated merely to be of " irregular amœba-like form." It is quite probable that HEATHCOTE was mistaken in his conception of their origin. It is suggested here as more probable that they are budded off from the dorsal parts of the somites. In all other Arthropoda certain cells at the apex of the somites become transformed into the muscle cells of the heart tube after the somites have grown dorsally and enclosed the cardiac cavity. The scattered cells of Julus would thus correspond to these cardioblasts, but, in this form, would be given off and separated completely from the mesodermal masses at a much earlier stage.

The method of heart formation in the Crustacea agrees, at least in the less specialised forms, with the typical Arthropodan type. It differs generally only in the absence of the cœlomic cavities in the segmental mesoderm masses. In Branchipus, the development of which was described by CLAUS (6), the mesoderm, divided into separate solid segmental masses, grows upwards on each side of the gut. The apices of these masses are formed by a row of cardioblasts. These assume a crescentic shape, so that a gutter is formed on each side. These gutters approach the mid-dorsal line and so include a tube which forms the cavity of the heart. The ostia are the persistent gaps between the boundaries of the mesoderm segments. This description would apply also to the method of heart formation of Limulus as described by KINGSLEY (16).

Here, however, cœlomic cavities appear in the mesoderm after the heart tube has been formed.

It is obvious that the method of heart formation exhibited by Estheria resembles very closely the typical Arthropodan type. It differs only in that the cardiac cavity appears as a split in a continuous mass of mesoderm that completely surrounds the gut, whereas in the typical Arthropodan type the mesoderm grows up on either side of the gut to include the mid-dorsal part of the primary body cavity. The development of Branchipus, and of other Crustacea such as Oniscus and Mysis, in this respect resembles more closely the usual type than does that of Estheria. In the latter, however, it is probable that if it were possible to work out the embryonic development of the nauplius, it would be found that the mesoderm originates as a ventral mass that grows up on either side of the gut, and so completely surrounds the gut by the time that the egg hatches. In support of this is the fact that in the earliest nauplius, while the mesoderm is definitely continuous around the gut, it forms a very thin layer in the mid-dorsal line, much thinner than elsewhere.

It must be mentioned here that NOWIKOFF (19), in a monograph on the anatomy of a form nearly related to Estheria, *Limnadia lenticularis* L., suggests a method by which the heart may be formed in which he assumes that the pericardial cavity is the primary body cavity, the cœlom remaining as the perivisceral cavity. This suggestion, however, is not based on any embryological evidence and will not be more than mentioned here.

While the method of heart formation in the Arthropoda shows a uniformity throughout the group, it cannot be said that the accounts of the subsequent fate of the dorsal parts of the cœlom pouches after the heart tube has been formed show any such agreement.

In Peripatus, omitting for the time being the consideration of those segments that form the gonads, it was shown by SEDGWICK'S careful work that the dorsal cœlomic cavities, after the formation of the heart, become wedged in the angle between the heart tube and the gut tube. As development proceeds they gradually dwindle in size until they finally disappear, their collapsed walls remaining as the pericardial floor.

In other Arthropoda the accounts differ. In most the fate of the dorsal cœlom is stated to be very similar to that shown in Peripatus, *e.g.*, Euscorpius (3), Scolopendra (13), Agalena (15), Donacia (14). But in some it is stated that the cœlom spaces, after the heart has been formed, enlarge and persist as the pericardial space, *e.g.*, Theridium (17). In fact, one of the most recent workers on Arthropodan embryology adopts the view that a true pericardium must originate from the cœlom, otherwise the space surrounding the heart is only topographically a pericardium (2).

Estheria must be added to that group of forms the development of which agrees with that of Peripatus. In Estheria there can be no doubt whatever that the cœlom pouches diminish in size and finally disappear, and that they do not in any way open out into the body cavity. It is significant that the forms whose development agrees, in this respect, with that of Peripatus are forms that can be considered in many ways

primitive forms, *e.g.*, Euscorpius, Scolopendra, and now Estheria. It suggests that possibly in the other forms this typical fate of the cœlom may still be present but masked by secondary developments.

With regard to the development of the gonads, the type of development exhibited by Peripatus is also shown by some of the higher Arthropoda, *e.g.*, Agalena (15), Scolopendra (13), and according to KINGSLEY (16), Limulus. Here the dorsal divisions of the cœlom of those segments from which the gonads develop fuse together, and from their walls the germ cells develop. In some forms, however, the primitive germ cells are segregated at a very early stage before the somites and their cœlomic cavities have appeared, *e.g.*, Donacia (14), Euscorpius (3). In Donacia the genital rudiment is formed at the time of blastoderm formation as a posterior thickening of that structure. Later on, when the cœlomic cavities have appeared, it migrates forwards as a loose group of cells that ultimately penetrate into the ninth segment and may reach as far forwards as the seventh segment. The remains of the cœlomic cavities persist as the cavities of the gonads.

Estheria clearly belongs to that group in which the genital cells segregate early in development. Here, however, instead of actively migrating into a cœlomic sac, the genital rudiment becomes surrounded by the down-growth of a cœlomic pouch—that belonging to the first trunk segment. In this respect Euscorpius probably resembles Estheria more closely than do other forms. In this form, according to BRAUER (3), certain cœlomic sacs press against the genital rudiment and ultimately the cells composing this rudiment pass into the sacs.

The down-growths of the pouches in Estheria form the lateral parts of the pericardial floor, so that ultimately, the genital rudiment becomes embedded in the pericardial floor. Here it grows back to form the primordium of the long tubular gonad. The cavity that subsequently appears in this primordium is, obviously, a cavity forming in the thickness of the pericardial floor, and, since the latter is formed by the collapse of the cavities of the cœlomic pouches, it is reasonable to assume that if a cavity is re-formed in that pericardial floor, that cavity is also a cœlomic cavity. If this argument is allowed, it brings Estheria completely into line with those forms in which the genital rudiment migrates into a cœlomic sac, the cavity of that sac remaining as the cavity of the gonad.

The development of the post-mandibular ventral mesoderm has been worked out in detail only in the maxillary and maxillulary segments. In the other segments it gives rise simply to the trunk limb muscles. It is only in the maxillary segment, however, that a cœlomic cavity appears in the ventral as well as in the dorsal mesoderm, and this cavity develops into the end sac of the maxillary gland and persists throughout life. Although these two cavities are not formed through the division of an originally single cavity, as is the case in the formation of the dorsal and ventral cœlom of Peripatus, the fact that the ventral cavity appears later in the segmental mass of mesoderm in continuity with the dorsal cœlom sac of the maxillary segment indicates, probably,

that it is strictly homologous with the ventral cœlom of Peripatus. This is entirely in agreement with the view that the maxillary gland of Crustacea is the homologue of the excretory organs of Peripatus. Although this view is adopted here only with certain reservations, it must be pointed out that, up to now, this interpretation of the maxillary gland has rested chiefly on adult anatomical comparisons. The development of the maxillary gland and its relation to the cœlom has not before been thoroughly worked out. From the work described here it is now seen that not only does this gland show a structure similar to that of the segmental organ of Peripatus, but that its development is also strictly comparable. The fact that in Estheria the connection between the dorsal and ventral parts of the maxillary mesoderm persists longer than it does in any segment of Peripatus may indicate a condition even more primitive than that found in the latter form.

With regard to the relation between the maxillary glands and the antennal glands, it can be safely stated that these two glands are usually taken as being homologous structures. There are certain points, however, that indicate that possibly this is not so, or, at least, that indicate that while the end sacs of both glands may be the remains of cœlomic cavities, the ducts may be of different origin, and hence not homologous. Until the development of the antennal gland has been fully worked out, it is not possible to settle this point with any degree of certainty.

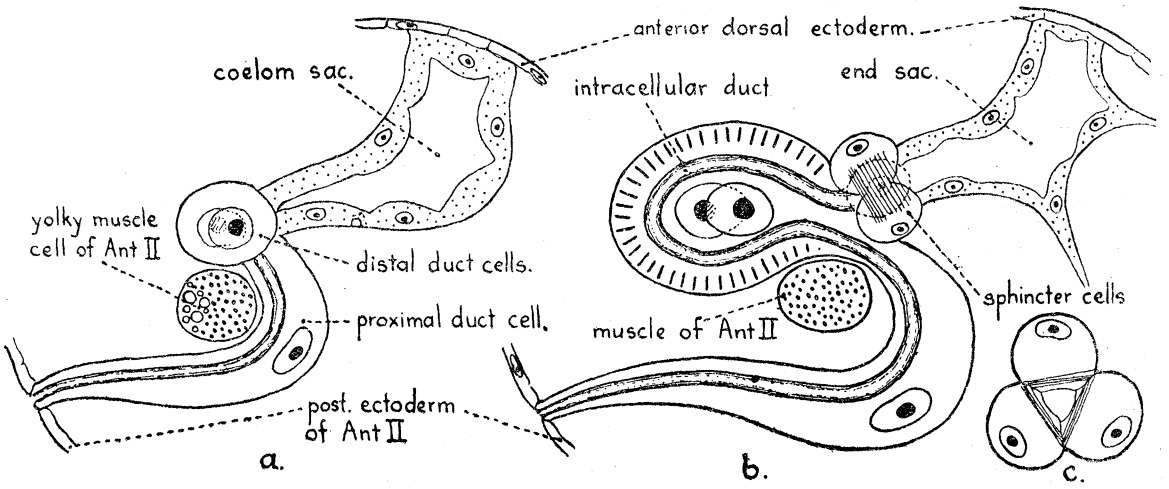
From a recent comparative account of the excretory glands of Crustacea (4) it appears that they can be divided into two groups, according to the nature of their efferent ducts. In the one group the duct is intracellular and consists of three, or at least very few, cells, while in the other it is intercellular and is composed of a large number of cells and may be a very complex structure. This fact, in itself, appears to the author sufficient to render it highly improbable that the excretory glands are homologous throughout the group.

In the earliest nauplius of Estheria the antennal glands are not fully formed, their ducts being still in a rudimentary condition. A comparison of the duct rudiments at this stage with certain ectodermal glands suggests that the duct portions of the glands are really ectodermal in origin, and hence not homologous with the duct portions of the maxillary glands.

Immediately after hatching, the antennal gland consists of a small end sac joined to the ectoderm by a duct system of three cells (*a*, text-fig. 5). The end sac is a thin-walled sac, and the general view will be adopted here that it is a cœlomic sac. The duct system consists of a long, thin, tubular duct cell, which can be called the proximal duct cell, that opens to the exterior on the posterior side of the base of the antenna. Internally it ends against two cells, which can be called the distal duct cells, that are apparently fused together into an oval mass resting against the posterior side of the end sac. The internal end of the lumen of the proximal duct cell lies in the plane in between the nuclei of the two distal duct cells. Soon after hatching, in this same plane, in the distal cells there appears a minute lumen in the form of a loop, one end being in

continuation with the lumen of the proximal duct cell and the other leading into the end sac. After the first moult the entrance of this lumen can be seen to be surrounded by three slightly yolky cells. On the inner side of these cells very delicate fibrils can with difficulty be seen to have developed (text-figs. 5 and 6). These fibrils do not persist long, the entrance cells becoming very soon indistinguishable from the other cells of the end sac.

This description agrees with that given by GROBBEN (9) for the antennal gland of a 0·5 mm. Estheria larva. He does not mention, however, the muscular cells that guard the entrance to the efferent duct. Probably in a larva as large as 0·5 mm. they had already disappeared. Similar cells have been frequently described, notably by VEJDOVSKY (23). Another author, ZAVADSKY (25), in a general account of this " guard sphincter," describes the muscle " ring " as being a differentiation of the " nephridial "



TEXT-FIG. 5.—Diagrams illustrating the anatomy of the antennal gland: A. The gland of the newly hatched nauplius. B. The gland of the early metanauplius. C. The three guard cells showing the arrangement of the fibrils.

wall, separate muscle cells being apparently absent. This is certainly not the case in the Estheria nauplius. There are three separate muscle cells, and, furthermore, the muscle fibrils are not accurately described as a ring. The fibrils develop on the lumen side of each cell as a flat layer, and these, at their ends, join the fibrils of the adjacent cells. In this way a triangular structure is formed rather than a ring of muscle fibrils (text-fig. 5, *c*).

In the metanauplius stages the coiled lumen in the distal cells develops considerably. It has apparently well-differentiated walls that are distinct from the surrounding cytoplasm, and, in fact, it may now be termed a distinct duct running through the two distal cells. The cytoplasm of the latter has now become full of deeply staining rods that radiate from a reservoir that has formed in between the nuclei of the two cells. This reservoir is thus almost completely surrounded by the coiled duct. As development proceeds it increases in size, but the coiled duct always lies along its periphery.

Ultimately, when the gland becomes effete and dwindles, there is a very considerable accumulation of secretion in the reservoir.

If the duct system of the antennal gland, apart from the end sac, is compared with the labral glands, there are seen to be some very marked morphological similarities. The labral glands of Estheria show the same arrangement as those of Chirocephalus that have been briefly described by the author (5). There are three conspicuous glands, each consisting of a pair of gland cells between which is a reservoir of secretion and a tubular duct cell leading from the reservoir to the exterior. One of the glands opens medianly and the others laterally.

The duct cell of the labral gland may be taken as corresponding to the proximal duct cell of the antennal gland. The two gland cells would then correspond to the two distal duct cells of the antennal gland. Such a comparison is supported by the fact that fluid of some sort does accumulate between the two distal duct cells, just as the secretion of the labral gland accumulates in a reservoir between its two gland cells. Further, the cytoplasm of the labral gland cells is filled with dark-staining rodlike bodies, just as are the distal duct cells of the antennal gland, although in the former case they are not arranged in any special manner as they are in the latter.

