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The Origin and Structure of the Mesoderm and the Formation of the Coelomic Sacs in Tenebrio Molitor L. [Insecta, Coleoptera]

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THE ORIGIN AND STRUCTURE OF THE MESODERM AND THE FORMATION OF THE COELOMIC SACS IN *TENEBRIO MOLITOR* L. [INSECTA, COLEOPTERA]

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[Plate 23]

CONTENTS

The present investigation was undertaken to fill in the gaps in the embryology of *Tenebrio molitor*, shown by previous work to be a relatively unspecialized beetle. The early development, which is typically coleopteran, is briefly described. Middle and lateral plates are delimited along the length of the germ band and the middle plate invaginates to give rise to the mesodermal inner layer. Invagination commences and is most pronounced posteriorly, and progresses anteriorly. Atypically for endopterygotes, the pre-oral mesoderm arises *in situ*.

Commencing in the anterior region of the protocorm the mesoderm spreads out laterally, between the ectoderm and the yolk. It segments prior to the ectoderm, from the labral to the tenth abdominal segment. Somites are formed laterally, in the typical manner. In the eleventh abdominal segment the undivided mass of mesoderm gives rise to the proctodaeal splanchnic musculature.

All the somites become excavated into coelomic sacs, of which there are three protocephalic, three gnathal, three thoracic and ten abdominal pairs. Labral coelomic sacs are described for the first time in the Coleoptera; this is the second recorded instance of their occurrence in the Endopterygota. The intercalary coelomic sacs, which are generally suppressed in the endopterygotes, are median and intersegmental in position and give rise to the suboesophageal body.

The structure of each cephalic coelomic sac is described. The labial and trunk sacs are similar, and resemble those in other Coleoptera. Mesodermal segmentation, especially in the cephalic region, is better developed in *T. molitor* than in the Coleoptera so far described. It is thus more suitable for head segmentation studies than the beetles hitherto subjected to such an investigation.

The *in situ* origin of the pre-oral mesoderm, the complete intersegmental separation of all but the terminal somites and the occurrence of well developed coelomic sacs in the protocephalon are to be regarded as primitive features in a holometabolan, indicating exopterygote affinities.

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Introduction

Although more than half a million insects are known to science and there is a vast literature on various aspects of their development, the embryology of hardly a dozen species has been described at all adequately. Thus although the flour beetle, *Tenebrio molitor*, has already formed the object of embryological investigations, the existing accounts of its development are fragmentary.

Henking (1892), in his study of maturation and fertilization phenomena in insects, gave a short account of this process in *T. molitor*. Selys-Longchamps (1904) described the structure and discussed the homology of the pleuropodia. Saling (1907) investigated the origin and fate of the germ cells in this insect, but failed to identify them with certainty prior to coelom formation. More recently Ewest (1937) has given a detailed description of the structure and early differentiation of the egg. The developmental phases are also outlined and organogeny very briefly mentioned.

The present investigation was undertaken primarily to fill in the existing gaps in the embryology, notably organogeny, of *T. molitor*. Thorough knowledge of normal development is desirable before experimental work, along the lines already begun by Ewest (1937), can be attempted. Secondly, in view of the renewed controversy over head segmentation in the Annelida and Arthropoda (Ferris 1947, 1948, and his school; Haget 1955; Du Porte 1957; Anderson 1959; Butt 1960; Manton 1960) a study of this problem in an unspecialized endopterygote seemed pertinent. The works of Seidel (1924), Ewest (1937), Krause (1939) and Weber (1954) have shown not only that the Coleoptera occupy an intermediate position between the 'higher' pterygotes, i.e. those which typically undergo a determinate, mosaic type of development, and the 'lower' pterygotes which undergo an indeterminate, regulative type of development, but also that the order Coleoptera represents, from the developmental aspect, a heterogeneous collection of forms, with *T. molitor* near the regulative end of the series. However, most of the descriptive work (e.g. Wheeler 1889; Lecaillon 1897, 1898; Hirschler 1909; Paterson 1931, 1932, 1935; Tiegs & Murray 1938) as well as the experimental work (Hegner 1908; Haget 1953) upon coleopteran embryology has been carried out with the Chrysomelidae and Curculionidae, which are the most specialized groups within the order and near the determinative end of the developmental series. In these forms many of the primitive features which would be of value for systematic and phylogenetic considerations have become obscured. In particular the segmentation of the cephalic mesoderm is largely suppressed, and thus these forms are unsuitable for head segmentation studies. Moreover, as stated by Eastham (1930*b*): 'The origin of the mesoderm of the cephalic segments in front of the mandibular has hitherto been obscure in all but the more generalised insects. . . . There is on the whole a lack of detailed information on the subject. . . mesoderm both in the matter of its development and its relation to appendages being incompletely investigated.' The investigation of *T. molitor*, an unspecialized holometabolan, therefore seemed likely to yield valuable results.

The work here presented is the first of an intended series of publications on organogeny in *T. molitor*. After a brief sketch of the early development, the present account will deal with the origin, segmentation and homologies of the mesoderm, together with the formation and breakdown of the coelomic sacs.

Materials and methods

The *Tenebrio molitor* culture was housed in tins 9 in. × 9 in. × 4 in., the lids of which were pierced to allow aeration to take place. Imagines, pupae and larvae were kept in separate containers, as the larvae are voracious and will attack the pupae. The food consisted of a 9:9:1 mixture of oats, middlings and yeast powder. The tins were kept at room temperature, between 18 and 28 °C. The relative humidity within the tins was maintained between 50 and 60% in the following way: glass tubes, 3 in. long, were filled with water and corked and from each a wick of thick string protruded through a hole in the cork. In addition damp pieces of filter paper were placed in each tin.

If the environment is kept constant, the females remain fecund throughout the year. If, however, the atmosphere is too dry, less than 40% R.H., egg-laying ceases.

The white eggs were collected by placing sheets of brown paper on the bottom of the tins, below the food. The beetles then lay and secure their eggs on to the paper, by a cement-like secretion of the collaterial glands. The method employed by Ewest (1937), in which eggs laid in cotton wool were collected, proved unsatisfactory since separation of the fragile, newly laid eggs from the cotton fibres, without distortion or damage, was both difficult and time-consuming. The sheets of paper, however, may be readily removed at appropriate time intervals. The eggs were allowed to develop to the desired age in an incubator at 27 °C and between 50 and 60% relative humidity. Wherever possible the eggs were left attached to the paper until required, in order to avoid damaging them. After the first day, when the chorion thickens, the eggs are easily manipulated.

The fixatives most successfully used were Smith's formol-bichromate, dioxane Bouin and aqueous Bouin, the latter being generally employed. The impermeable chorion was pierced with a fine tungsten needle, in order to allow the fixative to penetrate. The eggs were left in cold Bouin's fluid for 6 to 12 h, after which they were rapidly dehydrated, methyl salicylate being substituted for absolute alcohol; they were cleared in cedarwood or clove oil and then embedded in 54 °C paraffin wax for 6 to 12 h.

Alternatively Peterfi's celloidin-paraffin method was used, giving good results. From 95% alcohol the eggs were soaked in 1% methylbenzoate-celloidin for 1 to 2 h, cleared in benzene for 10 min and then embedded in 54 °C paraffin wax. Sections were cut at 2, 4, and 6 μm. To enhance adhesion of the yolky sections to the albumen-smeared slides, the latter, with their attached sections, were dipped into a solution of 0·05% celloidin; to the xylol and alcohol solutions subsequently used in the staining process, equal volumes of chloroform were added to harden the celloidin. Sections were stained in Heidenhein's iron haematoxylin and light green in 95% alcohol. The embryonic tissues showed little affinity for eosin, but a modification of Masson's ponceau-acid-fuchsin technique proved very successful. Following haematoxylin staining the sections were overstained in the fuchsin, after which they were very rapidly passed through 50 to 70% alcohols, differentiated in light green in 95% alcohol under the microscope, dehydrated, cleared and mounted.

The approximate numbers of specimens of various ages used, on which this description is based, is as follows: 15 eggs, from 0 to 19 hours; 8 eggs at each hour, from the 20th to the 29th hour; 10 eggs at 30 hours; 2 at 31 hours; 5 at 32 hours; 6 at 37 hours; 15 at 42 hours; 12 at 44 hours; 3 at 45 hours; 3 at 46 hours; 5 at 47 hours; 10 at 48 and 10 at 50 hours.

Observations

(1) *Outline of early development and formation of the germ rudiment*

The egg of *Tenebrio molitor* is oval in shape, frequently with a shallow concavity on one side which, however, bears no relationship to the orientation of the embryo. The zygote nucleus undergoes repeated divisions in the posterior third of the egg and, during the

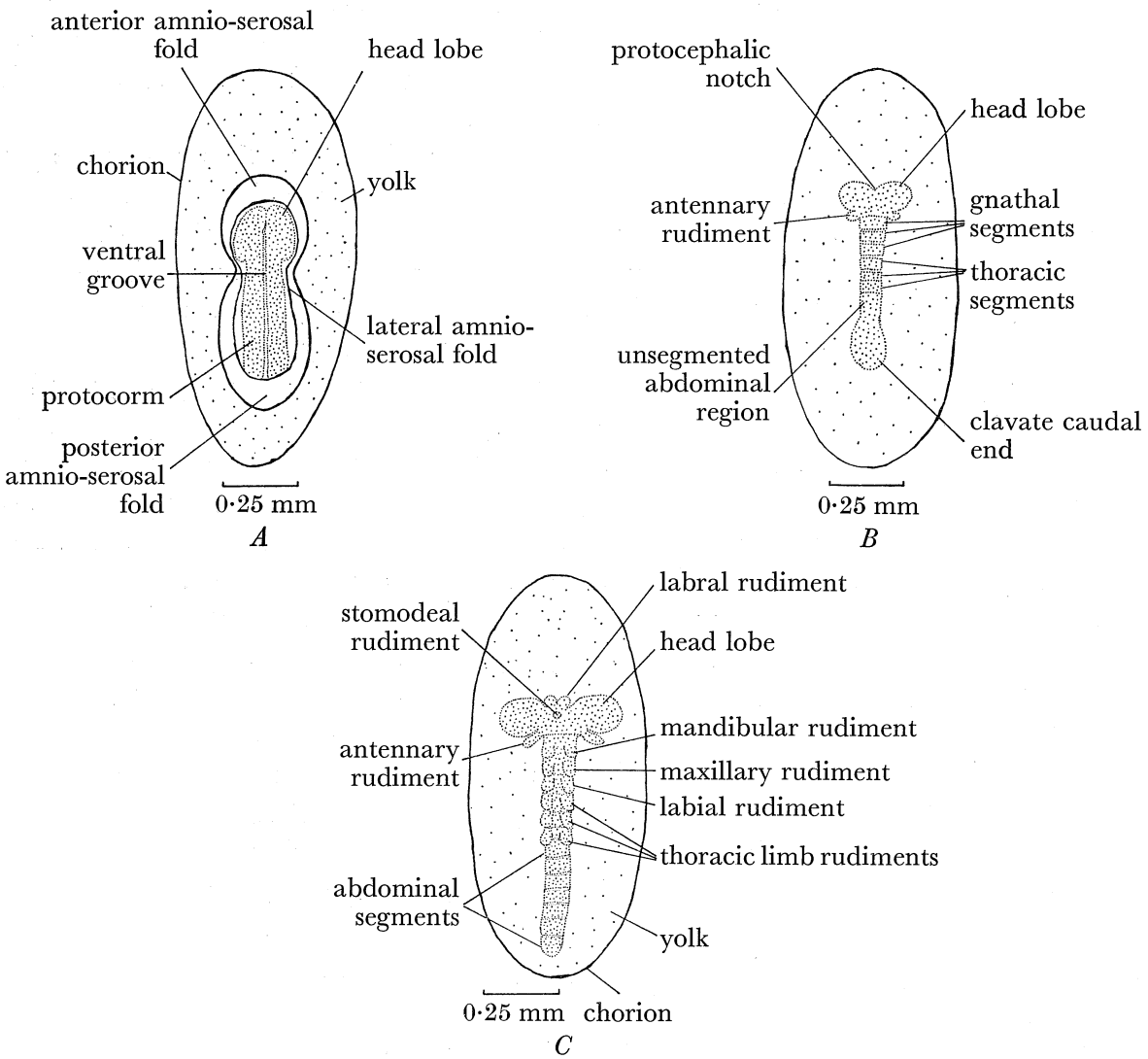

Figure 1. External views of embryos as seen from the ventral side. *A*, at 20 h, showing the overgrowth of the germ rudiment by the embryonic membranes; *B*, at 25 h, showing partial segmentation of the germ band; *C*, at 36 h, showing developing appendage rudiments. In *B* and *C* the embryonic membranes are not shown.

first 10 to 14 h after oviposition, the resulting nuclei migrate from the interior towards the periphery of the egg. Between the 15th and 16th hours the nuclei enveloped by their cytoplasmic 'halos' (cleavage energids) reach the yolk periphery, enter the periplasm and form the blastema (syncytial blastoderm). The development of cell walls around the nuclei transforms the blastema into the primary epithelium (cellular blastoderm). Some of the original cleavage nuclei never reach the periplasm, but remain within the yolk to form the

primary vitallophags, responsible for yolk liquefaction (Ewest 1937). The epithelium in the postero-ventral region of the egg thickens owing to the cells becoming columnar in shape. This portion of the primary epithelium forms the germ rudiment and will give rise to the embryo. The cells in the remaining portion of the epithelium become flattened and this region will give rise to the serosa, the outer of the two embryonic membranes. The inner