If it is allowed that this morphological similarity may have some homological significance, it appears to the writer possible to suggest a rational explanation of the two distinct types of excretory glands that occur in Estheria and in Crustacea generally. It can be taken either that the Crustacea are evolved from a form in which there was in each segment a pair of cœlomic sacs with their cœlomiducts and a pair of true ectodermal nephridia opening into them and acting as the excretory organs, or else that there were no true nephridia and the cœlomiducts functioned as the excretory ducts, as is the case in Peripatus. Whichever view is taken, during the development of such an ancestral form there would always be a stage when the cœlom sacs had produced cœlomiduct outgrowths that had not yet opened to the exterior in the ectoderm. At such a stage it is probable that the ectodermal glands would be precociously developed in connection with larval needs. It has been indicated earlier in this paper how the ectodermal labral glands are precociously developed in the newly hatched nauplius, most probably to function in the feeding mechanism of the larva. Further, the ectoderm at the base of the labrum consists almost entirely of large glandular cells. This can be seen more clearly in the nauplius of Chirocephalus. Now it is suggested that in some ancestral Crustacean some of the cœlomiduct outgrowths did not grow out as far as the ectoderm, but connected up with the inner ends of such ectodermal glands.

The ectodermal glands with which the cœlomiduct connected might have constituted distinct ectodermal glands such as are scattered over the body of most Crustacea, or they might have been simply a patch of glandular cells such as occur at the base of the labrum of the nauplii of Estheria or Chirocephalus or of adult Daphnids. In the former case a structure would be obtained agreeing closely with the young antennal gland. In the latter case the cœlomiduct would primitively open between the ectoderm cells,

as does the efferent duct of the maxillary gland of Estheria ; and it must be imagined that the two or three cells between which the duct opened to the exterior passed inwards, retaining at the same time their connection with the exterior by one of themselves or by another ectoderm cell acting as duct cell, just as the gland cells of the labral glands pass inwards from the ectoderm. Ultimately the tubular cœlomiduct outgrowth would have become unnecessary, and may have disappeared as such. It might very probably be represented in the muscular guard cells that occur transitorily around the exit from the end sac—that is, on the above hypothesis, at the junction of the mesodermal and ectodermal parts of the gland. In fact, although the sphincter guard cells have not yet been observed in the maxillary gland of Estheria, where the efferent duct is still clearly a cœlomiduct, it is very probable that the ancestral form did possess some sort of sphincter control to its cœlomiducts, and it is the remains of these that are seen in the antennal gland.

The secretory activity of the ectodermal part of the antennal gland would be represented in Estheria by the accumulation of fluid that occurs in between the distal duct cells, and by the occurrence of the deeply staining rods in the cytoplasm of these cells.

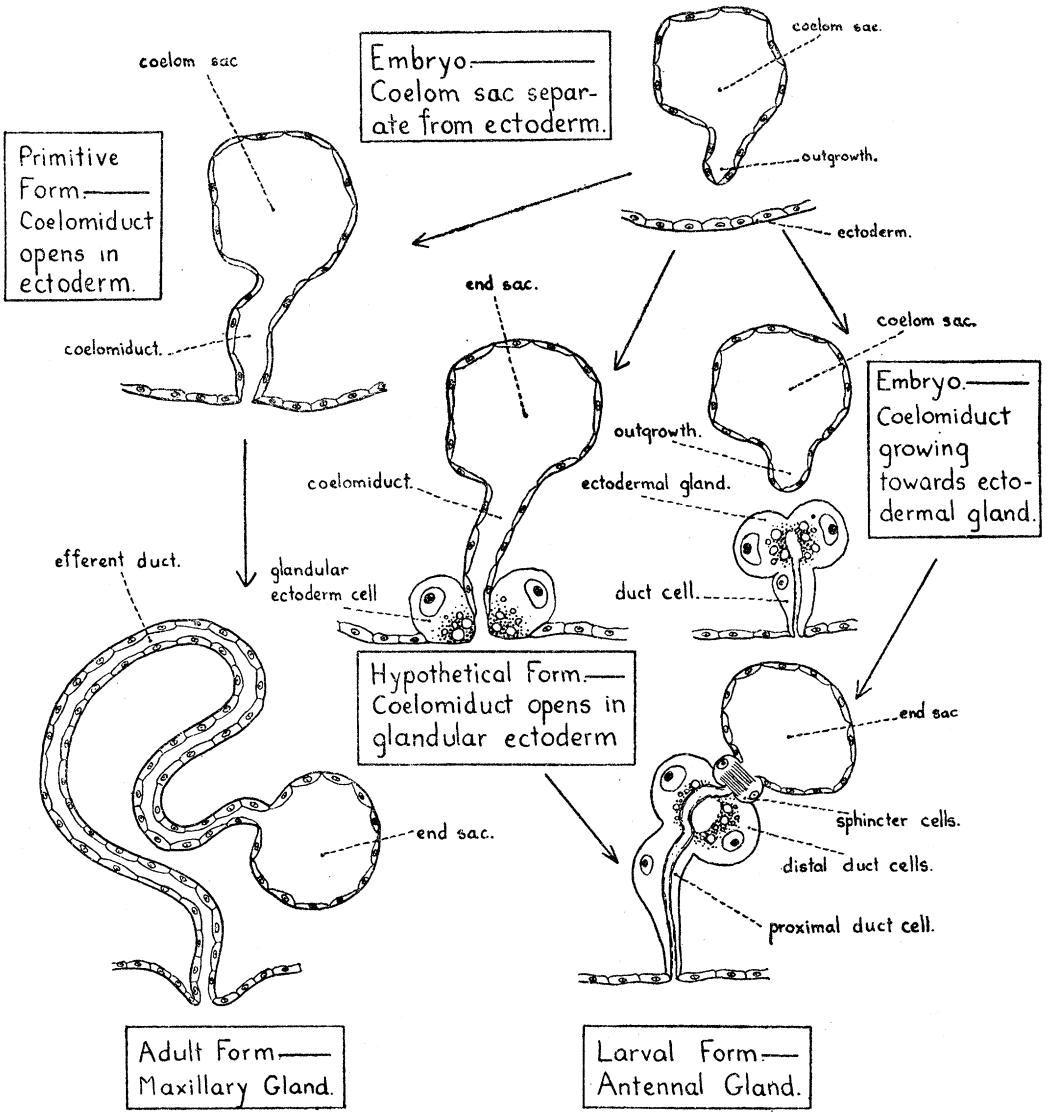
On such a scheme as this, which is figured in text-fig. 6, it is easy to understand why such diverse reports occur as to the germ-layer origin of the ducts of Crustacean excretory glands. A concise summary of the various opinions put forward by workers on this subject is given by Woodland (24). He comes to the conclusion that " the evidence for the ectodermal origin of the duct is preponderant." More probably most of the workers are correct in their statements, in some forms the duct being ectodermal and in others it is still the more primitive mesodermal cœlomiduct.

In Estheria there can be no doubt as to the cœlomiduct nature of the maxillary gland duct, while in the case of the antennal gland it is thought probable by the author that it represents the composite structure suggested above of a cœlom sac in connection with an ectodermal gland. It must be emphasised, however, that this is merely a suggestion based chiefly on morphological comparison, and only partly on developmental observations, and that, until the complete development of the antennal gland has been worked out, it will not be possible to settle its true homologies.

It is not maintained that all Crustacean excretory glands must be either of the composite nature suggested here or of the simple cœlomiduct type. The latter type may be associated with an ectodermal invagination, and may become extremely specialised structurally, but it is thought probable that all such glands can ultimately be traced back to these two types.

If the view suggested here is adopted, it follows not only that in any Crustacean the antennal gland and maxillary gland are not necessarily homologous, but that the antennal glands and the maxillary glands do not form two homologous structures throughout the Crustacea. Thus in Astacus the antennal gland is of the cœlomiduct type, and is thus comparable with the maxillary gland of Estheria. On the other

hand, however, in certain fresh-water Ostracods, according to BERGOLD (1), the antennal and maxillary glands are both present and are both of the same ectodermal gland type. Here, then, the maxillary gland is comparable with the antennal gland of Estheria. It is interesting to note also that in these forms BERGOLD observed a small



TEXT-FIG. 6.—Diagram illustrating the hypothesis suggested to explain the evolution of the two different types of excretory gland found in the Crustacea.

sac in the base of the first antenna, which stretched towards the ectoderm, but apparently did not open to the exterior. This would represent the stage mentioned above, where the cœlomiduct rudiment had grown towards the ectoderm but had not yet opened to the exterior.

Summary.

1. The larva of Estheria hatches as a true nauplius.
2. The mesoderm of the post-mandibular region forms a continuous sheet around the gut. This, together with the genital rudiment, fills up all the space between the endoderm and ectoderm, except for a small space in the most anterior ventral portion.
3. A split appears in the mesoderm along the mid-dorsal line. The mesoderm moves away from this split on either side, retaining at the same time its connection with both endoderm and ectoderm. The space so formed is the cardiac cavity.
4. At the same time a space appears between the more ventral mesoderm and the gut. This space is the perivisceral cavity.
5. The cardiac cavity and the perivisceral cavity are continuous through a space around the proctodæum.
6. The ventral mesoderm develops a transient segmentation that becomes obliterated by growth.
7. Later, the more dorsal mesoderm develops a series of seven pairs of cœlomic pouches.
8. From the walls of these pouches are developed the dorsal longitudinal extensor muscle of the trunk and from the first four the muscular heart tube. After these have been formed the cavities diminish in size. The space so formed between them and the ectoderm becomes the pericardial cavity. The lower edges of the sacs break connection with the more ventral mesoderm, except in the maxillary segment, and grow downwards to form the lateral portions of the pericardial floor. The collapsed remains of the sacs are finally represented by the pericardial floor and the epithelial covering to the muscular heart tube. The sacs do not in any way open into the general body cavity.
9. In the ventral mesoderm in connection with the first cœlom sac a cavity appears which becomes the end sac of the maxillary gland.
10. The development of the maxillary gland and of the adductor muscle are described. The homologies of the latter are discussed.
11. The genital rudiment is represented in the earliest nauplius by a paired mass of cells between the mesoderm and endoderm. It remains in this condition until the cœlom pouches grow downwards, when the portion on each side becomes included in the cœlom sac of the first trunk segment. It then grows backwards as a solid rod of cells in the pericardial floor. Later cavities appear in these rods that become the cavities of the gonads.
12. The antennal gland is described and compared with the labral glands. A suggestion is made to account for the occurrence of the two types of excretory glands found

in the Crustacea, the one with an intracellular duct and the other with an intercellular duct.

13. The mid-gut is devoid of musculature until a late stage, when circular muscle cells grow down from the pericardial floor from the region where the latter is in contact with the gut. The proctodæum becomes surrounded by circular muscle a few hours after hatching. These muscles send forwards fibrils that ramify over the surface of the gut and apparently persist throughout life.

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LIST OF ABBREVIATIONS USED.

a. = aorta.
b.c. = blood cell.
c.c. = cardiac cavity.
c.f. = carapace fold.
c. mes. = mesoderm covering muscular heart tube.
c.m.g. = cells of mid-gut.
c.mx.gl. = rudiments of coils of maxillary glands.
dors. sac 1, 2, 3, *etc.* = dorsal cœlomic sacs.
e.d. = efferent duct of maxillary gland.
e.s. = end sac of maxillary gland.
f. = longitudinal fibrils extending from circular muscles of proctodæum.
g.r. = genital rudiment.
i.a. = inner angle of dorsal cœlomic sac.
i.f. = inner face of dorsal cœlomic sac.
j. = junction between 1st dorsal cœlomic sac and rudiment of the maxillary gland.
l.a. = lower angle of dorsal cœlomic sac.
l.f. = lower face of dorsal cœlomic sac.
l.f.c.c. = lateral floor of cardiac cavity.
m.add. = adductor muscle.
m.a.e. = anterior extensor muscle of trunk segment.
m.c. = circular muscles of heart.
m.c.g. = circular muscles of mid-gut.
m.c.p. = circular muscles of proctodæum.
m.d.l. = dorsal longitudinal muscles of trunk.
m.dv.a. = anterior dorso-ventral muscle of trunk segment.
m.dv.p. = posterior dorso-ventral muscle of trunk segment.
mes. mx. 1, 2 = ventral mesoderm of the 1st and 2nd maxillary segments.
mes.p.mx. = post maxillary ventral mesoderm.
m.g. = mid-gut.
m.lat.mx. 1 = rudiment of lateral muscles of 1st maxilla.
m.l.sb. = sub-neural lateral muscles of trunk segment.
m.l.sp. = supra-neural lateral muscles of trunk segment.
m.mdb.d.v. = dorso-ventral muscles of mandible.
m.p.e. = posterior extensor muscle of trunk segment.
m.s.f. = shell fold muscle.
m.t.l. 1, 2, *etc.* = rudiments of muscles of 1st, 2nd, etc., trunk limbs.
m.v.l. = ventral longitudinal muscles of trunk.
mx. 1 = 1st maxilla.

mx. 2 = 2nd maxilla.
n.c. = nerve cord.
n.c.m. = non-cellular membrane.
n.c.r. = nerve cord rudiment.
o.f. = outer face of dorsal cœlomic sac.
ost. = ostium.
pc.c. = pericardial cavity.
pc.f. = pericardial floor.
pv.c. = perivisceral cavity.
tr.l. 1, 2, etc. = trunk limb, 1, 2, etc.
u.a. = upper angle of dorsal cœlomic sac.

All the figures are camera lucida drawings at a magnification ×1,154.

DESCRIPTION OF PLATES.

Plate 18.

Figs. 1–5 form a series of transverse sections illustrating the ventral segmentation at its maximum development. The planes of the sections are roughly indicated in text-fig. 2 by the arrows numbered 1–5.

Fig. 1.—A section through the maxillulary mesoderm and the first dorsal cœlom sac.

Fig. 2.—A section between the maxillulary mesoderm and the thickened columns of maxillary mesoderm from which arise the maxillary glands.

Fig. 3.—A section through the maxillary mesoderm.

Fig. 4.—A section in between the maxillary mesoderm and that of the first trunk segment. The genital rudiment is usually placed at the anterior side of the first trunk segment, and the section figured just grazed that rudiment.

Fig. 5.—A section through the second trunk segment showing the mesoderm complete ventrally as a thin, continuous layer of cells that is not divided in the middle line.

Plate 19.

Fig. 6.—A sagittal section a little to one side of the middle line of an embryo at about the same stage of development as the specimen from which figs. 1–5 are taken. Actually it is slightly younger, as only two segments have appeared behind the maxillary mesoderm. The position of the section is indicated by the arrow in text-fig. 2, *d*. The position of the genital rudiment is indicated by a dotted circle.

Figs. 7–9 illustrate the earliest development of the coil rudiment of the maxillary gland. The stage figured is that shown in text-fig. 2, *g*, and text-fig. 3, *a*, and the planes of section are indicated in the latter figure, by arrows marked 7–9.

Fig. 7.—A section through the maxillulary mesoderm at a level of the rudiment of the adductor muscle. It is simply a later stage of fig. 1.

Fig. 8.—A section passing just behind the adductor muscle rudiment and through the anterior part of the coil rudiment.

Fig. 9.—A section passing through the posterior part of the coil rudiment, and shows the connection between this and the first dorsal cœlom sac.

Plate 20.

Figs. 10 and 11 show a later stage in the development of the maxillary gland, and are comparable with figs. 8 and 9. They are drawings of the anterior focus and the posterior focus of the same section.

Fig. 10.—The first indication of the end sac rudiment.

Fig. 11.—This same rudiment and also the connection between it and the first dorsal cœlom sac.

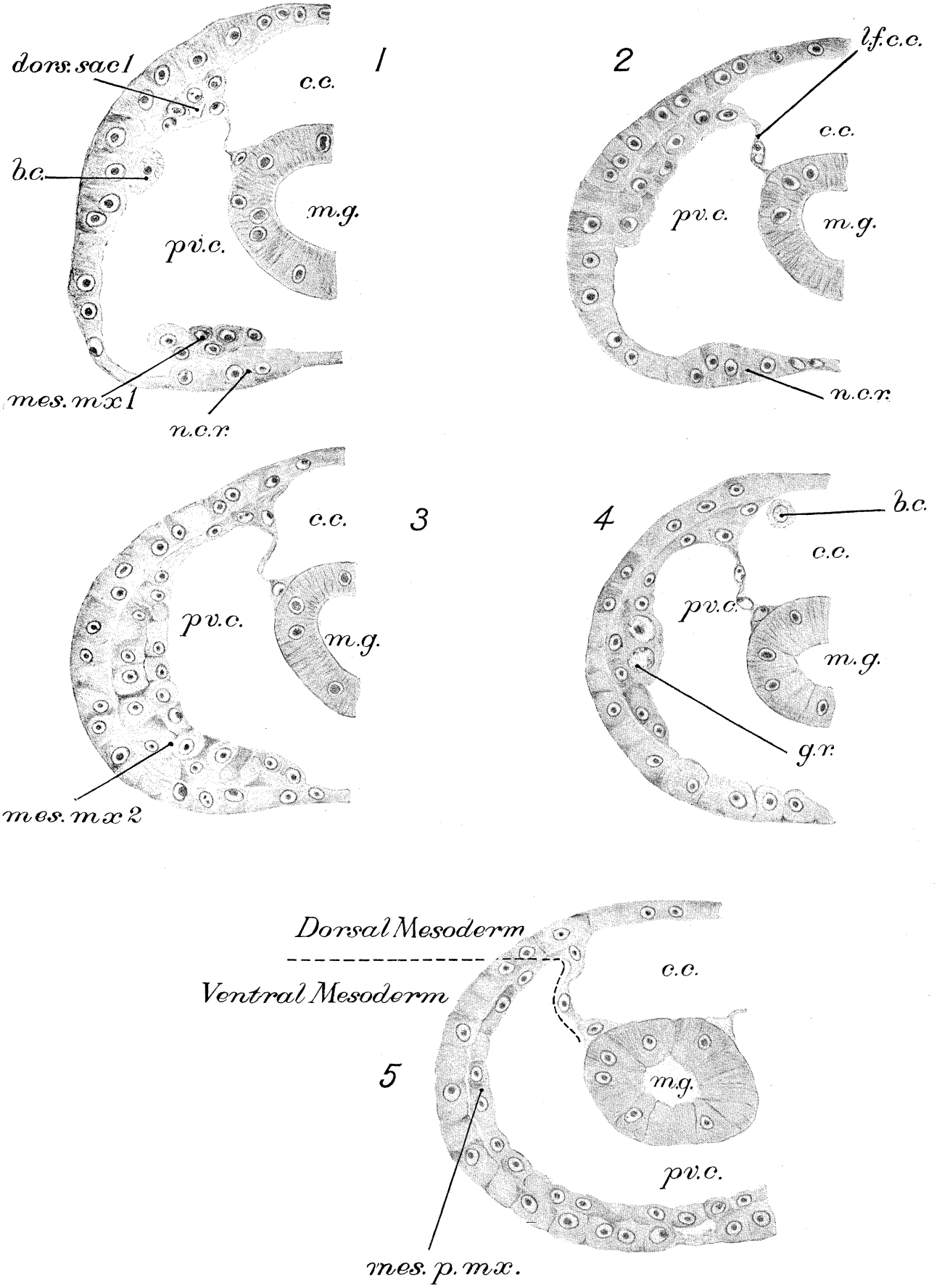
Fig. 12.—The maxillary gland differentiated into the circular mass of cells wedged in the carapace and a solid rod forming the rudiment of the efferent duct leading from this mass to end in the ectoderm of the maxilla.

Fig. 13.—A section through the maxillula showing the rudiment of the adductor muscle ending against the rudiment of the end sac and coil. It also shows the maxillulary mesoderm differentiated into the other muscles of the maxillula. The earliest indication of the shell-fold muscle can be seen at the lower corner of the section of the first dorsal cœlom sac.

Plate 21.

Fig. 14 is much later than fig. 13. It shows the adductor muscle ending just below the end sac, but not yet in contact with the ectoderm. The section passes behind the posterior attachment of the shell-fold muscle, and shows the posterior corner of the first dorsal cœlom sac in contact with the end sac. The inner wall of the cœlom pouch is seen to have differentiated heart muscle cells, while the outer has formed dorsal longitudinal muscles that are already partly separated from it.