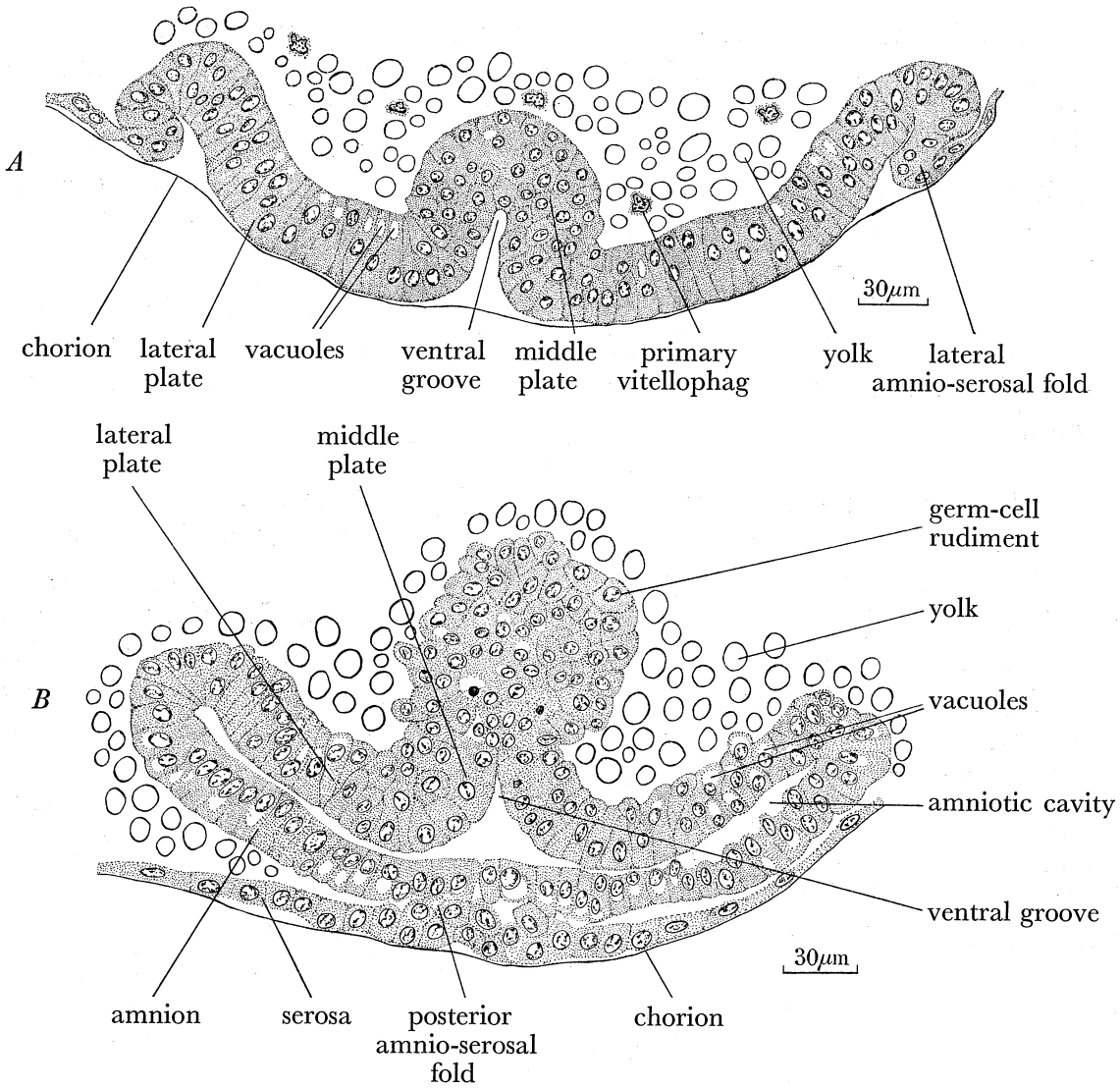

FIGURE 2. Transverse sections of a 20½ h-old germ band: *A*, through the protocorm, showing the developing amino-serosal folds and the invagination of the middle plate; *B*, through the penultimate segment, showing the germ cell rudiment.

membrane, the amnion, will develop from folds which arise from the edges of the rudiment, eventually to grow over the latter (figures 1*A*, 2 and 3). The amnion is thus derived from the germ rudiment. Simultaneously with these developments numerous cells migrate back into the yolk from the primary epithelium, especially from the embryonic region: these are the secondary vitellophags which differ markedly from the primary in shape (figures 2*A* and 3), though not apparently in function. In addition smaller cells (primary mesentoderm cells of Ewest (1937)) proliferate from the middle plate (p. 250) and

spread somewhat laterally. Unlike the secondary vitellophags, however, they do not enter the yolk but remain lodged between the latter and the germ rudiment. Some of these cells probably contribute to the inner layer, as stated by Ewest, but the majority seem to degenerate.

As the germ rudiment becomes more clearly defined from the extra-embryonic epithelium, it withdraws from the posterior pole and comes to lie wholly on the ventral side of the egg. It occupies about a quarter of the width and about two-thirds of the longitudinal axis of the egg. Between the 17th and 20th hours the anterior quarter of the rudiment expands into two small head lobes, forming the protocephalon, from which ultimately the brain and the anterior part of the head arises. The narrower posterior region constitutes the protocorm and gives rise to the gnathal and trunk segments (figure 1).

Between the 20th and 23rd hours a faint and narrow groove becomes visible along the midventral line of the germ rudiment, stretching forward from the caudal end and fading away in the head lobe region. This is the ventral (gastral) groove, and is formed by the invaginating middle plate (see below) (figure 2*A*). At the end of the first day the rudiment sinks slightly into the yolk, the posterior region curving dorsad, thus initiating the caudal flexure.

(2) *Invagination of the middle plate*

A little before or simultaneously with the development of the amniotic folds, the germ rudiment becomes differentiated into middle and lateral plates. A strip of primary epithelium, forming the middle region of the germ rudiment, flattens progressively postero-anteriorly. This strip, which is approximately a third of the width of the rudiment, constitutes the middle plate and is the region which will subsequently invaginate to form the inner layer. The epithelium on either side of this region forms the lateral plates of the rudiment. The cells of both middle and lateral plates are columnar, and vacuoles generally develop between the nuclei and the basement membrane.

Approximately between the 18th and 19th hours, and before the middle plate invaginates, a mass of cells is proliferated into the yolk from the caudal end of the rudiment. These do not wander into the yolk, as do the secondary vitellophags, but remain aggregated between the middle plate and the yolk, at the level of the future penultimate segment. Cytologically they resemble the epithelial cells from which they are derived, the protoplasm being coarsely granular and markedly basophilic. However, they differ in their oval or polyhedral shape. This cell mass, as suggested by Saling (1907), is in all probability the germ-cell rudiment (figure 2*B*).

At about the 19th hour, the invagination of the middle plate begins at the caudal end of the germ rudiment, this point being distinguishable as the primitive notch. The lateral plates thicken due to the enlargement of the vacuoles in the cells and a tendency for the plates to become multi-layered. The middle plate also thickens somewhat, as a result of compression by the lateral plates; it also becomes slightly curved towards the yolk, this being the first indication of the actual invagination.

As the median edges of the lateral plates approach each other, the middle plate comes to form a semi-cylindrical groove; by the 20th hour this ventral groove extends from the primitive notch to the protocephalic region. It is deepest at the posterior end; anteriorly it becomes progressively shallower, eventually fading away. In the protocephalic region,

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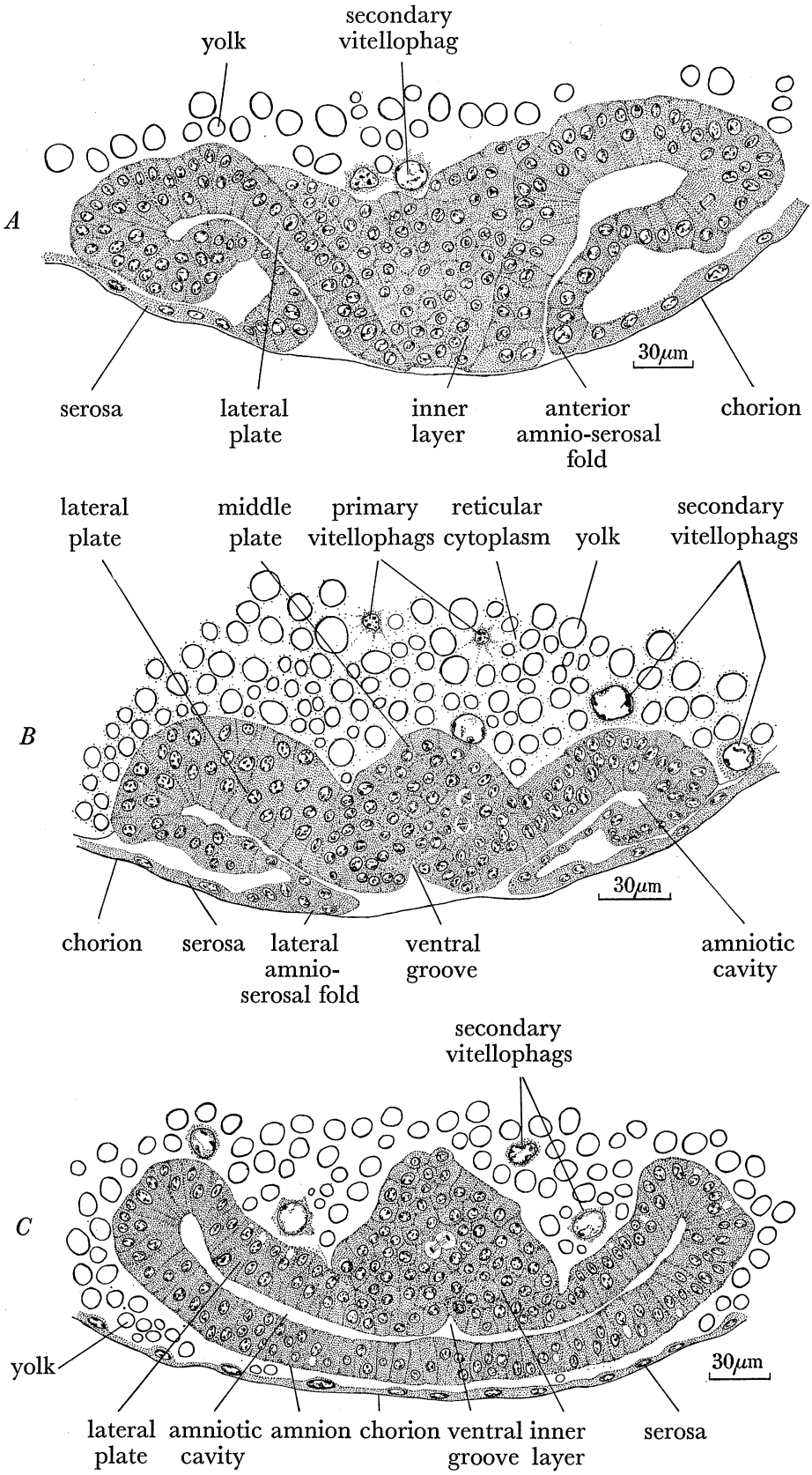

FIGURE 3. Transverse sections of a 20 h-old germ band: *A*, through protocephalon; *B*, through the mid-protocormic region; *C*, through the caudal region.

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the formation of the inner layer appears to be brought about, at least partially, by the overgrowth of the middle by the lateral plates (figure 3*A*).

In the cephalic and caudal regions the lateral edges of the germ rudiment become more deeply immersed in the yolk than in the intervening regions (figures 2*B*, 3*A*, *B* and *C*). At the caudal end this immersion is partly brought about by the incipient dorsal flexure; and partly by the penetration of yolk between the amnion and the serosa, which form precociously at the posterior end of the rudiment.

The lateral plates in the protocorm now meet and fuse in the midline, closing below the ventral groove. This process again, is initiated posteriorly and progresses anteriorly. Concurrently the invaginated middle plate gradually rolls up longitudinally, the lumen of the tubular structure first formed quickly disappearing due to the thickening and apposition of its walls. Subsequently the median plate breaks up into its constituent cells which now form the inner layer. This consists of a somewhat irregular strand, 4 to 6 cells deep, lying above the region formerly occupied by the ventral groove. The irregularity is caused by the inner layer being in slightly different stages of development along its length, due to progressive differentiation from behind forwards. There is no evidence to suggest that the inner layer undergoes segmentation prior to the lateral spreading which occurs later (see p. 254).

The inner layer cells are rounded or polyhedral in shape, with spherical nuclei; for a time some of these cells retain their vacuoles, but during the first half of the second day these disappear both from the inner layer and the lateral plates. The latter now constitute the ectoderm and neural tissues, the former the future mesoderm. The function of the vacuoles developed in the germinal epithelium at the time of invagination is possibly to increase the cell volume, and thus aid the morphogenetic processes which lead to invagination.