Fig. 15.—The adductor muscle after it has joined on to the lateral ectoderm of the shell-fold. It is bounded dorsally by the end sac and ventrally by one of the loops of the coil. The long-drawn-out connection passing above the shell-fold muscle between the first dorsal cœlom sac and the end sac can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pv.c.
m.g.
n.c.r.
3
c.c.
pv.c.
m.g.
mes. mx 2
4
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c.c.
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Dorsal Mesoderm
Ventral Mesoderm
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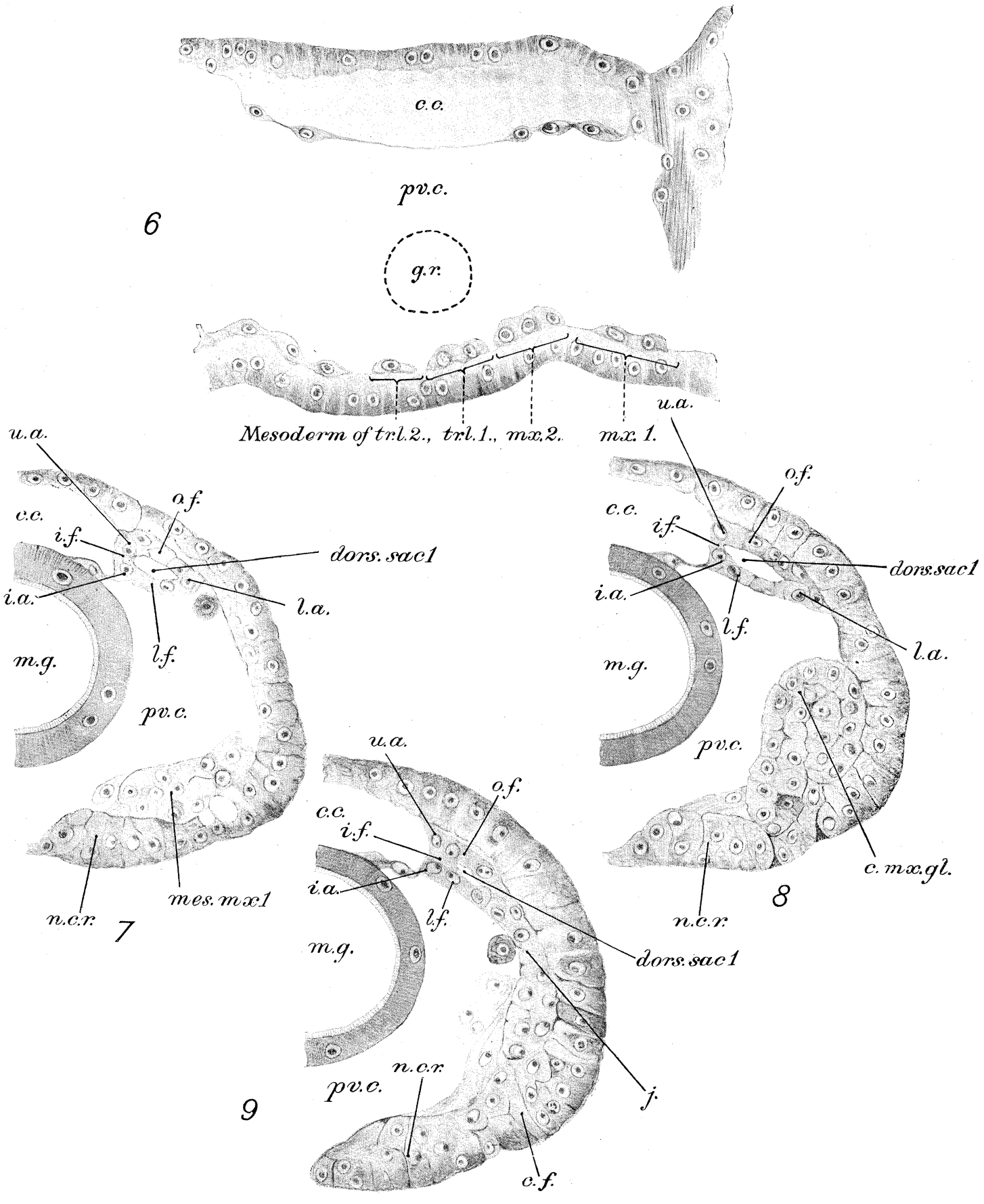
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Mesoderm of trl.2., trl.1., mx.2.
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o.f.
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dors.sac1
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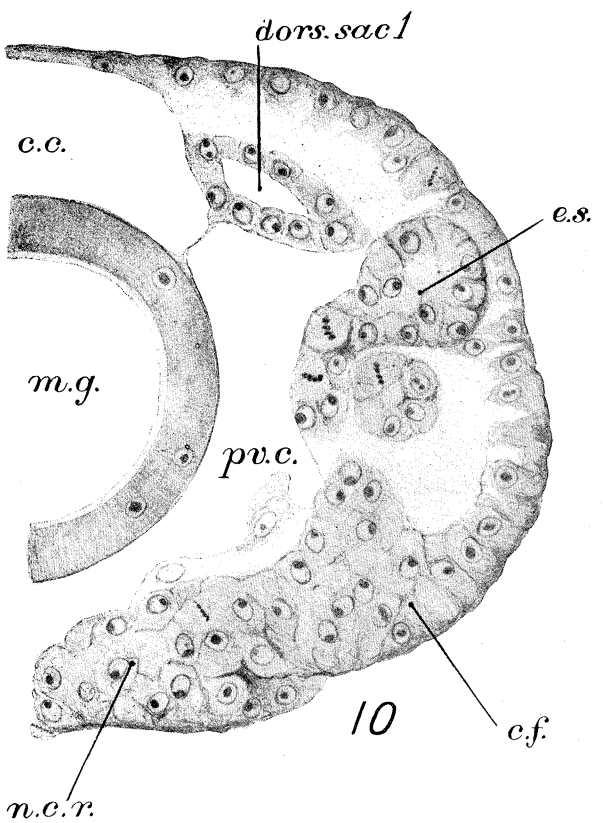
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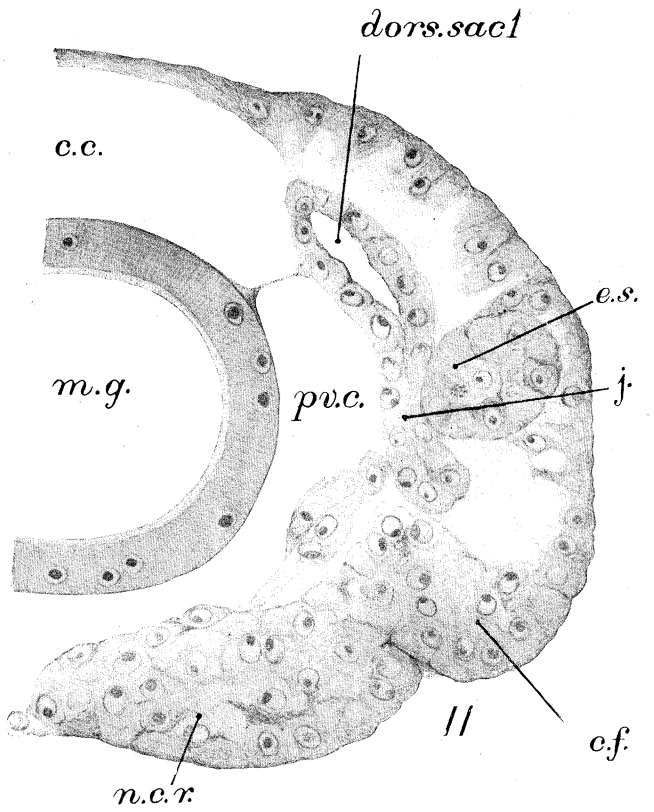
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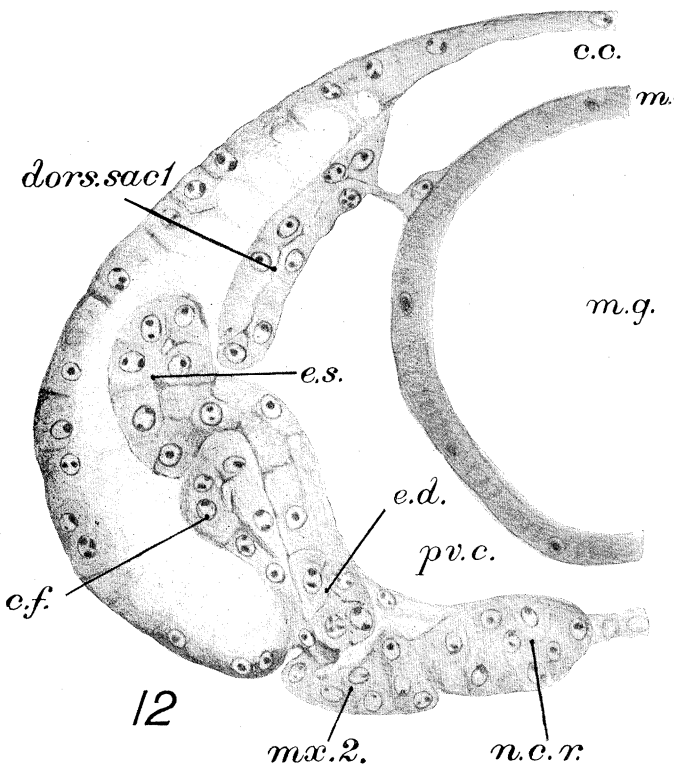
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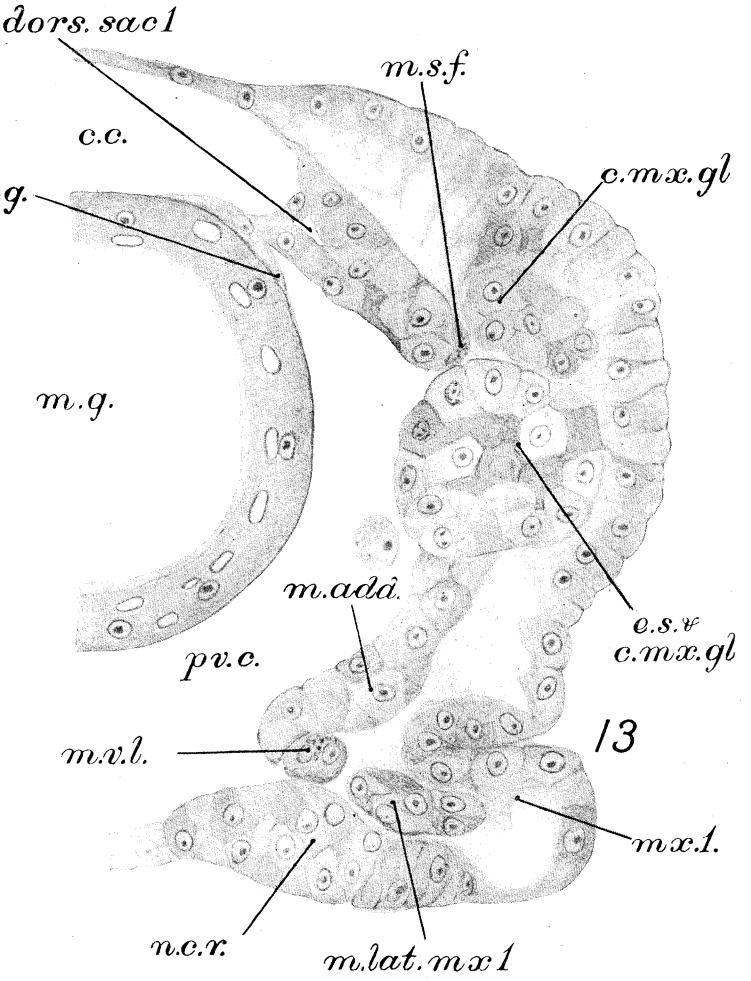
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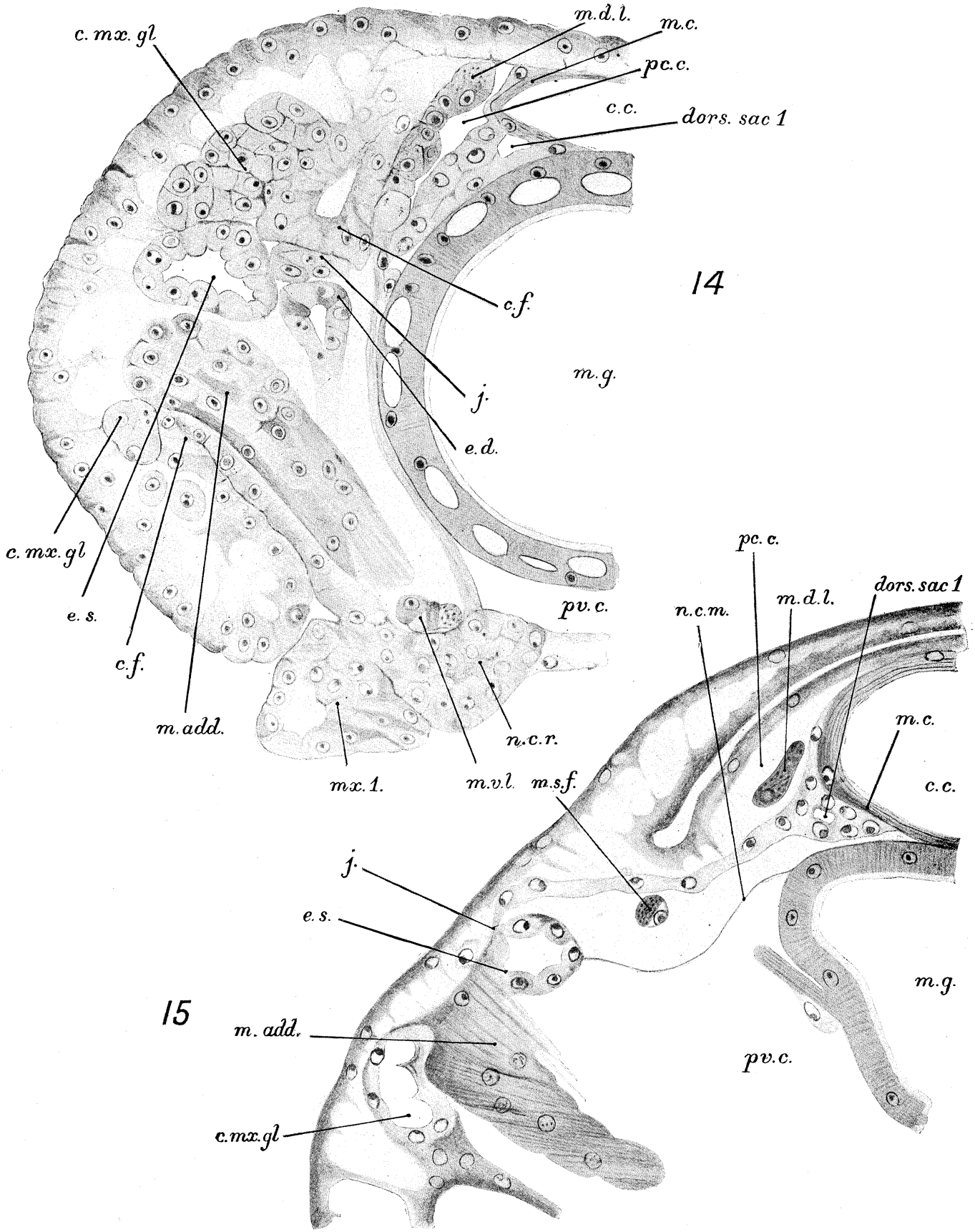
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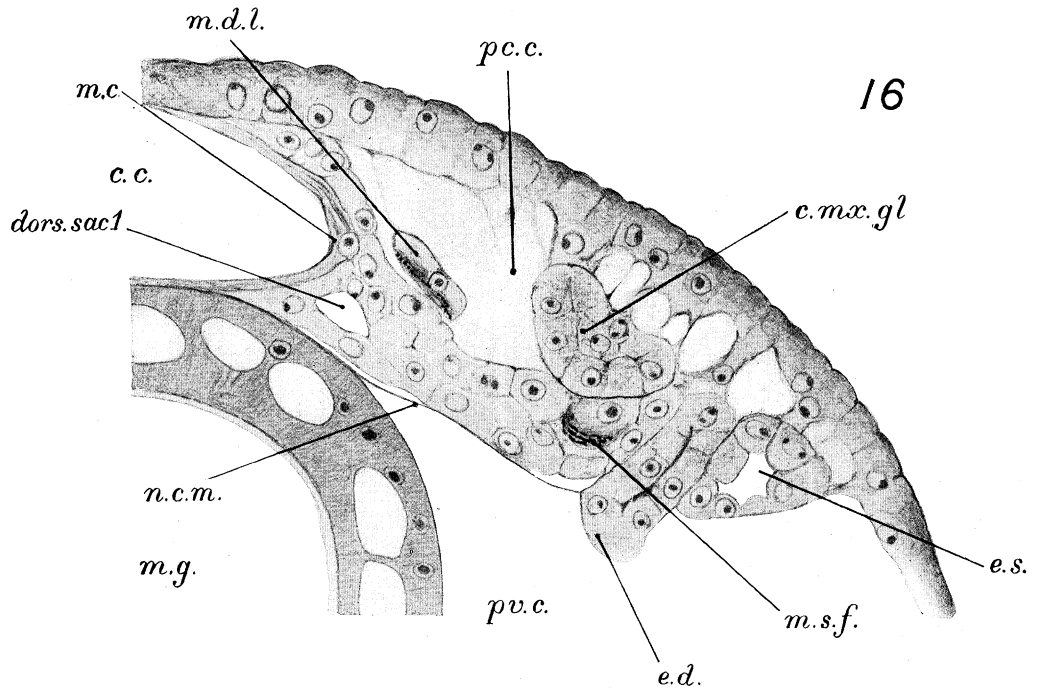
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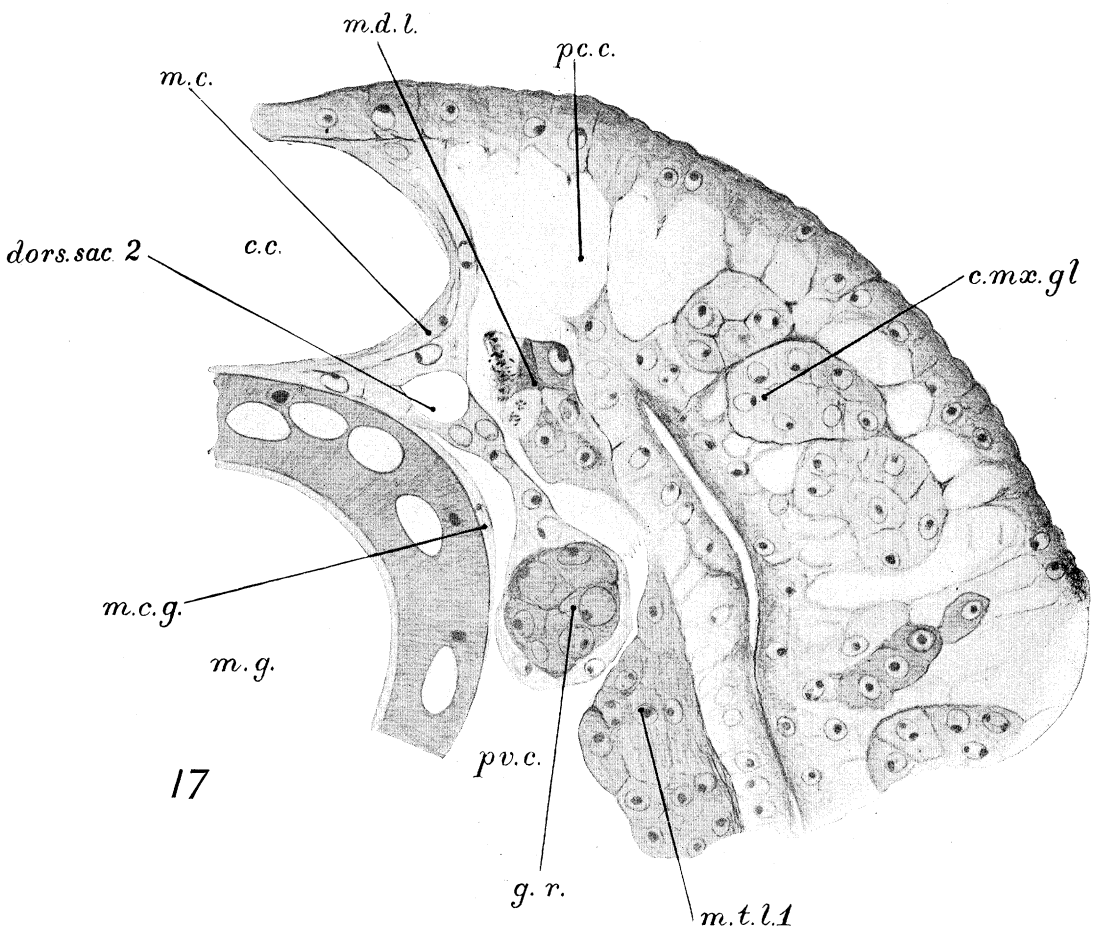
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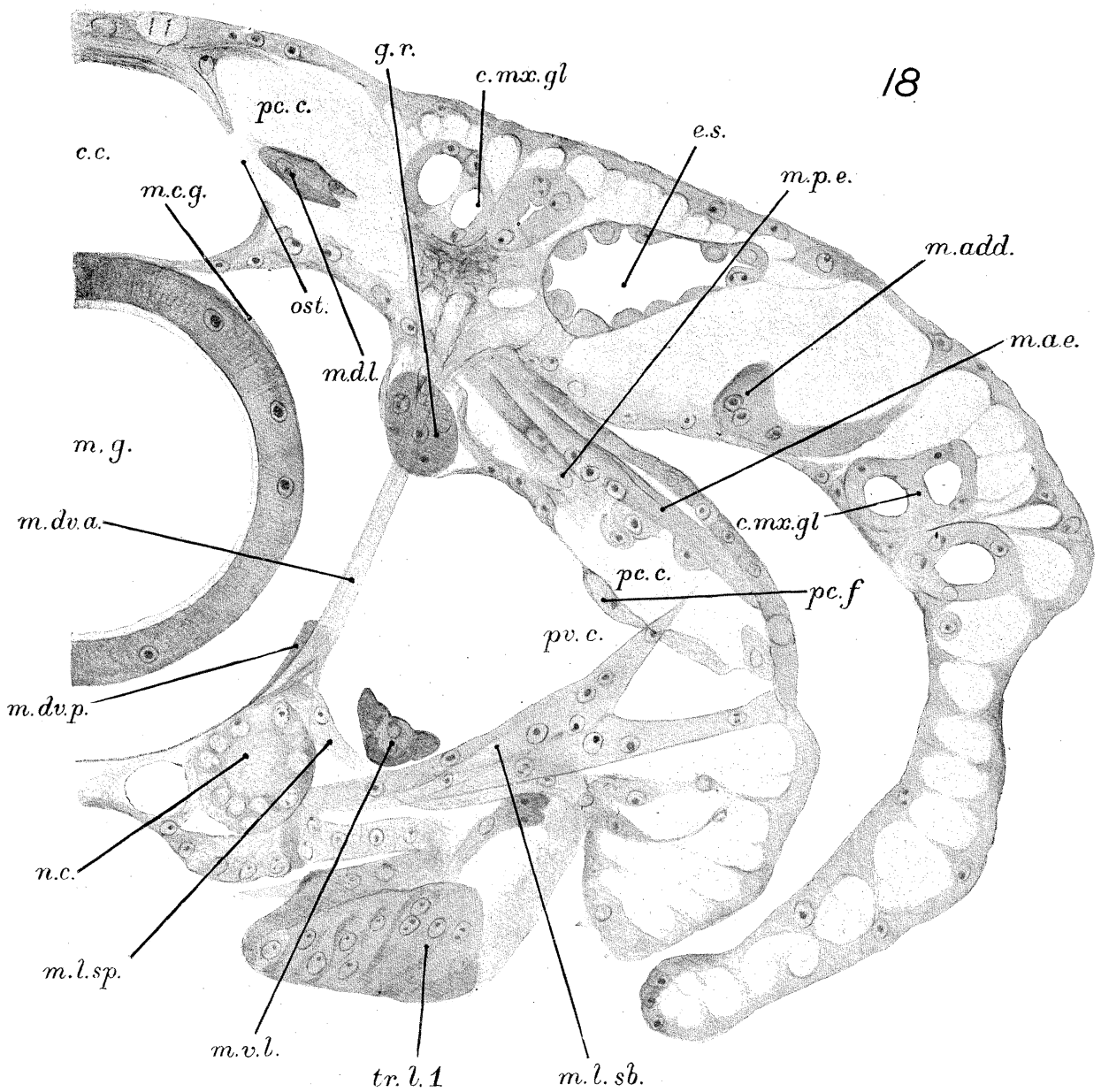
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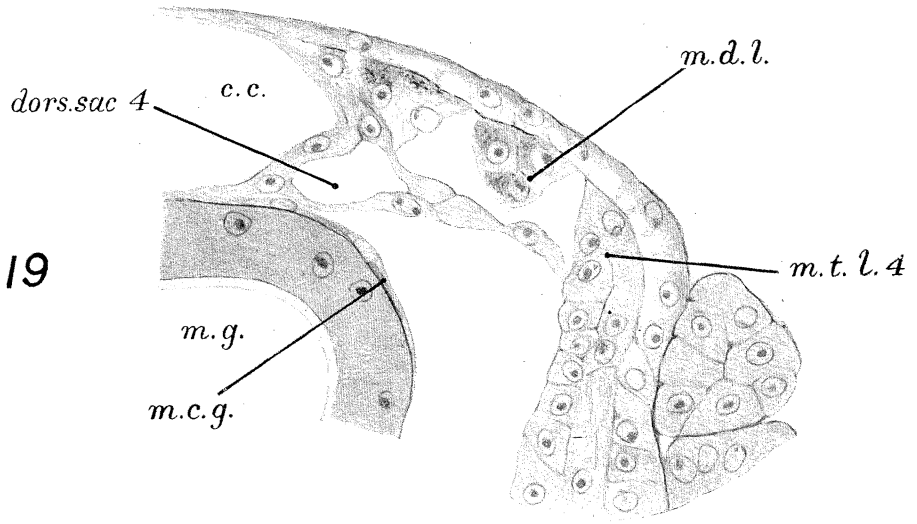
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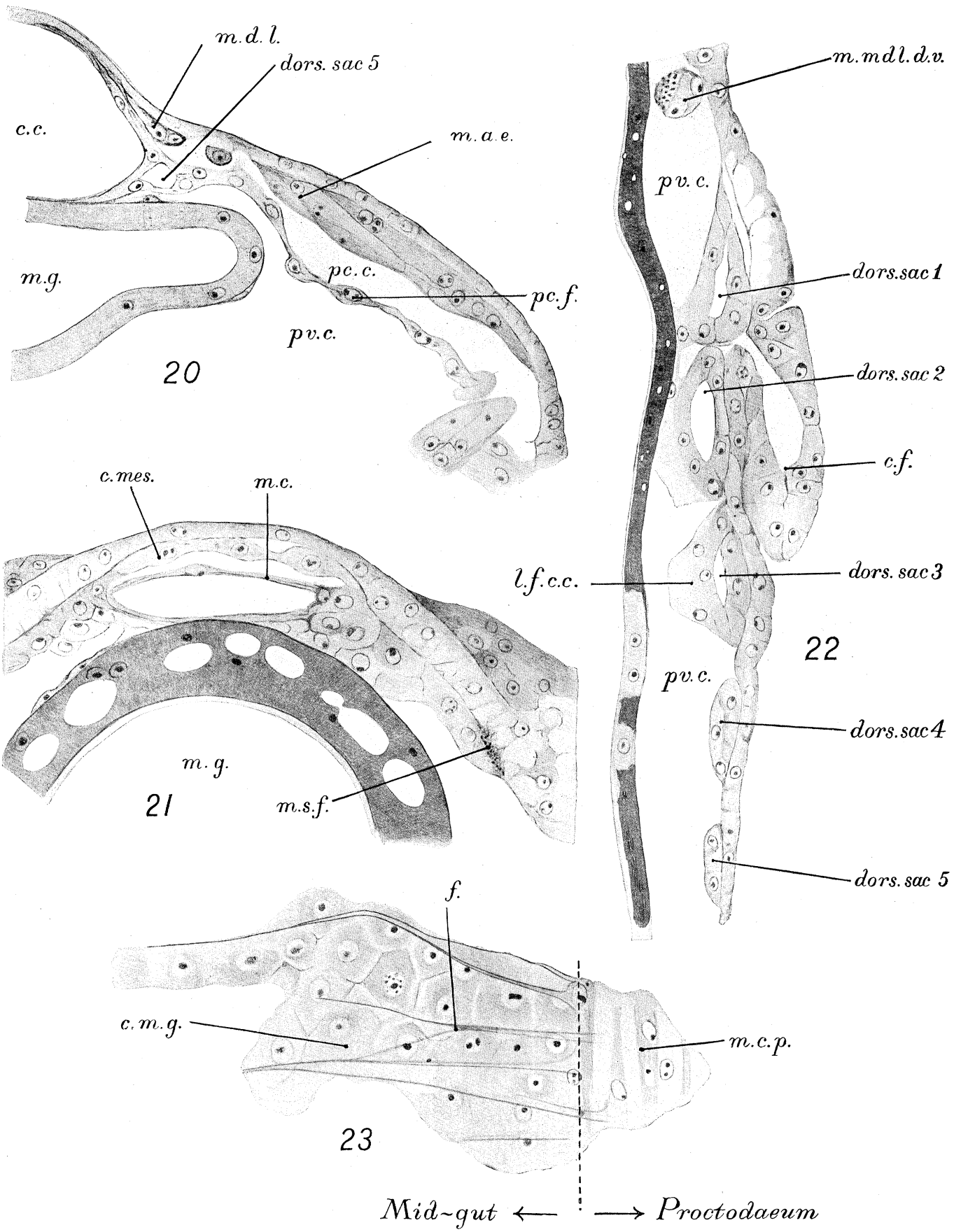
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dors. sac 2
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l.f.c.c.
dors. sac 3
22
pv.c.
dors.sac 4
dors. sac 5
f.
c.m.g.
m.c.p.
23
Mid-gut ← → Proctodaeum