With the formation of the inner layer, the germ rudiment becomes transformed into the germ band. Strictly speaking this term 'represents the stage in development when the primary germ layers are established' (Counce 1961), although it has been used by numerous authors to describe earlier stages as well.

(3) *Differentiation of the germ band*

(*a*) *Structure of the outer (ectodermal) layer of the germ band*

By the end of the first day there is a conspicuous difference in width between the protocephalon and protocorm, the former being approximately twice as wide as the latter (figure 4). The anterior extremities of the head lobes may be regarded as lateral plate ectoderm which has bulged forward in front of the median protocephalic notch. The latter represent the morphologically anterior end of the germ band, i.e. the anterior end of the invaginated middle plate. The head lobes begin to curve dorsally, around the yolk, and will eventually form part of the precociously developed head (figure 5*A*).

The narrower protocormic region of the germ band lies more or less parallel to the ventral surface of the egg. Variations in shape also occur along the length of the protocorm of the same embryo. At different levels the protocorm is flat, curved or gutter-shaped in section (figures 6, 7 and 8), the latter being characteristic of the posterior quarter of the

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germ band, which is slightly expanded. By the 25th hour the edges of the germ band become curved dorsally, eventually to form an ectodermal trough filled by mesoderm (figure 8).

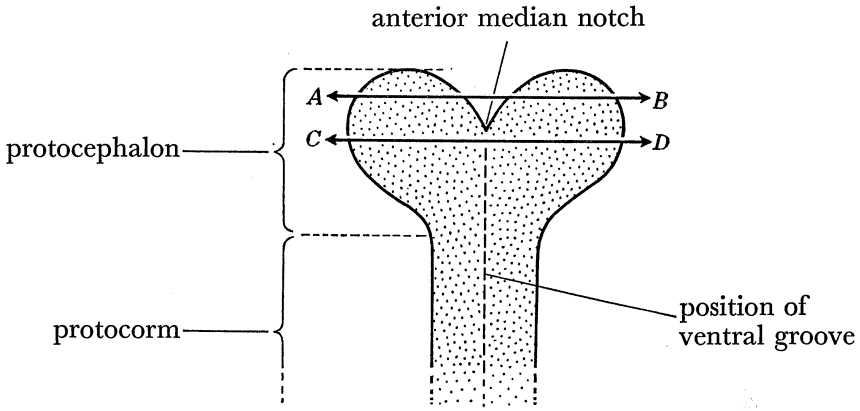

FIGURE 4. Diagram of the anterior region of a 25 h-old germ band.

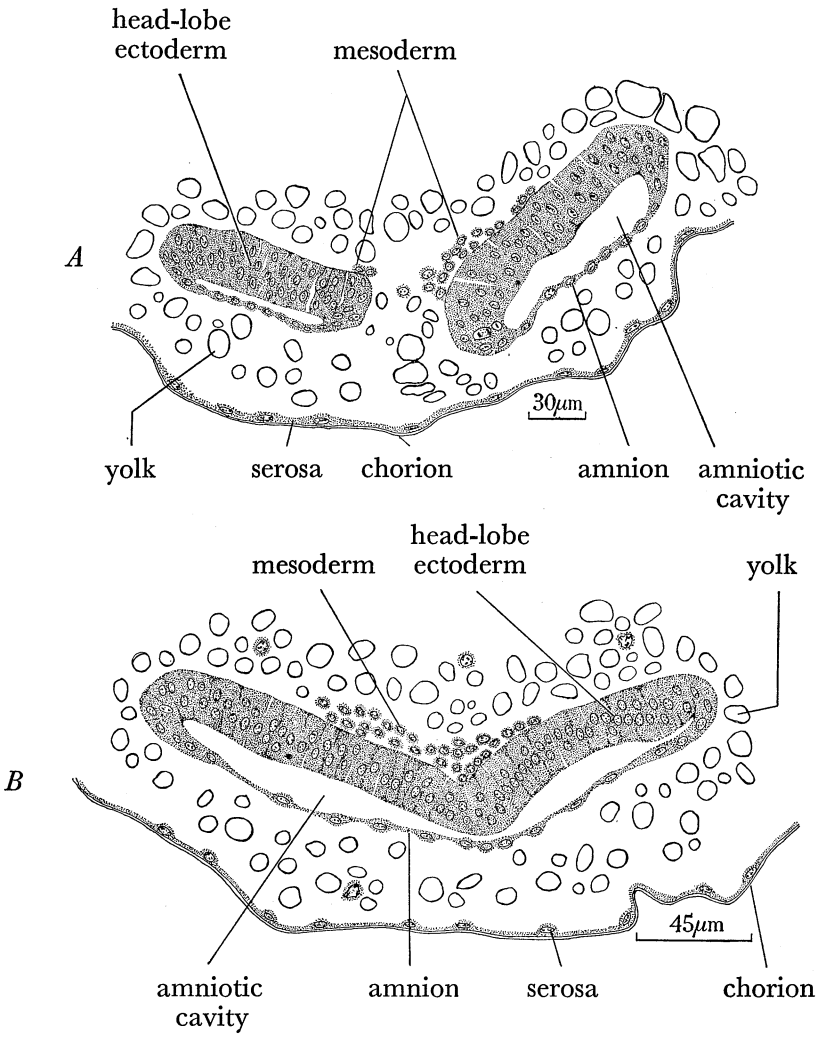

FIGURE 5. Slightly oblique transverse section of the head lobes of a 25 h-old germ band; *A*, through the anterior protocephalic region at the level *AB* in figure 4; *B*, just behind the median protocephalic notch, at level *CD* in figure 4.

(*b*) *Lateral spreading and segmentation of the inner (mesodermal) layer of germ band*

By the end of the first day invagination has been completed and the inner layer now lies between the ectoderm and the yolk (figures 5 and 6). As in *Tenebrio molitor* the inner layer makes no contribution to the midgut epithelium, it may be regarded as exclusively mesodermal in nature (see Discussion, p. 268).

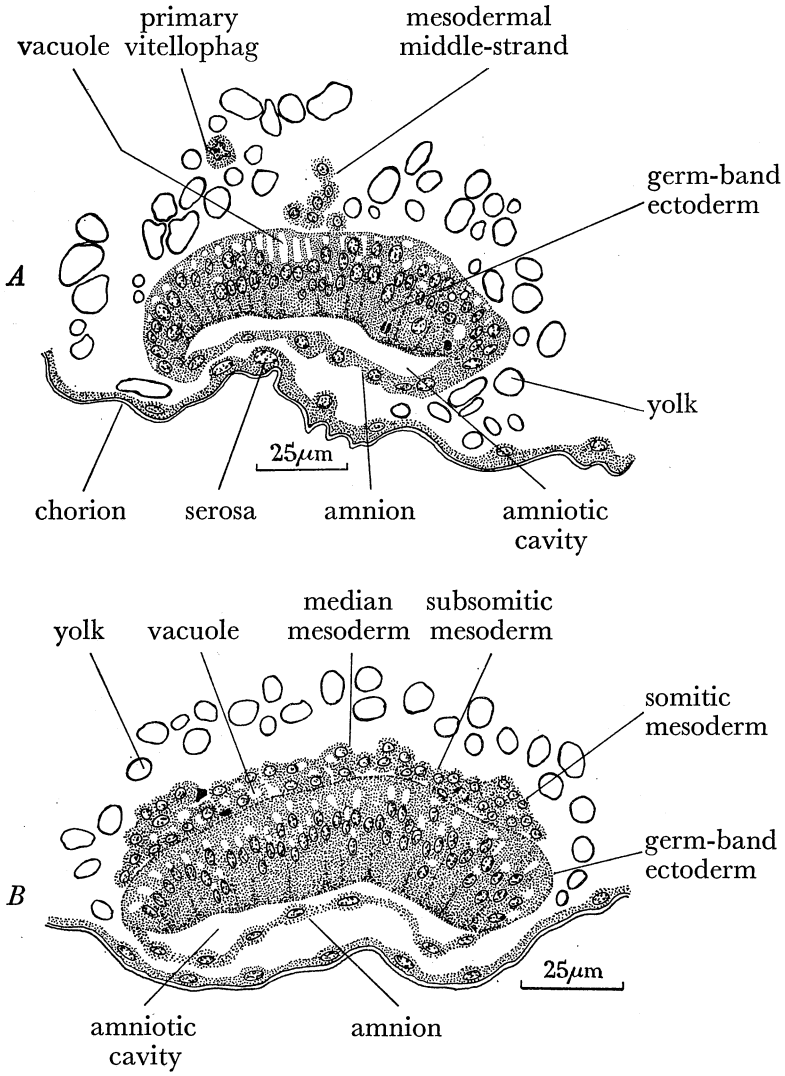

FIGURE 6. Transverse section of a 25 h-old germ band: *A*, through the intersegmental region of the protocorm; *B*, through the segmental region of the protocorm.

Histologically the mesoderm is composed of polyhedral cells with rather dense, basophilic cytoplasm which stains somewhat more deeply with iron haematoxylin than does that of the ectoderm. The spherical nuclei are densely populated by granules. The presence of numerous mitotic spindles, which occur at random throughout the strand, is indicative of the intense cell proliferation which takes place at this time. The amount of mesoderm is thus increased and, after the 24th hour, the cells begin to spread out laterally and, except for certain regions of the head lobes (p. 255), eventually come to cover the yolkside of the ectoderm. The spreading, however, is not a uniform process so that, between

the 24th and 25th hours, the mesoderm presents an irregular appearance. Transverse serial sections show local cell accumulations in some regions of the protocorm, whilst in other regions the cells have migrated laterally and levelled out to form a layer approximately two cells deep.

At about the 25th hour mesodermal cell accumulations, 3 to 4 layers deep, begin to form segmentally at the lateral edges of the germ band: these are the future somites (figure 6*B*). At this stage the cells composing the somite rudiments do not, as yet, show an epithelial arrangement.

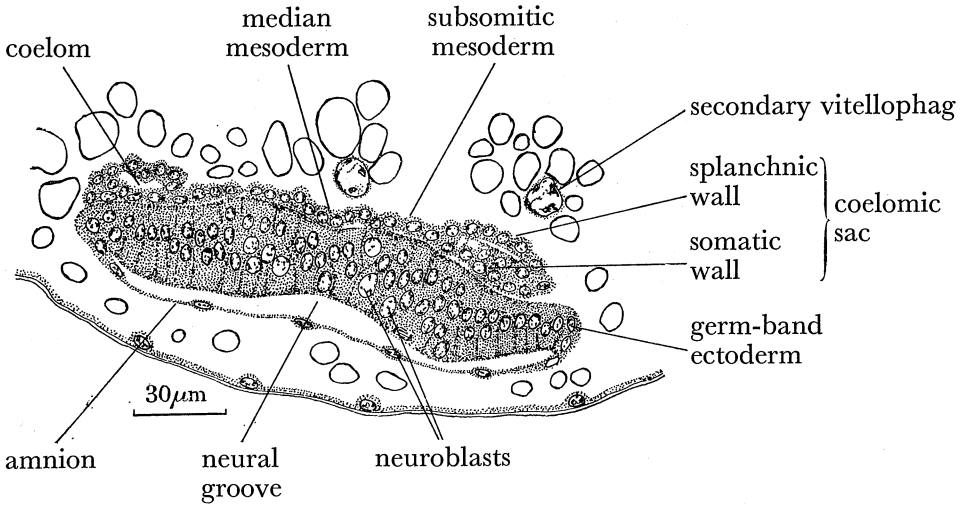

FIGURE 7. Transverse section through the segmental region of the protocorm of a 28 h-old embryo. The coelomic sacs are beginning to form.

Intersegmentally the somitic mesoderm eventually becomes severed, so that adjacent somites are no longer connected. Thus, when segmentation is completed, mesoderm is no longer found laterally in the intersegmental zones. As seen from figure 6*A*, the median strip of mesoderm remains unsegmented. Segmentation of the mesoderm, which precedes that of the ectoderm, begins near the anterior region of the protocorm, whence it progresses anteriorly and posteriorly.

The foregoing description refers to the early stages of mesodermal differentiation, as it occurs in the thoracic and the majority of abdominal segments. Mesodermal development in the head and caudal regions deviates somewhat from this basic pattern.

In the protocephalon the spreading mesoderm never reaches the lateral edges of the germ band. The development of the mesoderm in this region is best understood from a study of transverse sections. Figure 5*A* represents an oblique section through the anterior region of the head lobes. As already mentioned, this region of the protocephalon probably represents a forward bulge of the lateral plate ectoderm in front of the protocephalic notch, for there is no middle plate in front of the latter, i.e. at level *AB* in figure 4. The section drawn in figure 5B transects the germ band at *CD* in figure 4 where the mesoderm is more abundant than further anteriorly. Comparison of such sections indicates that the anterior region of the head lobes, i.e. the portion lying in front of the notch, is invaded by mesoderm from behind.