be seen, and also the thin non-cellular membrane that this sheet of cells has formed on its surface ending against the end sac. The dorsal longitudinal muscles are completely separated from the outer face, and the cavity of the cœlom sac has shrunk considerably.

PLATE 22.

Fig. 16.—The cells of the first dorsal cœlom sac passing both above and below the shell-fold muscle rudiment, that it has formed along its lower edge, to end against the end sac rudiment. The dorsal longitudinal muscles, the heart muscles, and the non-cellular membrane of the maxillary segment can be seen clearly.

Fig. 17.—The genital rudiment after the first trunk cœlom sac has grown down and surrounded it. The rudiment of the limb muscles is seen growing up on the outer side of the genital rudiment. A circular muscle cell can be seen on the outer side of the gut growing down from the cœlom sac.

PLATE 23.

Fig. 18.—A section through the first trunk segment at a later stage than that shown in fig. 17. In this section it has been attempted to figure all the important features that occur in the thickness of the section, and not the view of that section at one particular focus, as has been done in the other figures. Thus the anterior dorso-ventral muscle is seen completely, while behind it is the genital rudiment enclosed in the second dorsal cœlom pouch that has now grown down as far as the base of the limb to form part of the pericardial floor. The gap in the heart tube wall is the first ostium. The nerve cord is now completely separated from the ectoderm. All the chief limb muscles are figured.

Fig. 19.—The lower angle of the cœlom sac of a posterior trunk segment growing down on the inner side of the rudiment of the trunk muscles. The circular gut muscles are seen growing down on the outside of the gut wall. The cavity of the cœlom sac is comparatively large, as can be seen by reference to figs. 17 and 16, which are sections through the first trunk segment and through the first dorsal cœlom sac respectively of the same specimen.

PLATE 24.

Fig. 20.—A section through a posterior trunk limb of the same specimen as that from which fig. 15 is taken. It shows the lower angle of the cœlom sac grown down completely to the base of the limb to form part of the pericardial floor. The anterior extensor muscle can be seen wholly external to it.

Fig. 21.—A section through the most anterior part of the heart tube showing the muscle cells separated from the investing mesoderm cells. It shows the anterior attachment of the shell-fold muscle, and is from the same specimen as figs. 16, 17 and 19.

Fig. 22.—A horizontal section through the dorsal cœlomic sacs of one side of a larva at a stage when the rudiment of the maxillary gland consists of a solid coil of cells with only one twist. The level of the section is indicated by an arrow in text-fig. 2, *g*.

Fig. 23.—A slightly oblique longitudinal vertical section that just grazes the gut wall at the junction between the mid-gut and proctodæum. It shows the circular muscle cells around the latter sending forwards fibrils over the surface of the former. The whole thickness of the section has been figured here.

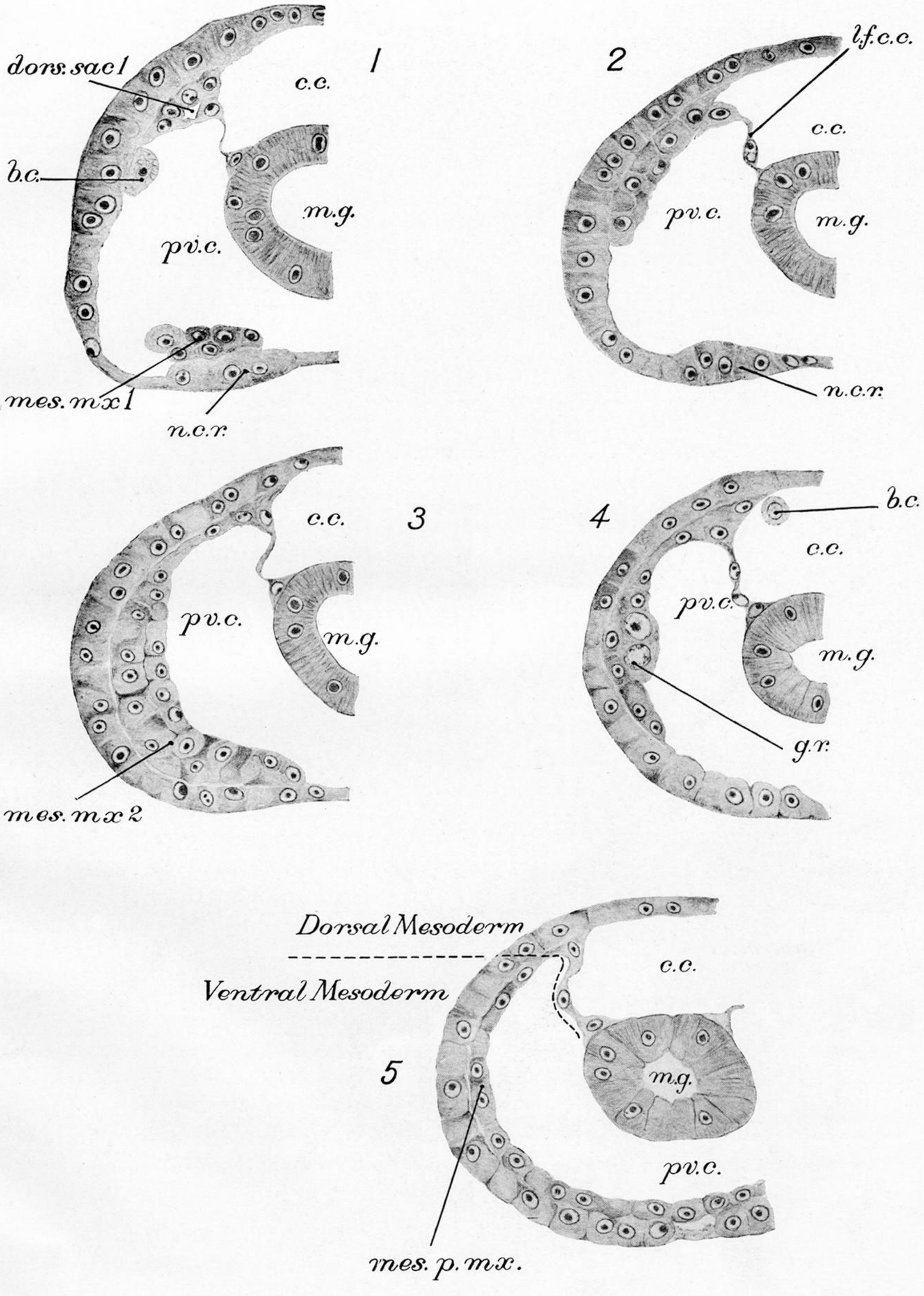


PLATE 18.

Figs. 1–5 form a series of transverse sections illustrating the ventral segmentation at its maximum development. The planes of the sections are roughly indicated in text-fig. 2 by the arrows numbered 1–5.

Fig. 1.—A section through the maxillulary mesoderm and the first dorsal cœlom sac.

Fig. 2.—A section between the maxillulary mesoderm and the thickened columns of maxillary mesoderm from which arise the maxillary glands.

Fig. 3.—A section through the maxillary mesoderm.

Fig. 4.—A section in between the maxillary mesoderm and that of the first trunk segment. The genital rudiment is usually placed at the anterior side of the first trunk segment, and the section figured just grazed that rudiment.

Fig. 5.—A section through the second trunk segment showing the mesoderm complete ventrally as a thin, continuous layer of cells that is not divided in the middle line.

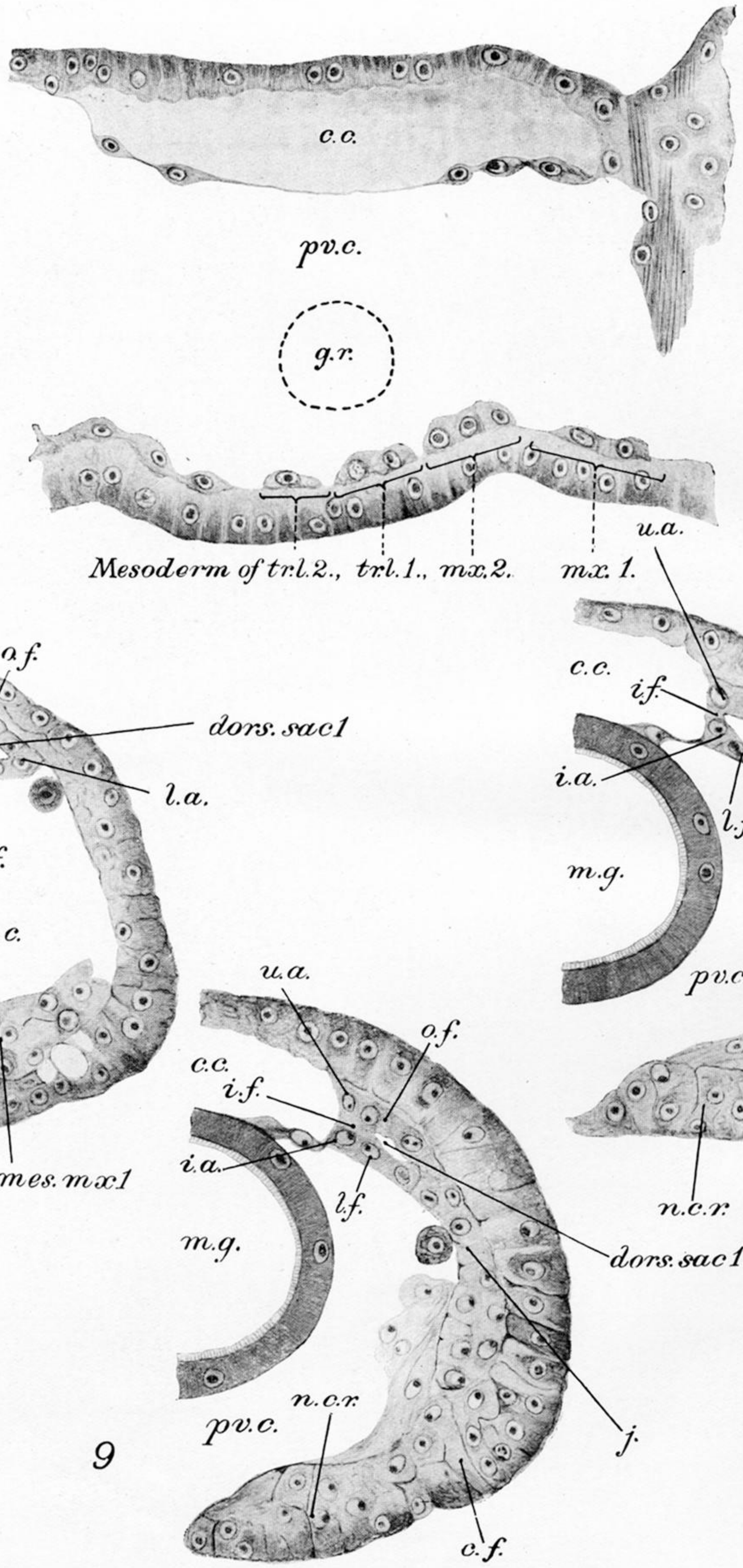


PLATE 19.

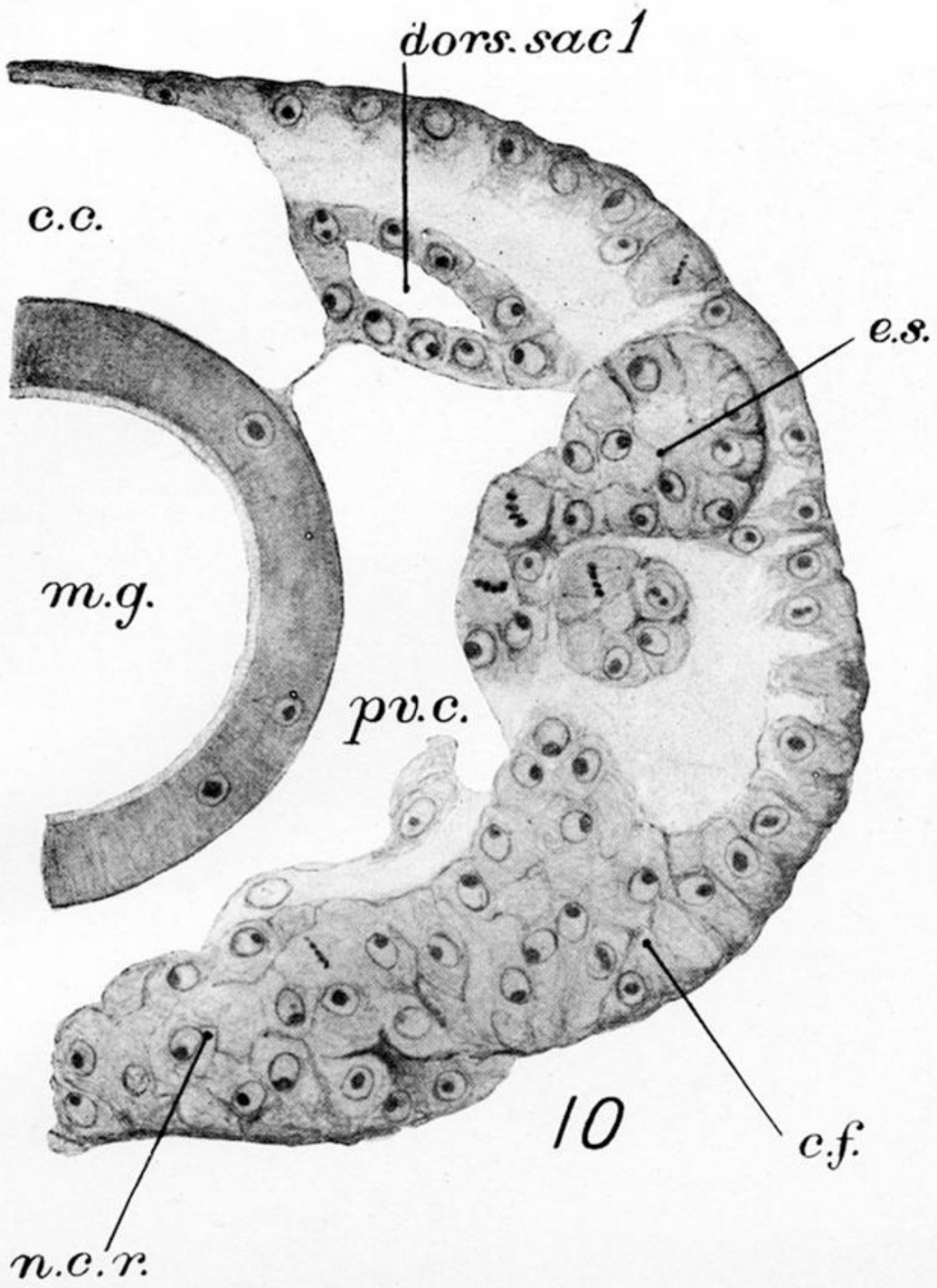
Fig. 6.—A sagittal section a little to one side of the middle line of an embryo at about the same stage of development as the specimen from which figs. 1–5 are taken. Actually it is slightly younger, as only two segments have appeared behind the maxillary mesoderm. The position of the section is indicated by the arrow in text-fig. 2, *d*. The position of the genital rudiment is indicated by a dotted circle.

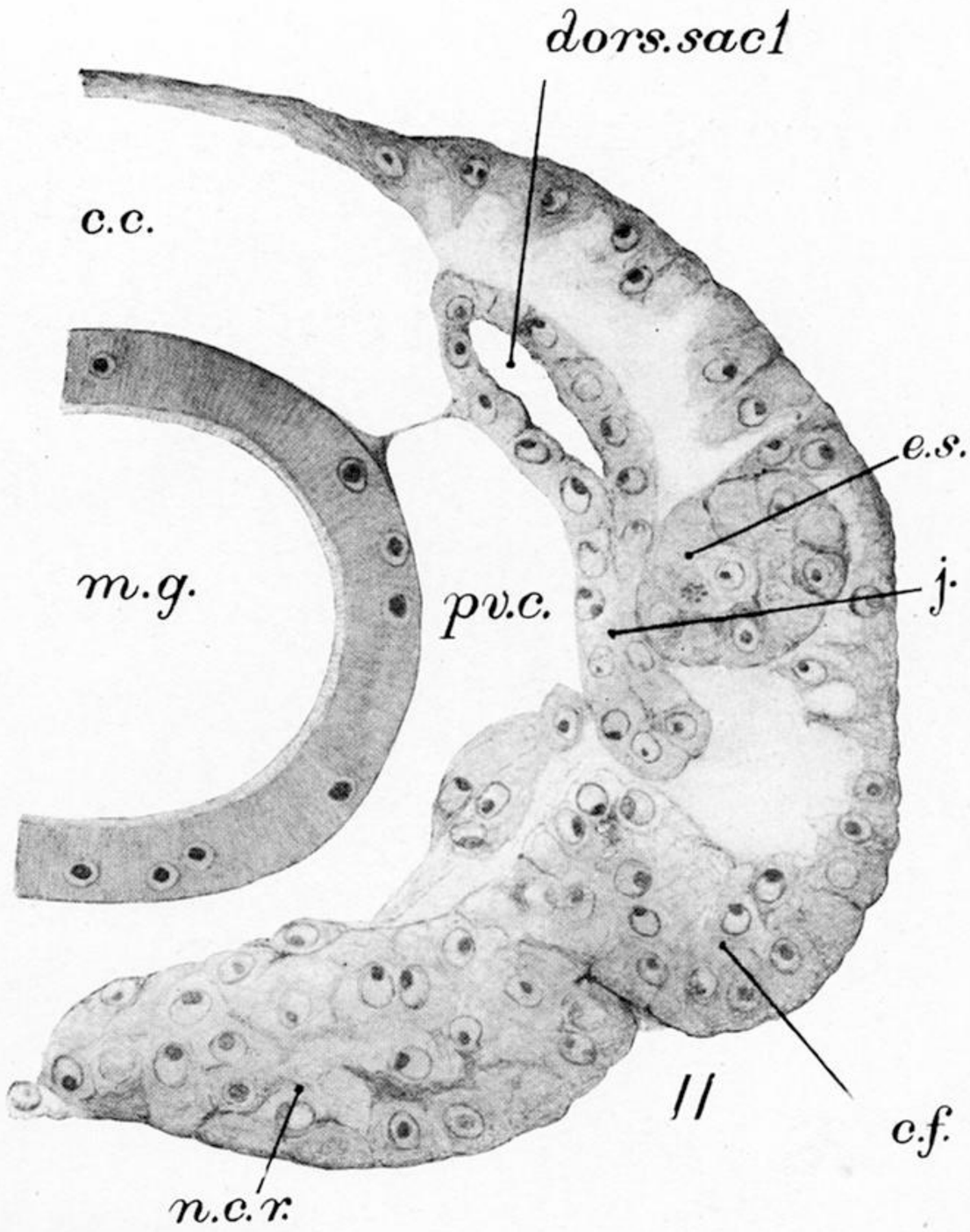
Figs. 7–9 illustrate the earliest development of the coil rudiment of the maxillary gland. The stage figured is that shown in text-fig. 2, *g*, and text-fig. 3, *a*, and the planes of section are indicated in the latter figure, by arrows marked 7–9.