In the caudal region there is a greater accumulation of mesoderm than elsewhere in the protocorm. As mesodermal differentiation takes place progressively posteriorly, this region

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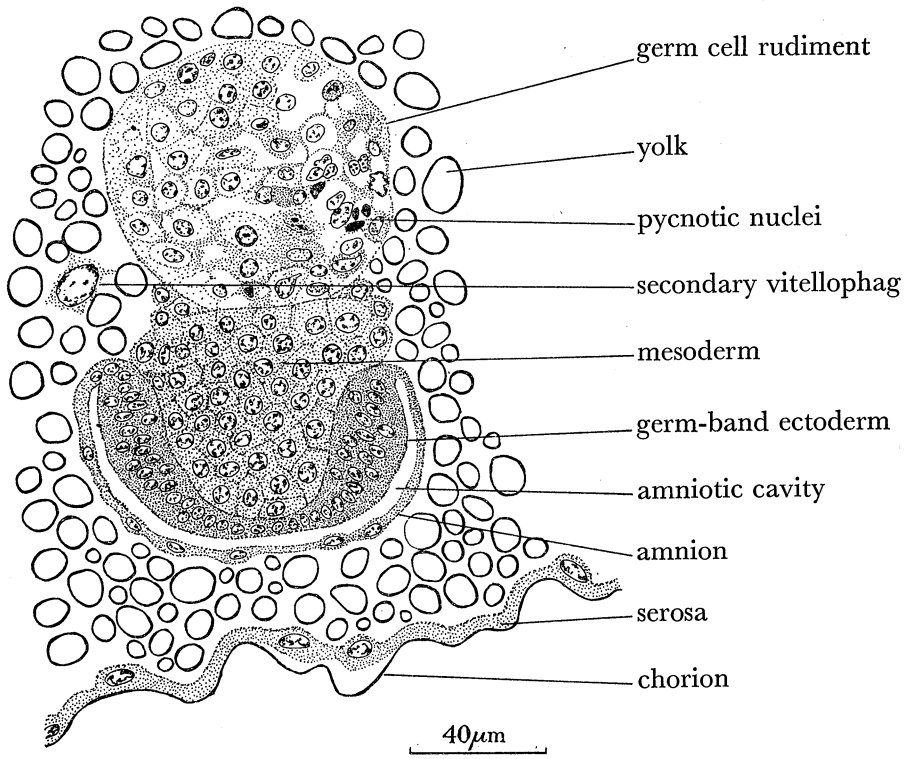

FIGURE 8. Transverse section through the germ-cell rudiment of a 26 h-old embryo.

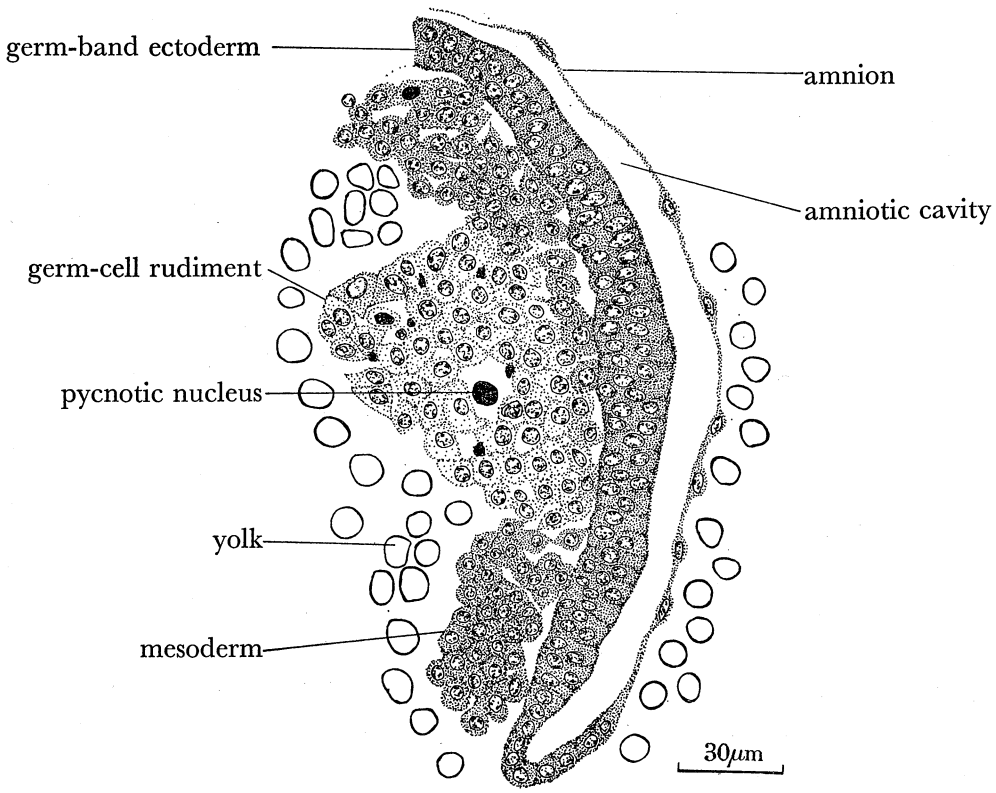

FIGURE 9. Longitudinal section through the germ-cell rudiment of a 30 h-old embryo.

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is the last to become segmented. With the invagination of the middle plate, the germ cell rudiment is pushed deeply into the yolk. The relationship of the rudiment to the ectoderm and mesoderm is illustrated by figures 8, 9 and 20.

(4) *Formation of the coelomic sacs*

(*a*) *General account*

The next stage in the differentiation of the mesoderm is the formation of the coelomic sacs within the somites. This process is initiated between the 26th and 27th hours though in the caudal region, due to the retardation of segmentation, it may be somewhat later. The processes of segmentation and coelom formation, however, are generally completed by the 30th hour.

Somites occur in the labral (primary head segment), antennary, intercalary, three gnathal, three thoracic, and ten abdominal segments. The validity of the labral segment will be discussed in a future communication. In the 11th abdominal segment paired somites are not developed; the undivided mass of mesoderm applies itself to the future ventral side of the proctodaeal invagination and gives rise to the splanchnic muscle coat of the hind gut.

In a typical segment, such as one in the thorax or abdomen, the segmentation of the lateral mesoderm is facilitated by the appearance and deepening of the intersegmental ectodermal furrows and the somites thus come to lie in ectodermal pockets.

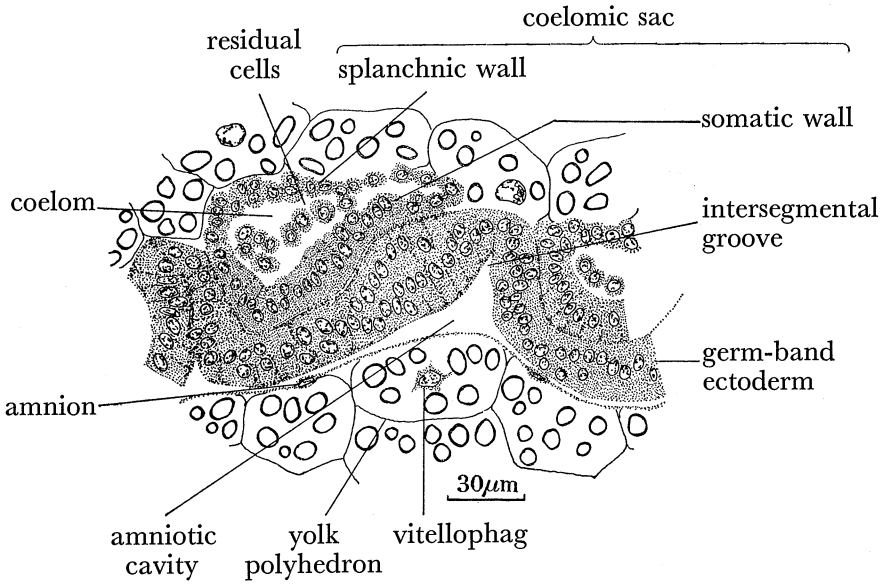

FIGURE 10. Longitudinal section through an abdominal somite of a 30 h-old embryo. Note the partially formed coelom and the residual cell mass within it.

The strip of intrasegmental mesoderm which connects the somites of a segment becomes transformed from the multi-layered condition into a single-cell layer. The increasing width of the germ band, which is expanding laterally to cover the ventral yolk surface at this time, is probably directly responsible for this. To faciliate description and to emphasize the differing fates of the various regions, the segmental mesoderm may be divided into median, subsomitic and somitic portions, as has been done by Eastham (1930*b*).

The median mesoderm is that portion which lies above the future nerve cord and this is the only part which remains unsegmented and continuous (figure 6*A*). The subsomitic mesoderm is that portion which lies between the somite and the median strip. The somitic and subsomitic portions undergo segmentation and are absent intersegmentally. The median and subsomitic mesoderm are histologically indistinguishable and it must be emphasized that these subdivisions are arbitrary.

With the completion of segmentation coelom formation is initiated. A rearrangement of the cells of each somite now takes place. At the beginning of this process the somites are composed of 2 to 3 layers of cells. Those on the periphery aline themselves to form an epithelium and small spaces appear beneath the cell layer which is adjacent to the yolk; this is the first indication of coelom formation. The spaces increase in size, unite and eventually form a single cavity within the somite (figures 7 and 10).

In many specimens loose mesoderm cells are encountered within the partially differentiated coelomic sac; in others such residual cells are absent, but the somatic wall may locally be composed of more than a single layer (figure 7). As, however, in the fully formed coelomic sac the walls are single-layered and the cavity is devoid of cells, these conditions are simply transitional stages occurring during differentiation. The single-layered condition of the sac wall is restored, in all probability, by the insinuation of these extra cells in the epithelium.

The coelomic sacs first appear in the anterior segments and their formation progresses posteriorly. The simple, single-layered condition of the sacs lasts till about the 40th hour. During the remainder of the second day the coelomic sacs enter on a phase of activity, which manifests itself in cell divisions which are especially numerous in the splanchnic walls of the sacs. Most of the spindles lie adjacent and parallel to the lumen.

Simultaneously the formerly cubical cells elongate, so that by the 44th hour the splanchnic walls are transformed into a columnar epithelium, with the oval nuclei alined at right angles to the lumen. Consequently the splanchnic walls thicken and in places become two-layered. The somatic walls remain relatively unaltered, though occasional divisions occur, especially at the junction with the subsomitic mesoderm.

Late on the second day the coelomic sacs attain their maximum size, measuring approximately 100 μm in length and 35 to 40 μm in diameter at their widest point. The epithelium composing the walls is about 10 μm thick and that of the splanchnic walls is rather thicker than the somatic. The enlargement of the sacs is probably aided by the accumulation of fluid, as something akin to a fine coagulum, which stains with light green, is commonly to be observed in the lumen. Such an uptake of fluid by the coelomic sacs is postulated for *Carausius morosus* by Wiesmann (1926) and for *Calandra oryzae* by Tiegs & Murray (1938).

All the thoracic and abdominal sacs are essentially similar in structure and conform to the foregoing account. Those belonging to the definitive head, however, deviate to varying extents from the typical condition. The individual peculiarities of the head coelomic sacs will now be described.

(*b*) *Coelomic sacs of the head*

(i) *The labral coelomic sacs*. The most anterior pair of coelomic sacs in the protocephalon belong to the labral segment. They are first observed in the 27th hour, when the only

appendage rudiments present are the antennary. At this time the labral rudiments have not begun to develop and the sacs are situated in line with the succeeding somites. They do not, however, coincide with the lateral edges of the germ band as do the thoracic sacs (e.g. figure 7). This is because the protocephalon is much wider than the protocorm. The

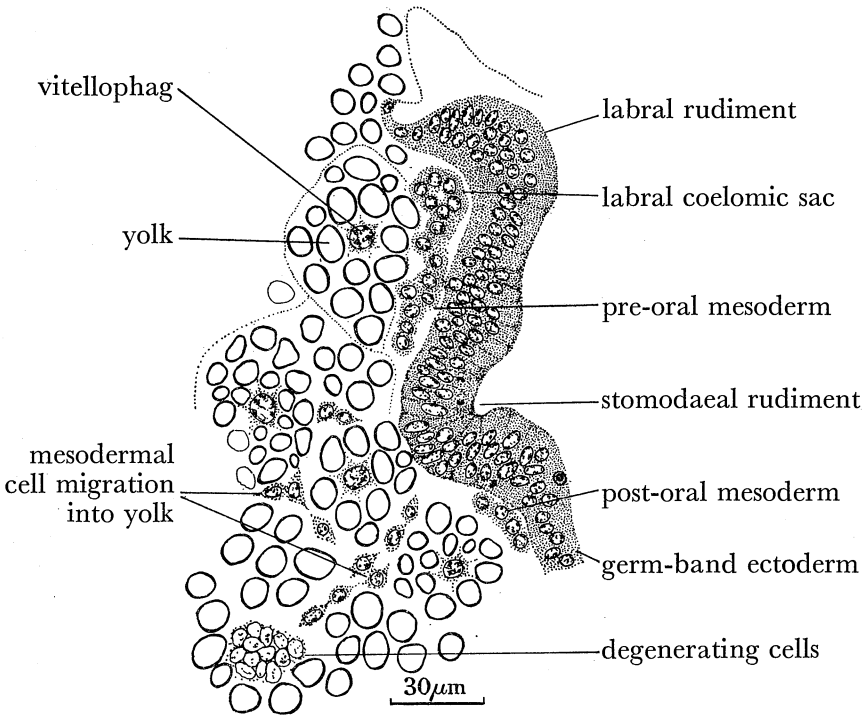

FIGURE 11. Longitudinal section of a 30 h-old embryo through the stomodaeal and labral rudiments. Note the labral coelomic sac and the mass of mesodermal cells migrating into the yolk, there to degenerate. The stomodaeum has broken through the mesodermal layer and abuts on to the yolk.