Fig. 7.—A section through the maxillulary mesoderm at a level of the rudiment of the adductor muscle. It is simply a later stage of fig. 1.

Fig. 8.—A section passing just behind the adductor muscle rudiment and through the anterior part of the coil rudiment.

Fig. 9.—A section passing through the posterior part of the coil rudiment, and shows the connection between this and the first dorsal cœlom sac.





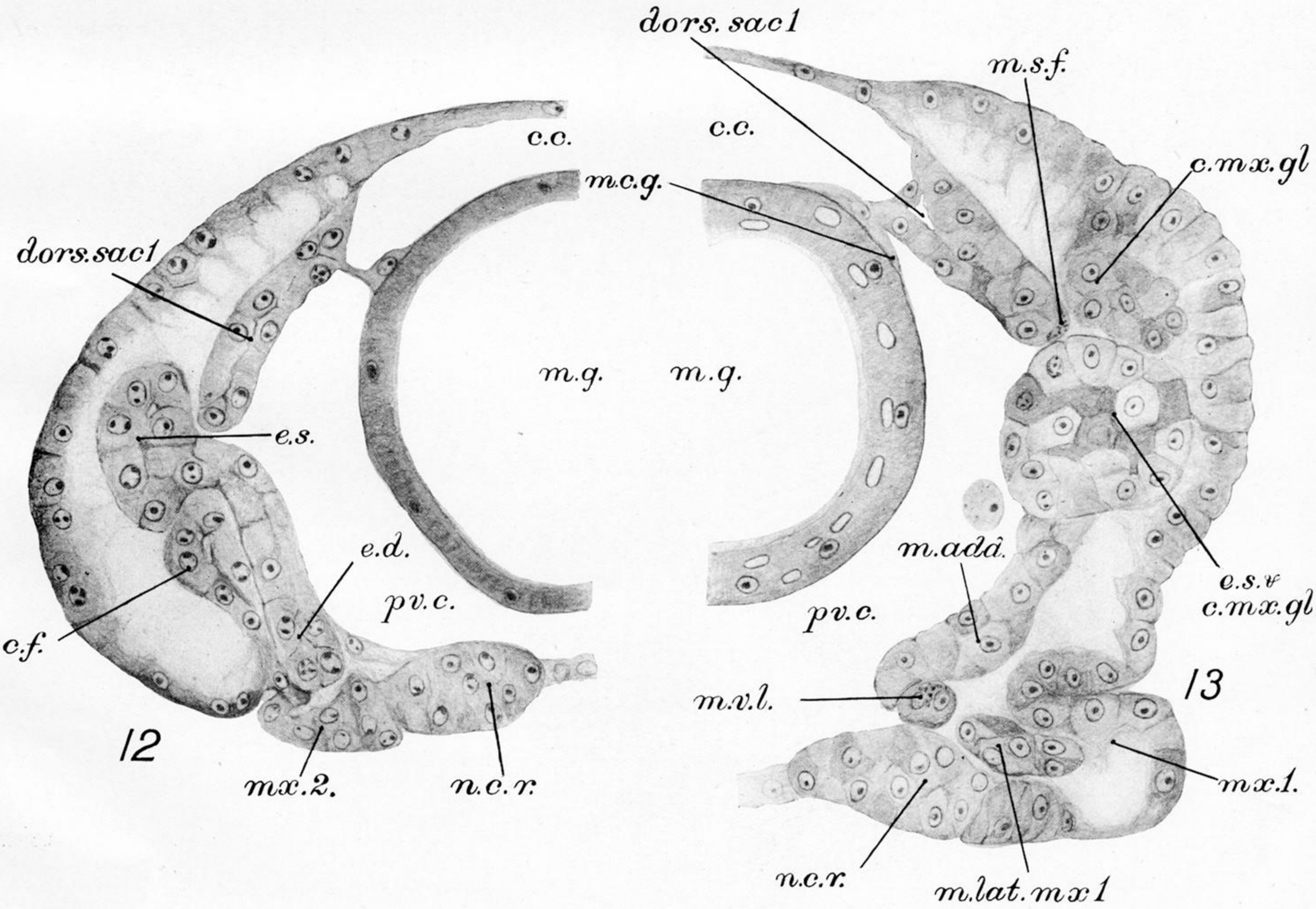


PLATE 20.

Figs. 10 and 11 show a later stage in the development of the maxillary gland, and are comparable with figs. 8 and 9. They are drawings of the anterior focus and the posterior focus of the same section.

Fig. 10.—The first indication of the end sac rudiment.

Fig. 11.—This same rudiment and also the connection between it and the first dorsal cœlom sac.

Fig. 12.—The maxillary gland differentiated into the circular mass of cells wedged in the carapace and a solid rod forming the rudiment of the efferent duct leading from this mass to end in the ectoderm of the maxilla.

Fig. 13.—A section through the maxillula showing the rudiment of the adductor muscle ending against the rudiment of the end sac and coil. It also shows the maxillulary mesoderm differentiated into the other muscles of the maxillula. The earliest indication of the shell-fold muscle can be seen at the lower corner of the section of the first dorsal cœlom sac.

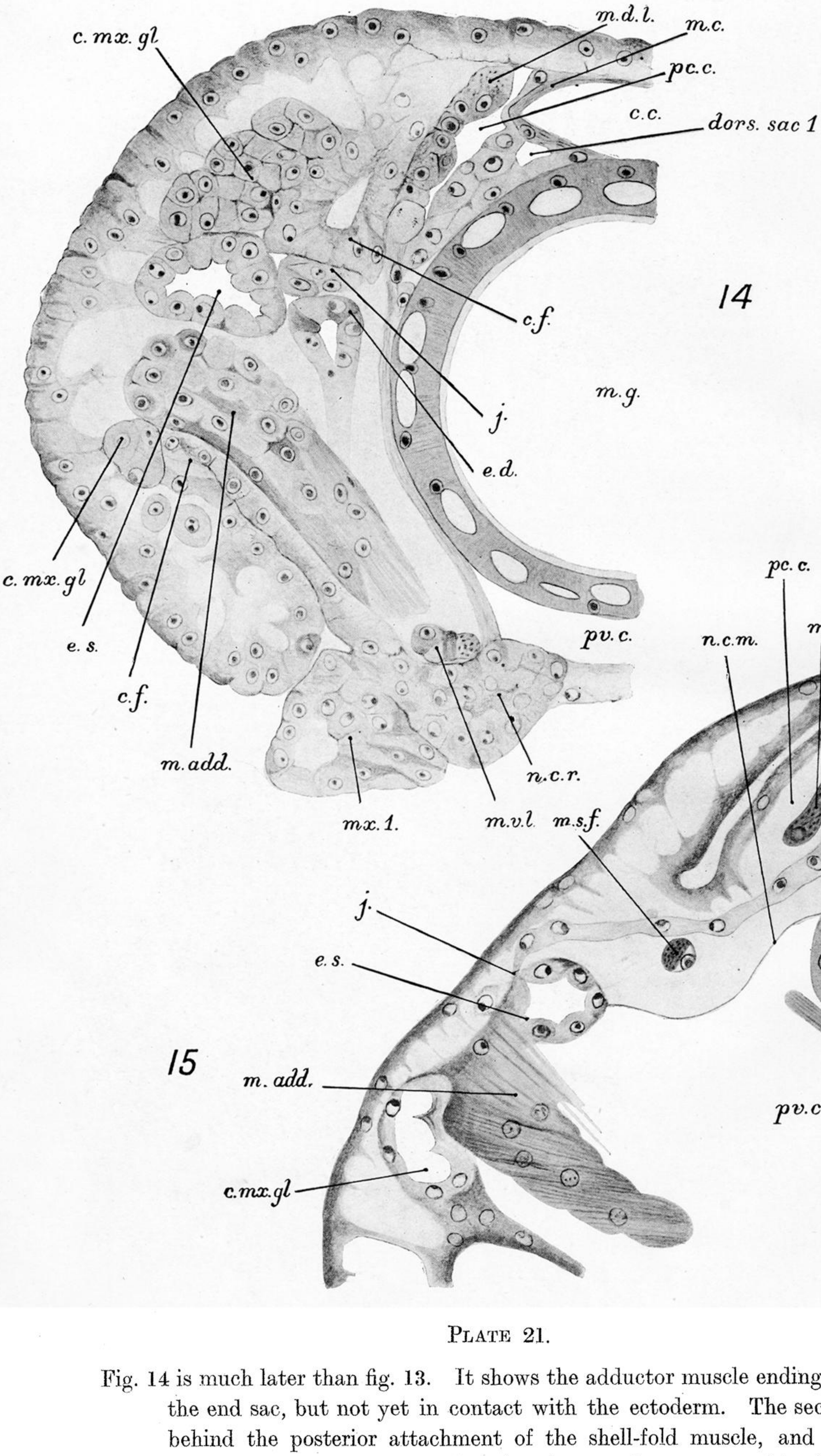
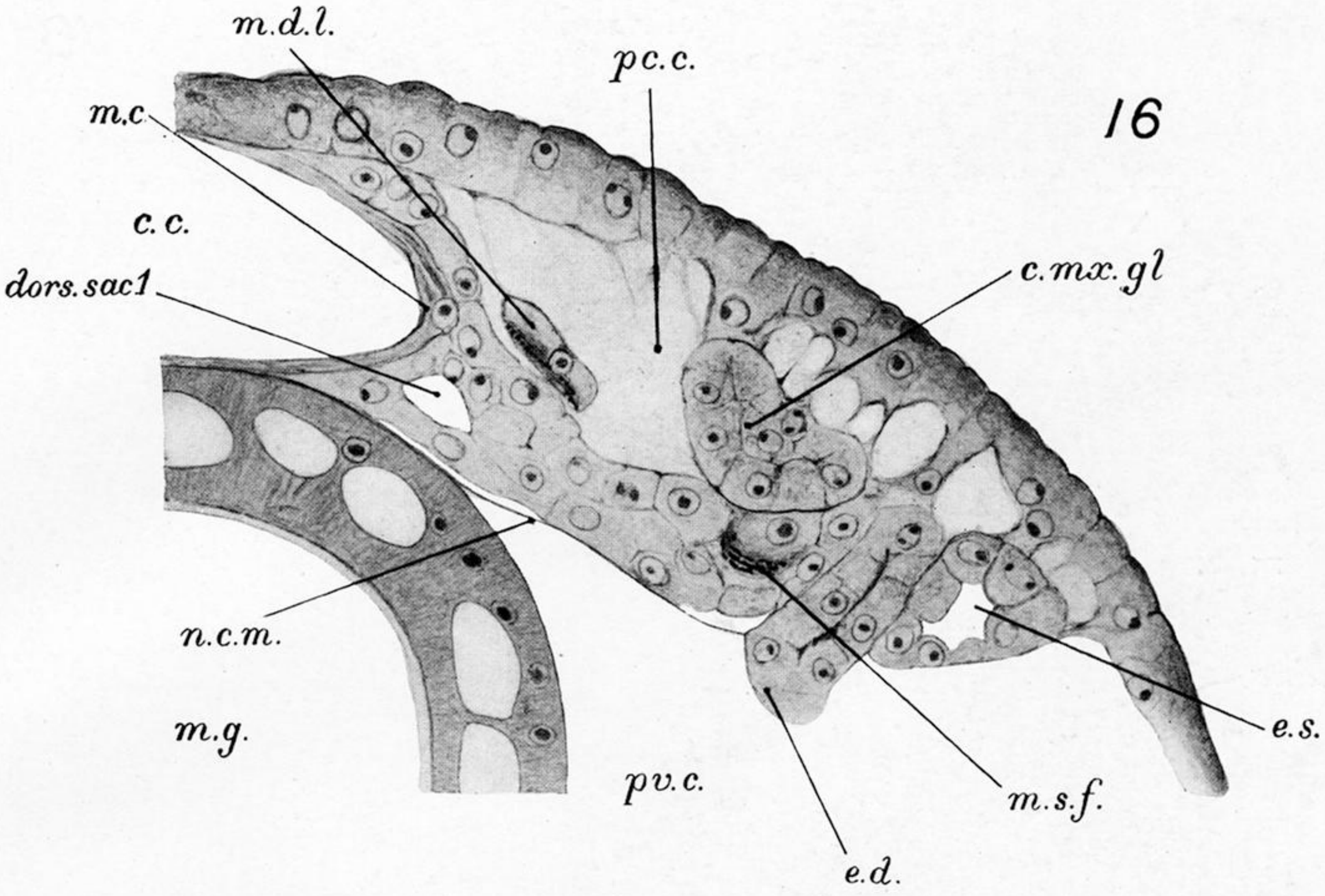


PLATE 21.

Fig. 14 is much later than fig. 13. It shows the adductor muscle ending just below the end sac, but not yet in contact with the ectoderm. The section passes behind the posterior attachment of the shell-fold muscle, and shows the posterior corner of the first dorsal cœlom sac in contact with the end sac. The inner wall of the cœlom pouch is seen to have differentiated heart muscle cells, while the outer has formed dorsal longitudinal muscles that are already partly separated from it.

Fig. 15.—The adductor muscle after it has joined on to the lateral ectoderm of the shell-fold. It is bounded dorsally by the end sac and ventrally by one of the loops of the coil. The long-drawn-out connection passing above the shell-fold muscle between the first dorsal cœlom sac and the end sac can be seen, and also the thin non-cellular membrane that this sheet of cells has formed on its surface ending against the end sac. The dorsal longitudinal muscles are completely separated from the outer face, and the cavity of the cœlom sac has shrunk considerably.



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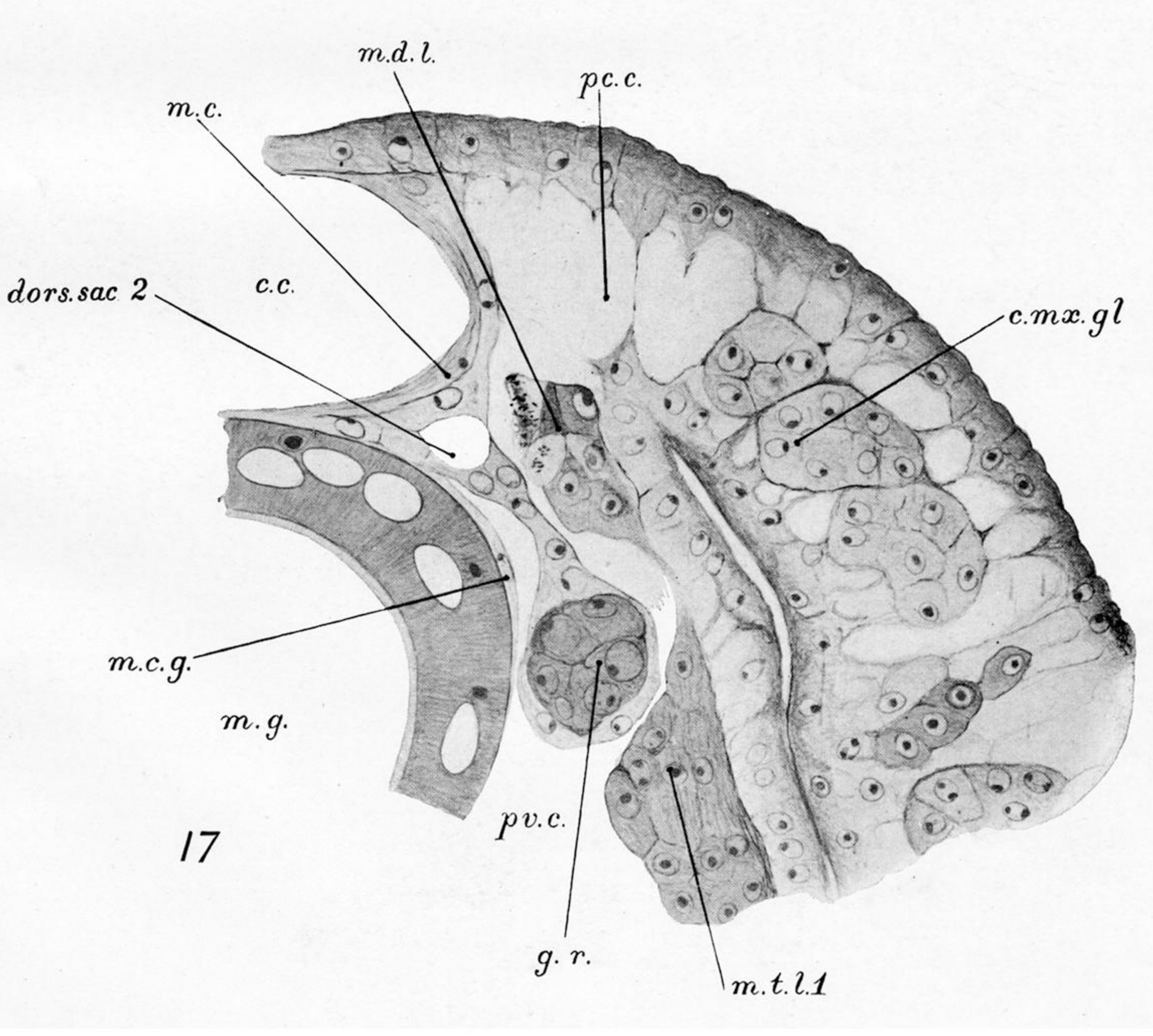

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PLATE 22.

Fig. 16.—The cells of the first dorsal cœlom sac passing both above and below the shell-fold muscle rudiment, that it has formed along its lower edge, to end against the end sac rudiment. The dorsal longitudinal muscles, the heart muscles, and the non-cellular membrane of the maxillary segment can be seen clearly.

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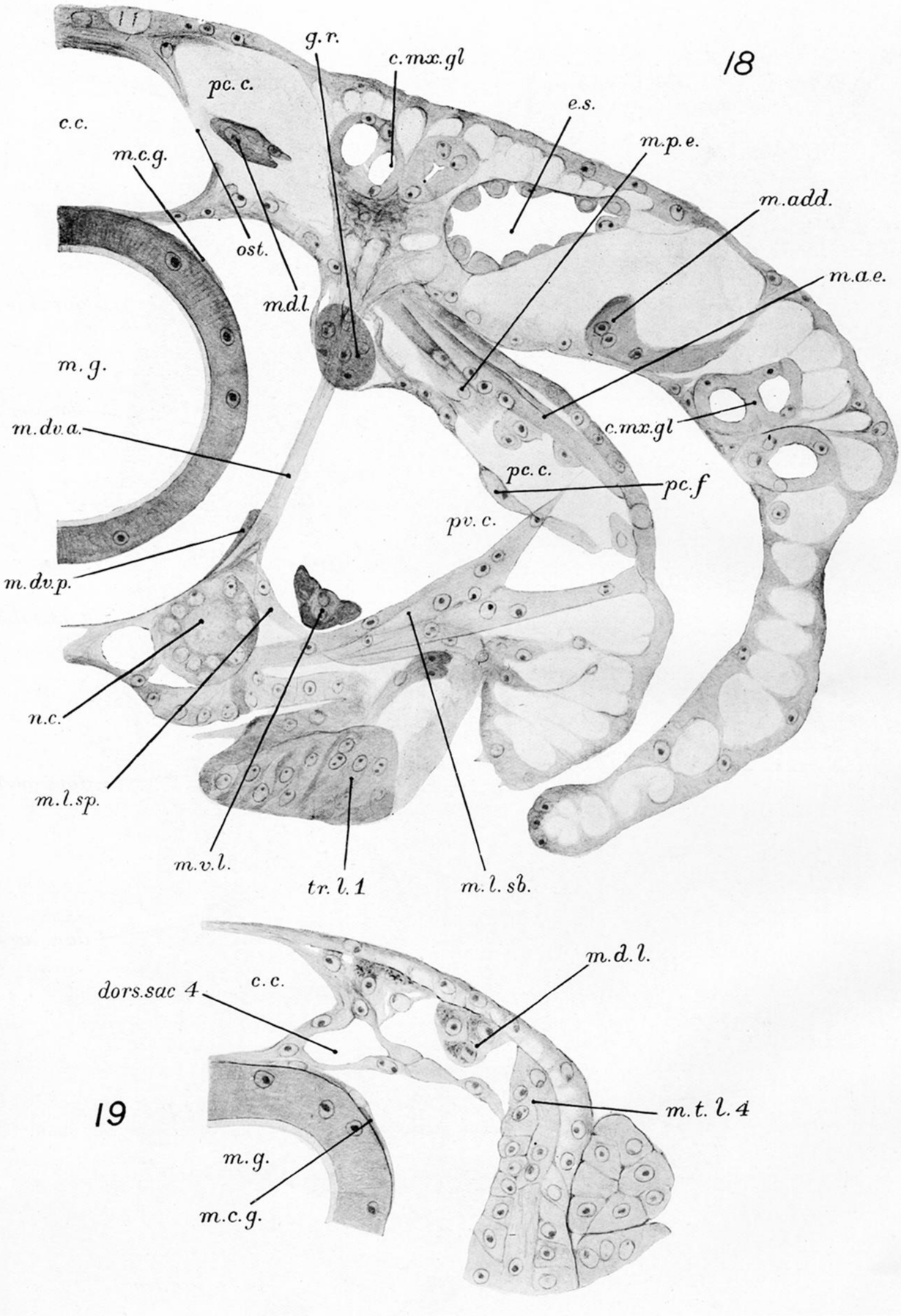

PLATE 23.

Fig. 18.—A section through the first trunk segment at a later stage than that shown in fig. 17. In this section it has been attempted to figure all the important features that occur in the thickness of the section, and not the view of that section at one particular focus, as has been done in the other figures. Thus the anterior dorso-ventral muscle is seen completely, while behind it is the genital rudiment enclosed in the second dorsal cœlom pouch that has now grown down as far as the base of the limb to form part of the pericardial floor. The gap in the heart tube wall is the first ostium. The nerve cord is now completely separated from the ectoderm. All the chief limb muscles are figured.

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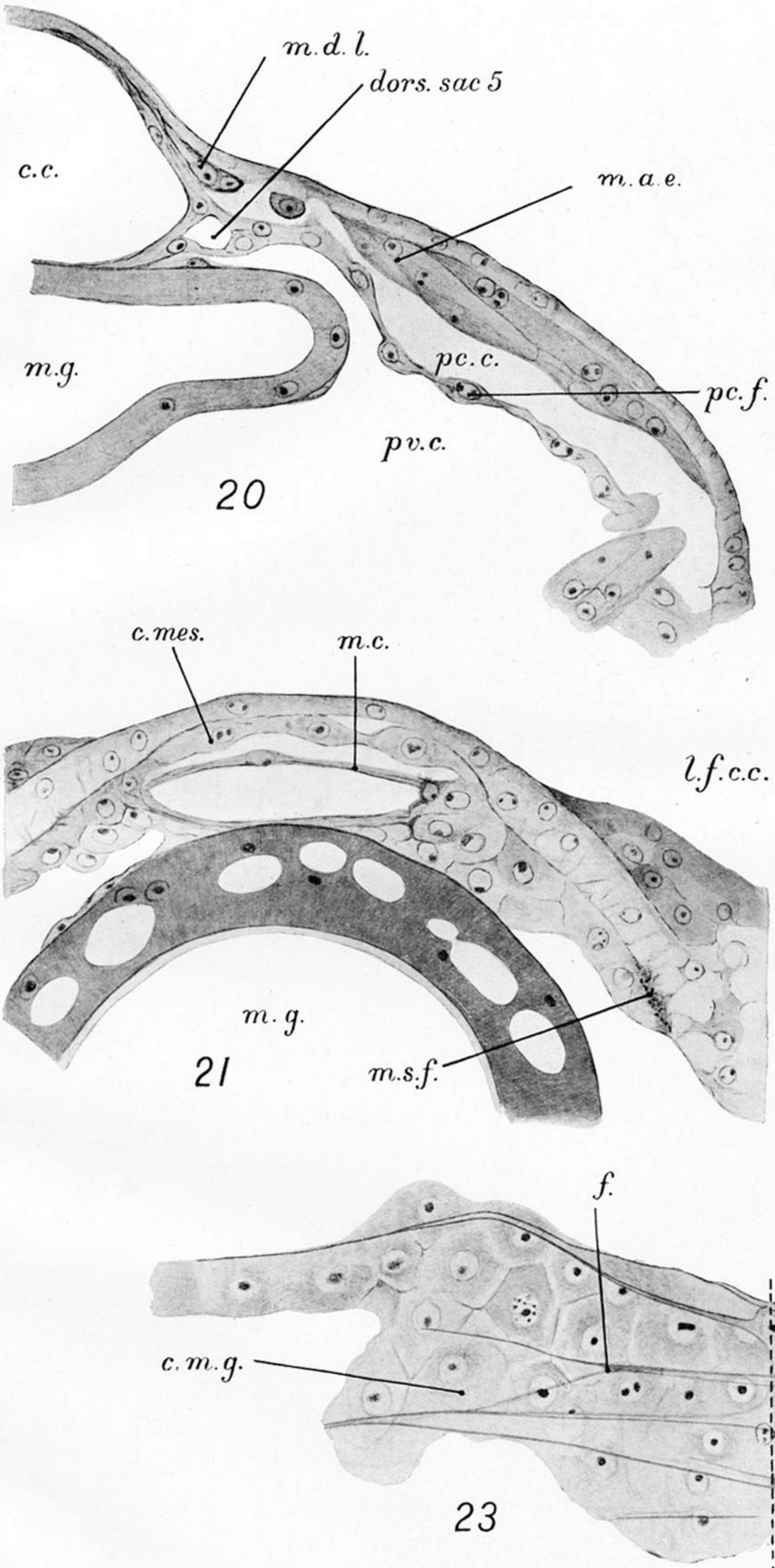

PLATE 24.

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Fig. 21.—A section through the most anterior part of the heart tube showing the muscle cells separated from the investing mesoderm cells. It shows the anterior attachment of the shell-fold muscle, and is from the same specimen as figs. 16, 17 and 19.

Fig. 22.—A horizontal section through the dorsal cœlomic sacs of one side of a larva at a stage when the rudiment of the maxillary gland consists of a solid coil of cells with only one twist. The level of the section is indicated by an arrow in text-fig. 2, *g*.

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