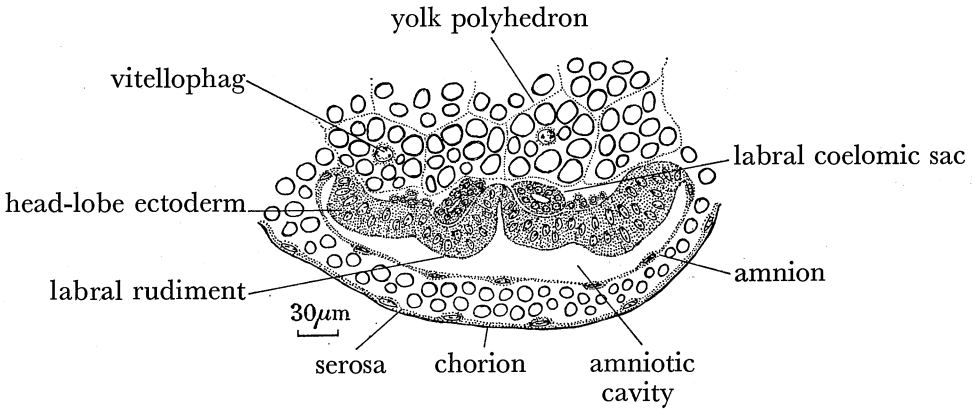

FIGURE 12. Transverse section through the labral region of a 28 h-old embryo.

lateral edges of the head lobes, the bulk of which gives rise to the protocerebral lobes of the brain, are never lined by mesoderm.

At about the 28th hour, before the gnathal and thoracic appendages have begun to develop, the labral rudiments make their appearance. There is no distinct acron present (figure 11), and the rudiments form a pair of medially placed, hollow, ectodermal protuberances (figure 12). Laterally they are not sharply demarcated from the head lobes,

but medially they are separated by a deep and narrow cleft. The latter becomes insinuated between the labral coelomic sacs which, as the cleft invaginates, consequently migrate towards the midline. The sacs thus come to be situated within the cavity of the labral rudiments. At first somewhat dorsoventrally flattened, the sacs expand with the enlarging rudiments and line them almost completely.

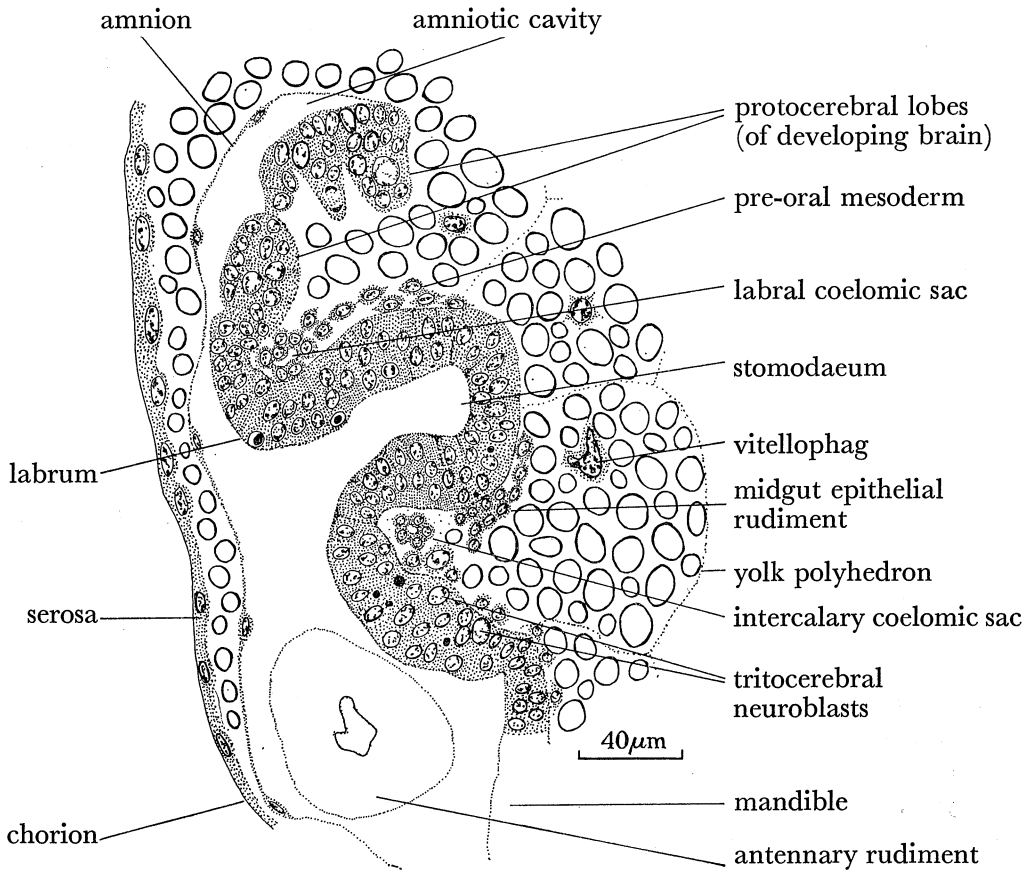

FIGURE 13. Parasagittal section through the anterior region of a 43 h-old embryo. Note the labral and intercalary coelomic sacs.

Two factors appear to contribute to the displacement of the sacs from their original position. These are the development of the labral rudiments themselves and the stomodaeal (foregut) invagination. With the development of the latter the labral somites, which at first lie close to the antennary, become displaced somewhat anteriorly. The formation of the labral rudiments which result from the invagination of the median cleft causes, as already indicated, the sacs to approach each other medially.

Between the 30th and 36th hours the labral rudiments develop into prominent rounded structures which project anteriorly from the protocephalon. The walls of the rounded coelomic sacs are formed of a single-layered, more or less cubical epithelium (figure 13). Between the 44th and 48th hours spaces develop between the ectoderm of the rudiments and the sacs, which eventually come to lie in a cavity which is continuous behind with cephalic haemocoel. Posteriorly the sacs are continuous with the pre-oral mesoderm which is quite small in amount and stretches backwards on either side of the stomodaeum to join the post-oral mesoderm in front of the antennary sacs.

The labral rudiments gradually approximate and fuse progressively, from the base. The median ectodermal walls of the rudiments withdraw to the exterior again, and the coelomic sacs come to lie within a single cavity, that of the upper lip or labrum. At the end of the second day the epithelium of the sacs break down into a loose collection of mesoderm cells which give rise to the bulk of the stomodaeal splanchnic musculature; in all probability they also contribute to the dorsal 'pharyngeal' extrinsic muscles.

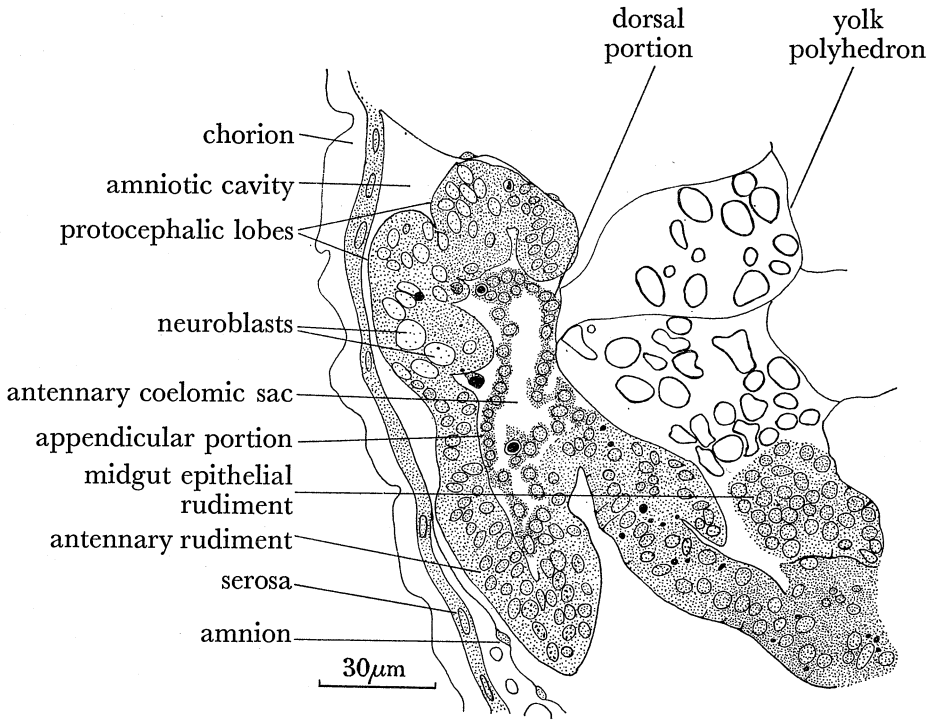

FIGURE 14. Transverse section through the antennary segment of a 43 h-old embryo.

The labral sacs differ from the typical coelomic sacs in their almost spherical shape, eventual median location and histological uniformity. The epithelial walls do not show the regional differentiation exhibited by the typical coelomic sacs. Since, however, the splanchnic muscle of the foregut and, probably, also the extrinsic muscles are derived from the walls of the labral coelomic sacs, these also apparently give rise to both splanchnic and somatic muscles; and in this feature they resemble the thoracic and abdominal sacs.

(ii) *The antennary coelomic sacs.* The second pair of protocephalic coelomic sacs, the antennary, are first observed at the 27th hour, at the base of the very rudimentary antennary appendages. As the latter elongate the small sacs, composed of a single layer of epithelial cells, extend into them. In the fully formed antennary sacs two regions are distinguishable: a dorsal portion which lies above the limb base, between the ectoderm and the yolk; and a ventral, appendicular portion, situated within the antennary cavity. As with the labral sacs, the walls do not differentiate into splanchnic and somatic regions (figure 14). From the 40th hour onwards the dorso-lateral portion of each sac grows posteriorly.

Between the 44th and 50th hours the antennary rudiments, together with their coelomic sacs, migrate pre-orally. The sac walls undergo histological differentiation, at the same

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time greatly increasing in size due to the posterior growth (the anal diverticulum) of the dorsolateral region. By the 45th hour this diverticulum reaches back to the middle of the mandibular segment, and lies laterally above the mandibular mesoderm (figure 24), while the appendicular portion is rapidly elongating to form an attenuated cylindrical sac within the cavity of the growing antenna (figure 22).

Figure 16, plate 23, is a horizontal section through the protocephalon of a 44 hour old embryo. At the base of the antennary rudiments, the dorsal portion of the sacs is seen, beginning to grow backward. The posterior wall is beginning to undergo histological differentiation, the epithelial cells becoming drawn out antero-posteriorly. This differentiation of the antennary coelomic sacs continues between the 45th and 50th hours. Ultimately the anal lobe comes to be formed of a pavement epithelium, and eventually gives rise to the cephalic aorta. The appendicular portion of the coelomic walls remains composed of a cubical epithelium.

(iii) *The intercalary coelomic sacs.* At about the 30th hour, when the majority of coelomic sacs has already been formed, an accumulation of mesoderm is to be observed situated immediately behind the incipient stomodaeal invagination and between the bases of the still post-orally situated antennary rudiments. This mass, which is, as yet, undivided and consists of typical polyhedral mesodermal cells with spherical nuclei, belongs to the partially suppressed premandibular or intercalary segment.

Between the 30th and 40th hours this intercalary mesoderm becomes partly divided into two; thus a pair of somites is formed, which is not in alinement with the others. Whereas the typical somites are usually compact oval structures, the intercalary somites

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Explanation of plate 23

Abbreviations: *a*, amnion; *a.c.s.*, antennary coelomic sac (dorsal portion); *ab.c.s.*7, 7th abdominal coelomic sac; *c.*, coelom; *ch.*, chorion; *c.h.*, cephalic haemocoel; *e.*, ectoderm; *g.b.*, germ band; *g.c.r.*, germ-cell rudiment; *i.c.s.*, intercalary coelomic sac; *l.c.s.*, labral coelomic sac; *l.r.*, labral rudiment; *m.*, mesoderm; *md.r.*, mandibular rudiment; *m.g.e.r.*, midgut epithelial rudiment; *p.a.c.*, posterior amniotic cavity; *pr.l.*, protocerebral lobes; *s.*, serosa; *s.o.b.*, suboesophageal body cell; *sp.w.*, splanchnic wall; *s.w.*, somatic wall; *st.*, stomodaeum; *y.*, yolk; *y.n.*, yolk nucleus.

Figure 16. Horizontal section through the protocephalon of a 44 h-old embryo. Note the dorsal pouch of the antennary coelomic sacs.

Figure 17. Transverse section through the antennary-mandibular intersegment of a 42 h-old embryo. Note the position of the antennary and intercalary coelomic sacs.

Figure 18. Longitudinal section through the labral rudiment of a 45 h-old embryo. Note the labral coelomic sac. The large cell below the stomodaeum belongs to the intercalary mesoderm.

Figure 19. Transverse section through the labral rudiment and coelomic sacs of a 45 h-old embryo.

Figure 20. Transverse section through the posterior region of a 28 h-old egg. The dorsally flexed germ band is sectioned twice. From the dorsally situated caudal end of the embryo the germ-cell rudiment is seen to project into the yolk. Morphologically the rudiment lies above the underlying, still undifferentiated, mesoderm but it projects into the yolk to such an extent that it impinges on the mesoderm associated with the ventrally situated portion of the germ band.

Figure 21. Horizontal section through the abdominal coelomic sacs and germ cells of a 44 h-old embryo. Note the variable thickness of the splanchnic wall of the sacs.

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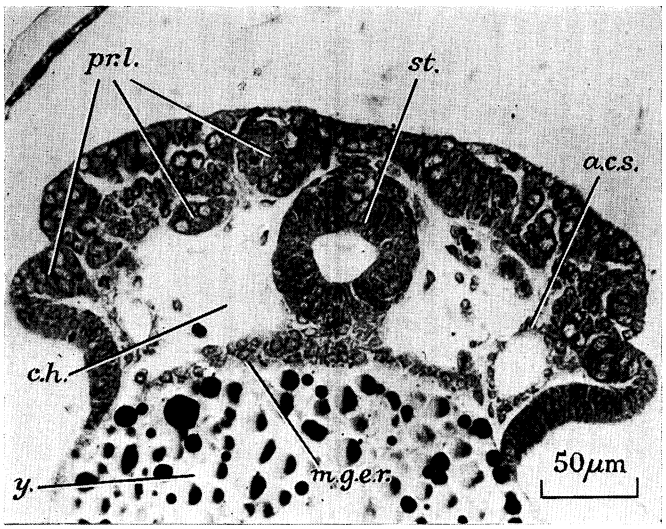

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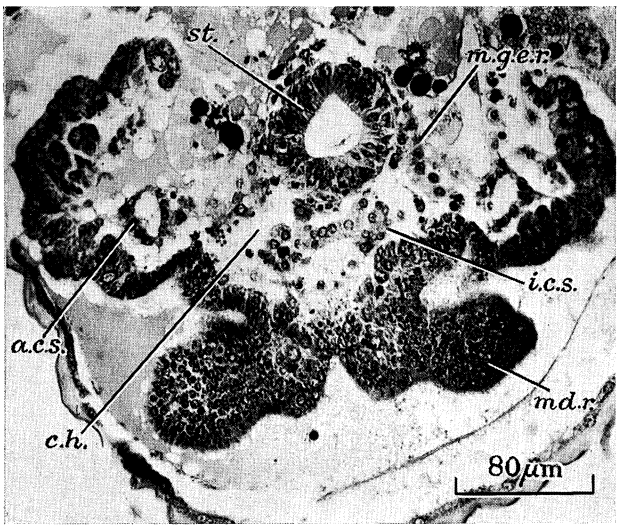

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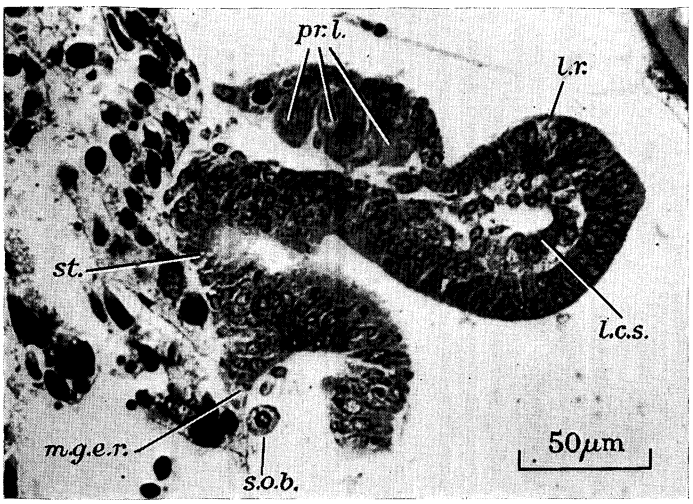

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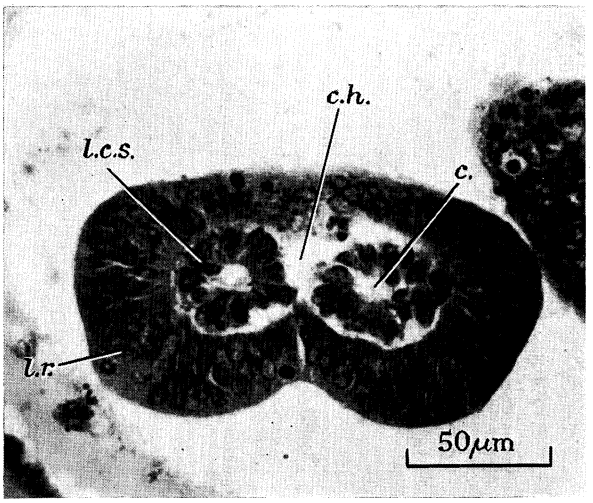

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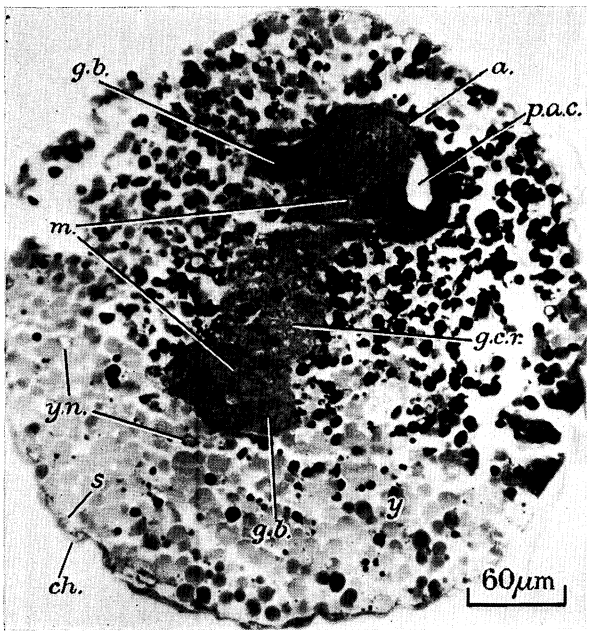

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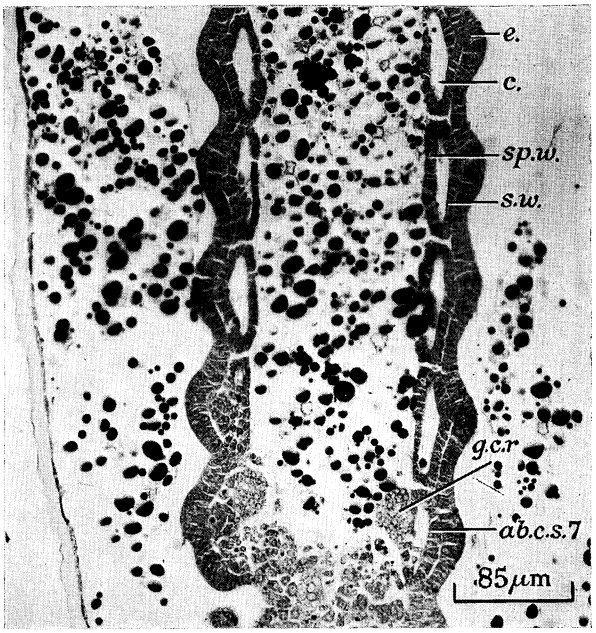

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tend to form irregular cell masses which are medially connected by a single-layered strand of about 3 to 4 more or less cubical cells. In the middle of the second day the somites become transformed into coelomic sacs, roughly oval in shape and lie medially to the antennary sacs. Their longitudinal axes lie parallel to that of the body and the sacs extend into the intersegmental region behind. As the antennary-mandibular 'intersegment' is approached, from each intercalary sac a short solid strand, composed of a variable number of polyhedral cells, extends laterally to impinge on the anal diverticulum of the antennary sac of its side. It may be noted that in *Locusta migratoria* (Roonwal 1937) the posterior part of the intercalary mesoderm is connected to the mandibular ectoderm by a cell strand. The coelomic cavity traversing the length of the somite is approximately between 7 to 10 μm in diameter.

At an early stage the intercalary mesoderm can be distinguished by its large, pale, polyhedral cells. The cytoplasm is more abundant than in the other mesodermal cells, and stains less intensely with basophil stains, a property which facilitates its identification.

The backward growth of the anal diverticula of the antennary sacs, into the lateral regions of the segment behind, appears to be responsible for the median displacement of the intercalary sacs (figure 17, plate 23). As the intercalary somites develop later than those of the antennary segment, at the time of their appearance the lateral position, typical of somites, is already occupied.

When the yolk is withdrawn from the anterior end of the embryo, approximately between the 42nd and 45th hours, the stomodaeum, formerly sandwiched between the mesoderm and the yolk, is lifted from the underlying tissues, to lie freely in the cephalic haemocoel (figures 13 and 24). As the intercalary sacs maintain their contact with the ventral side of the stomodaeum, they too are raised with the organ. At the same time they are carried slightly posteriorly with the rapidly elongating stomodaeum; concurrently the antennary rudiments migrate anteriorly. Thus these two structures, which at first were situated at the same level, become separated.

Of all the coelomic sacs of the body the intercalary are the most short lived: they develop after the others, and begin to break down in the 45th hour. The cells then form loose masses which extend on either side of the stomodaeum, from the antennary to the mandibular segment (figures 23 and 24). The products of disintegration are traced in later stages with facility, due to the peculiar staining properties of the tissue. The cells continue to enlarge (figure 18, plate 23), become plump and oval, drawn out into a short process at each end, and soon they all become binucleate. The most conspicuous feature of these cells, which give rise to the suboesophageal body, are their large size, poor affinity for stains and binucleate condition.

(iv) *The mandibular coelomic sacs.* In the mandibular segment a pair of somites is developed by the 27th hour, above the region where the appendage rudiment will arise. The somites are thus placed closer together medially than in the trunk segments, where they lie dorso-laterally to the limbs (p. 266, figures 7 and 25). They do not, however, approximate to the same degree as the intercalary somites. By the 30th hour a coelom, oval in transverse section, is developed within them. In the middle of the second day the mandibular appendage rudiments begin to develop behind the antennary. When first

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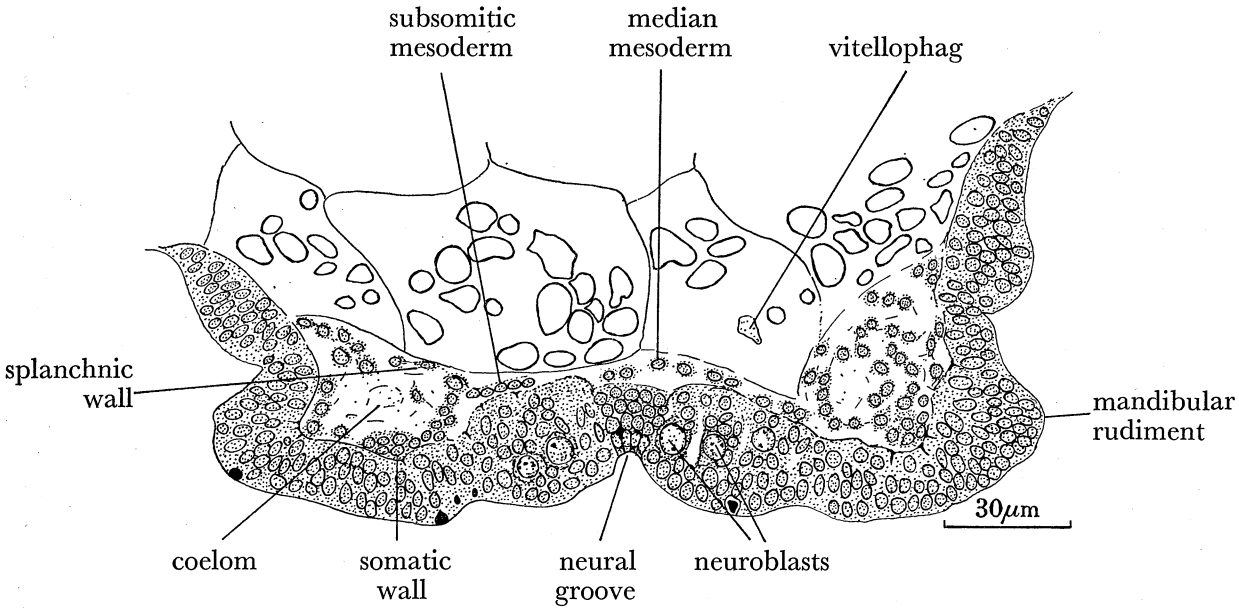

FIGURE 15. Transverse section through the mandibular segment of a 43 h-old embryo.

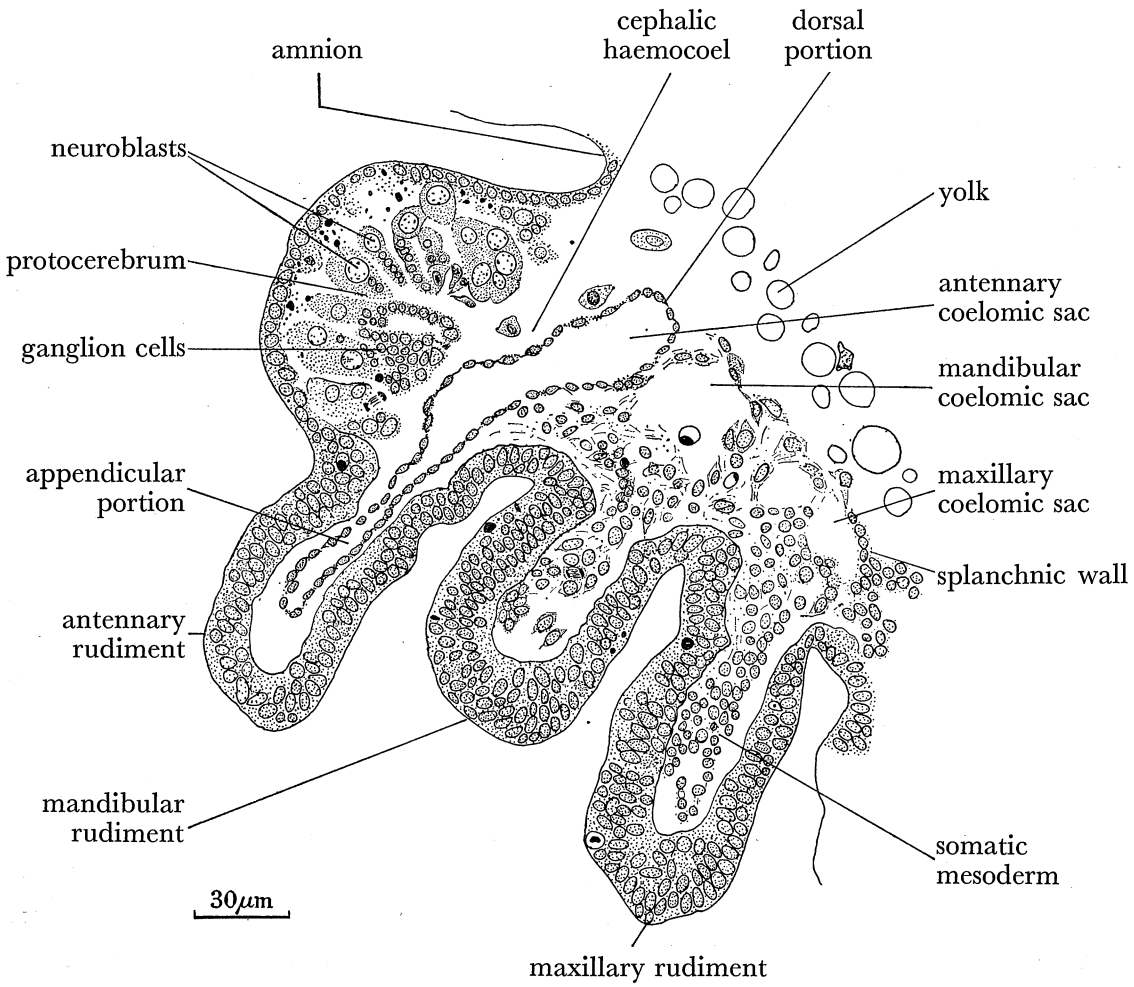

FIGURE 22. Parasagittal section through the antennary and gnathal segments of a 45 h-old embryo.

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formed they are small, postero-laterally directed, hollow, rounded prominences of the multi-layered germ-band ectoderm. The coelomic sacs gradually sink into them.

During the latter half of the second day the mandibular appendages increase in size and the somatic walls of the sacs, adhering to the ectodermal basement membrane, extend into them to form a lining. The splanchnic wall of the sac does not penetrate the appendage, and is in continuity with the single-layered subsomitic and median mesoderm which extend ventrally to the yolk, across to the sac of the opposite side. Figure 15 shows the atypical appearance of the mandibular coelomic sacs. The delicate structure of the cell wall, easily damaged by sectioning, and its early breakdown, considerably hinder the observation of coelom formation in this segment.

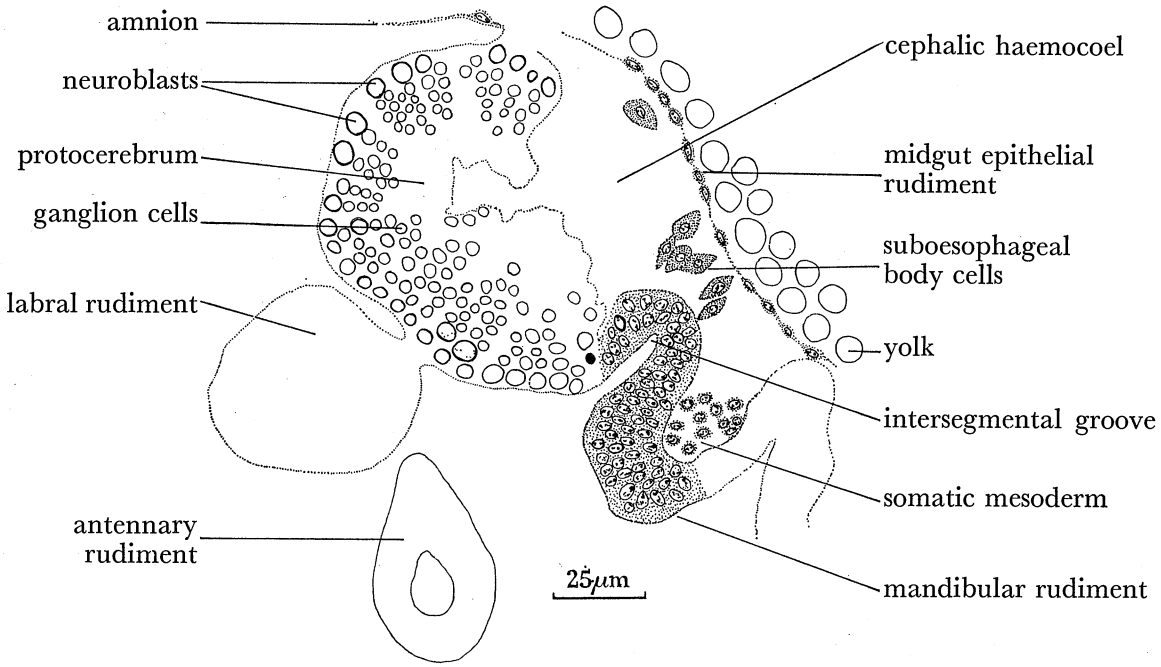

FIGURE 23. Parasagittal section through the protocephalon and mandible of a 45 h-old embryo. Note the suboesophageal body cells in the intersegmental region.

As the rudiment increases in length, the sac epithelium gradually becomes disrupted. Within the coelom there appears to be a coagulum in the form of an irregular meshwork. This is probably derived from the mesodermal cells and may be associated with the breakdown of the sacs. The amount of somatic mesoderm increases due to mitoses, and the cells form a clump within the rudiment. These cells, as seen from figure 24, are dorso-ventrally orientated and ultimately give rise to the mandibular muscles. As already mentioned, the intercalary mesoderm impinges on the mandibular segment and this gives the false impression that the suboesophageal body cells arise from the mandibular mesoderm.

(v) *The maxillary coelomic sacs*. The maxillary somite occupies the same relative position within its segment, as that of the mandibular, whose structure and development it closely resembles. The coelom arises within the somites in the typical manner and then, as the maxillary rudiments develop, the somatic walls of the sacs keep pace with their development. In figure 22 the structure of the maxillary sac can be seen, in longitudinal section.

(vi) *The labial coelomic sacs*. The last of the gnathal segments bears the labial appendages. The coelomic sacs belonging to this segment are noteworthy in that they are quite different in structure and position from those of the two preceding segments. Instead, the labial sacs resemble, in form and orientation, those of the thorax (see below). The spacious sacs, which are elongate structures, are oval in transverse section.

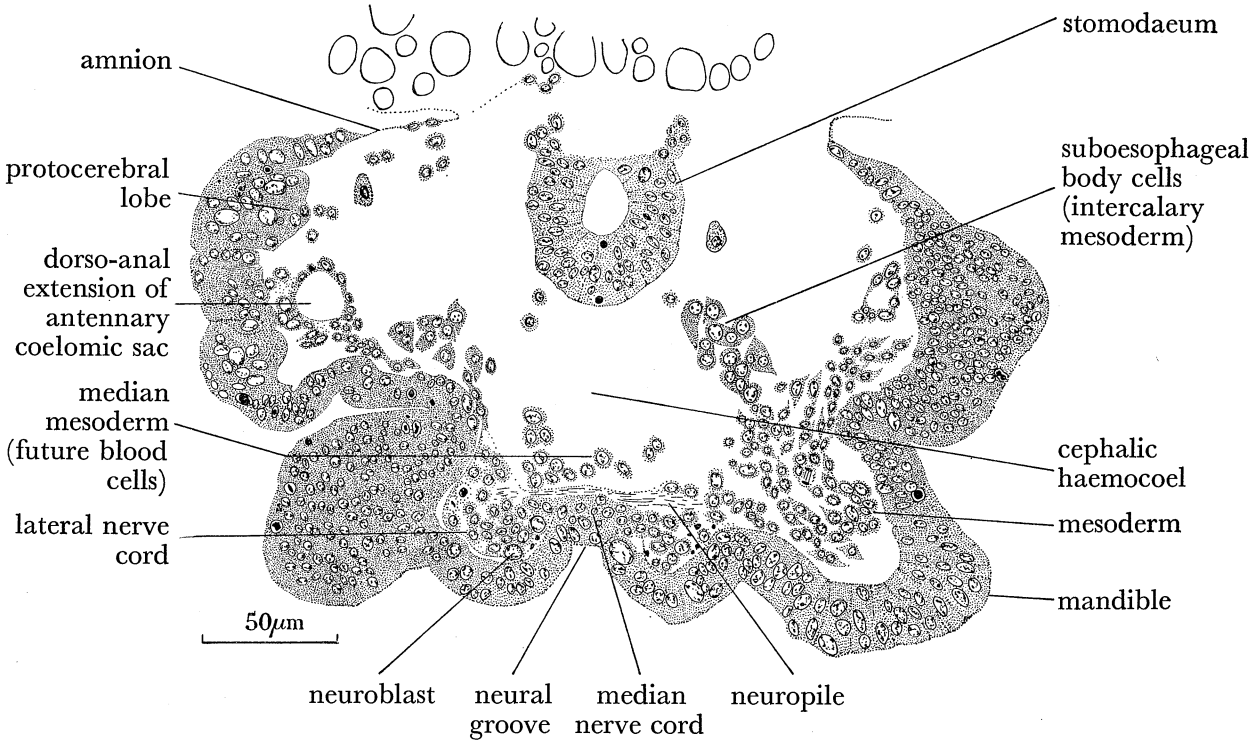

FIGURE 24. Slightly oblique transverse section through the antennary-mandibular intersegment of a 45 h-old embryo.

(*c*) *The thoracic and abdominal coelomic sacs*

There are three thoracic and ten abdominal coelomic sacs. The labial, thoracic and first six abdominal sacs resemble each other histologically and in their elongate, oval form. They tend to expand a little in each segment, the somatic wall bulging somewhat towards the ectoderm, as seen from figure 21, plate 23. The subsomitic mesoderm is closely applied to the underlying ectoderm. When the thoracic and first abdominal appendage rudiments develop, this contact between the ectoderm and mesoderm is maintained; thus the rudiments come to be lined by a layer of mesoderm two to three cells thick (figure 25). Whereas the appendages of the cephalic segments, except the labial, are lined by the somatic wall of the sac, the thoracic and first abdominal appendages are lined by the subsomitic mesoderm, and the sacs are not involved.

The seventh abdominal coelomic sacs have the germ-cell rudiments attached to their median, splanchnic walls; they are accordingly slightly modified, the lumen being somewhat restricted in this region (figure 21, plate 23). The seventh, eighth and ninth abdominal sacs are shorter and broader than those anterior to them (p. 258), being 60 to 70 μm in length and 40 to 45 μm in breadth when fully developed. This change in proportions is directly due to the dorsal flexure of the germ band which causes the sacs to become

compressed longitudinally and hence broader transversely. The tenth abdominal sacs are the smallest of all. By the 45th hour they may be found on either side of the distal end of the proctodaeum. As the midgut epithelial ribbons develop, from the distal ends of the stomodaeum (figure 16, plate 23) and proctodaeum, they become applied to the splanchnic walls of the sacs. The splanchnic mesoderm of the abdominal, thoracic and labial coelomic

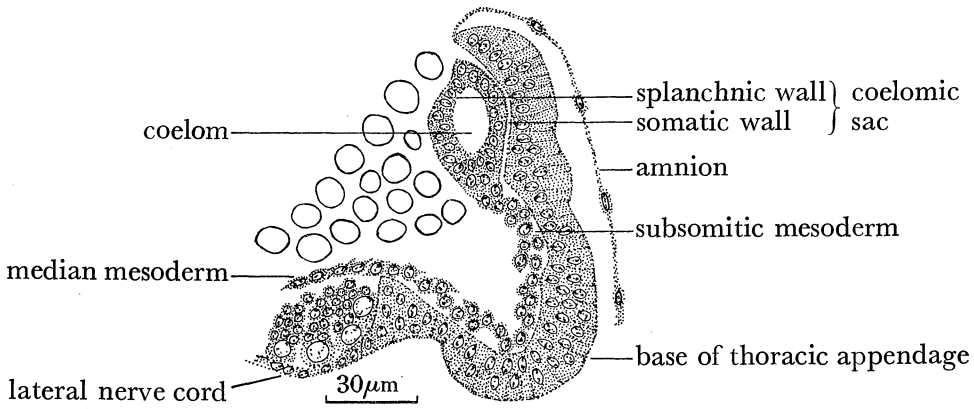

FIGURE 25. Transverse section through the right side of a thoracic segment of a 45 h-old embryo. Note the dorso-lateral location of the coelomic sac, with respect to the appendage rudiment; the latter is lined by the subsomitic and not by the somitic mesoderm, as is the case with the head appendages (excluding the labial).

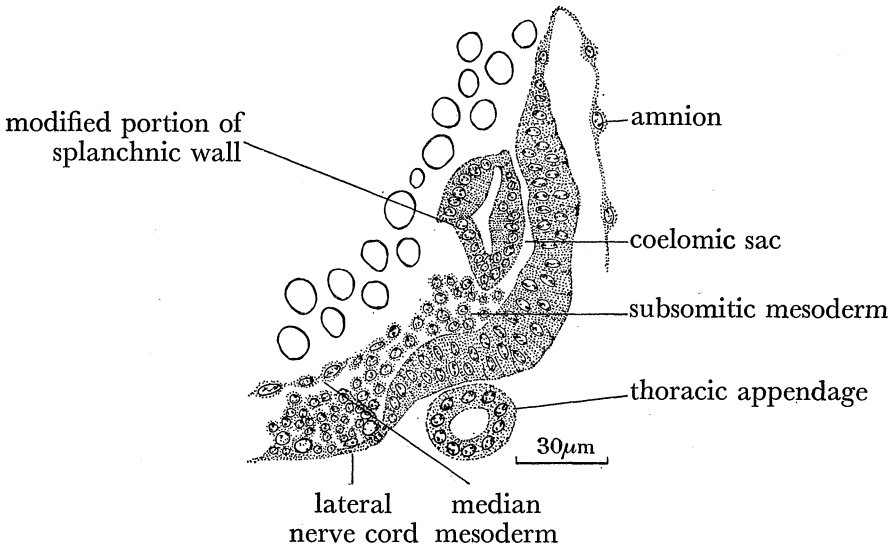

FIGURE 26. Transverse section through the right side of a thoracic segment of a 45 h-old embryo. Note the atypical appearance of the splanchnic coelomic wall.

sacs contributes to the intrinsic musculature of the midgut. The subsomitic mesoderm of the tenth abdominal somite seems to contribute to the mesodermal sheaths of the Malpighian tubules.

In the eleventh abdominal segment no somites are formed. The undivided mass of mesoderm lying ventral to the proctodaeal invagination grows around it and supplies the splanchnic mesoderm of the hindgut.

(5) *The disintegration of the coelomic sacs*

In embryos aged between 44 and 48 hours a peculiar specialization of the splanchnic wall of the coelomic sacs is often seen. A strip along the ventral portion of the splanchnic wall thins out, the cells here becoming cubical again; this process may begin at the anterior, middle or posterior end of a sac, and then spreads along its length (figure 21, plate 23). These changes herald the later disintegration of the coelomic sacs and are most conspicuous in the abdominal sacs (figure 26). The subsomitic mesoderm associated with these segments is about 4 to 5 cells thick, but in the appendicular segments it is much thinner. This is because in the latter the subsomitic mesoderm lines the appendage rudiments, and thus spreads out over a larger surface area (figure 25).

Between the 45th and 48th hours small spaces develop in the subsomitic mesoderm. Simultaneously the epithelium of the ventral wall of the sacs breaks down; this region, which lies adjacent to the subsomitic mesoderm, forms the fat body anlage. This rudiment histodifferentiates rapidly, the cells becoming vacuolated early in the third day; the cytoplasm stains more lightly than before. Some of the adjacent cells of the subsomitic mesoderm also appear to contribute to the fat body anlage. The latter forms a compact cell mass in each segment, from the prothoracic to the tenth abdominal. As the cells enlarge they push into the coelom, thus partially occluding it. At the same time the anterior and posterior midgut epithelial ribbons extend towards each other from the blind ends of the stomodaeum and proctodaeum. As they do so, they become applied to the splanchnic wall of the sacs. Simultaneously the midgut ribbons extend also medioventrally around the yolk, and as the splanchnic mesoderm adheres to the ribbons, the tension causes the sacs to rupture dorsal to the fat body anlage.

Early in the third day the somatic walls of the sacs undergo numerous cell divisions producing a mass of cells about four layers deep, flanking the sides of the germ band. This mesoderm forms the lateral myoblast plate, which becomes divided into a dorsal and a ventral portion. From these cells the body wall musculature is developed.

Mitotic divisions at the dorsal junction of the splanchnic and somatic walls proliferate a small group of cells, the cardioblasts, which give rise to the vascular anlage. By the 60th hour the coelomic sacs have broken down completely, and the various anlagen now proceed to histodifferentiate.

Discussion

The inner layer

Of special interest in the embryology of *Tenebrio molitor* is the development of the mesoderm, which shows a number of primitive features. Ewest (1937) refers to the inner layer as 'entomesoderm', but this terminology is incorrect inasmuch as the inner layer makes no contribution to the midgut epithelium at all and gives rise to mesodermal structures only.

The process of invagination of the middle plate, regarded by many authors (Eastham 1930*a*; Paterson 1935; Roonwal 1937) as a modified act of gastrulation, generally takes place rapidly and the point at which it is initiated varies among the Coleoptera. Thus in *T. molitor*, where the ventral groove is well developed, its initiation and the subsequent phases of inner layer formation commence at the posterior end and progress anteriorly. In

the beetles *Donacia crassipes* (Hirschler 1909), *Euryope terminalis* and *Corynodes pusis* (Paterson 1931, 1935) the ventral groove is first formed in the middle, then in the posterior and finally in the anterior region of the germ rudiment. On the other hand, in *Doryphora decemlineata* (Wheeler 1889) and *Calandra oryzae* (Tiegs & Murray 1938) the groove and inner layer formation begin at the anterior end, whence they progress posteriorly.

It has been shown for a number of insects (Krause 1939) that the point at which groove and inner layer formation begin coincides with the differentiation centre, 'which is invariably associated with the presumptive prothorax region' (Counce 1961). Taking the hind pole of the egg as 0% and the anterior pole as 100%, Ewest (1937) was able to identify the differentiation centre in the young *Tenebrio molitor* egg as between 12 and 26%. At this stage the germ primordium is still a shield-shaped structure embracing the hind pole of the egg, and the differentiation centre is located rather posteriorly. Later the germ band migrates forward on the ventral side, eventually to occupy the region between 0 and 66% of the egg length. The new position of the differentiation centre is at 50%, and from this region differentiation of the segments and organ systems is said to commence.

It thus appears that the activity of the differentiation centre in *T. molitor* is biphased. It exerts its effect first on the yolk system in early development, while it is still posteriorly located, and inner layer formation is subsequently initiated in this region, i.e. between 12 and 26%. At the time of invagination, however, this region is occupied by the posterior portion of the germ rudiment and here groove formation begins. The second phase of the activity of the differentiation centre occurs later in ontogeny, when it is located at 50%. The variation in the position at which invagination begins in the Coleoptera may perhaps be related to the location of the differentiation centre, which probably varies with the type of germ band possessed.

Insect germ bands vary in size relative to the egg dimensions, and two basic types may be distinguished, with numerous intermediate forms (Krause 1939; Weber 1954, p. 34). The 'lower' pterygotes, the Hemimetabola, are generally characterized by small 'head germs' (Kopfkeim) which represent the head end of the future larva, the trunk segments subsequently proliferating from a posterior segment-forming centre (Segmentbildungzone). The Holometabola, on the other hand, generally possess larger 'trunk-germs' (Rumpfkeim) which more or less represent the larval proportions and little subsequent extension takes place. The Coleoptera occupy an intermediate position in this scheme, there being a transition from the small to the large type of germ band within the order.

T. molitor has a relatively short germ rudiment with an initially posteriorly located differentiation centre and in this feature shows affinities with the Hemimetabola (see Weber 1954, p. 34). Whether the subsequent growth of the germ band is due to a segment centre or to the overall growth of the germ band is not clear and, as pointed out by Krause (1939), further investigations are necessary to determine the proportions of the presumptive segments. *Calandra oryzae* (Tiegs & Murray 1938) has a relatively large germ rudiment, the presumptive prothorax and thus the differentiation centre being anteriorly located, and at this point invagination begins.

Whereas in *Doryphora decemlineata* (Wheeler 1889), *Donacia crassipes* (Hirschler 1909), *Pieris rapae* (Eastham 1927), and most other insects the ventral groove has its anterior limit in the middle of the protocephalon at the future site of the stomodaeal invagination, in

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T. molitor it appears to extend almost to the anterior limit of the germ band. The inner layer is certainly produced along its whole length, though at the anterior extremity it seems to arise from the middle plate by overgrowth of the latter by the lateral plates rather than by invagination (figure 3*A*).

Thus in *T. molitor* the pre-oral mesoderm arises *in situ*, as in *Carausius morosus* (Orth.) (Wiesmann 1926), and does not migrate forward from an originally post-oral position, as it does in *D. crassipes* and other Coleoptera. Tiegs & Murray (1938) state that in *C. oryzae* the pre-oral mesoderm 'has, as in all higher insects, a post-oral origin'. The '*in situ*' origin of the pre-oral mesoderm is to be regarded as the more primitive method of formation, and the forward migration, from an initially post-oral position, as a secondary state. The condition found in *T. molitor* is therefore both interesting and significant, for it represents a primitive process not apparently retained by other endopterygotes.

A trough-like ventral groove, giving rise to the inner layer as in *T. molitor*, is commonly found in the Coleoptera. In *Gastrophysa*, *Agelastica* (Lecaillon 1898) and *Meloe violaceus* (Czerski 1904), however, no groove is formed and the inner layer arises by cell proliferation. In *Meloe proscarabaeus* (Nusbaum 1888) an intermediate condition obtains, a ventral groove being present in the mid and hind regions of the germ rudiment, while in the anterior region the inner layer is produced by cell multiplication. In *T. molitor*, similarly, the anterior extremity of the inner layer appears to arise by cell proliferation and overgrowth, without the formation of a ventral groove. In *C. oryzae* although a ventral groove is present the inner layer never forms a tube, but consists of a solid strand of cells; the condition in *T. molitor*, where a transitional tube is formed, is probably more primitive. Contributions to the inner layer from the lateral plates, such as occur in *D. crassipes*, have not been observed in *T. molitor*.

Insect embryologists are divided in opinion as to whether cell proliferation or invagination is the more primitive method of inner layer formation. In the Orthoptera this generally arises by cell proliferation along the midline of the germ band. In the Hymenoptera the inner layer is usually formed by overgrowth of the middle by the lateral plates. The Lepidoptera exhibit an intermediate condition between the Coleoptera and Hymenoptera in that the ventral invagination is accompanied by overgrowth. As, however, the process of invagination occurs sporadically in a number of orders among both the Hemimetabola (e.g. Orthoptera, Hemiptera) and Holometabola (e.g. Lepidoptera, Hymenoptera, Diptera), no phylogenetic significance can be ascribed to groove formation, as pointed out by Hirschler (1924).

Among investigators there is much diversity of opinion as regards the origin and nature of the midgut epithelium in insects, and whether or not the inner layer contributes to its formation (Johannsen & Butt 1941). The actual observations themselves are also disputed. Thus Wiesmann (1926) derives the midgut epithelium in *Carausius morosus* from the median strip of mesendoderm, the 'Blutzellenlamellae'; while Thomas (1936), working on the same insect, claims that it arises from bipolar cell proliferations.

Most of the ways in which the insect midgut epithelium may originate appear to be represented in the Coleoptera. In *D. crassipes* the inner layer is mesendodermal in nature; its endodermal portions are represented by bipolar rudiments situated at the site of the future stomodaeal and proctodaeal invaginations. Certain cells along the midventral line,

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homologous to the 'Blutzellenlamellae' of *C. morosus*, are also said to contribute, in *D. crassipes*, to the formation of the midgut epithelium. In *T. molitor* and *C. oryzae* the midgut epithelium is formed exclusively from cell proliferations at the blind ends of the ectodermal stomodaeal and proctodaeal invaginations; the inner layer, which makes no contributions to it, is therefore purely mesodermal.

The condition in the beetles *C. pusis* and *E. terminalis* (Paterson 1935), in which a mesendoderm occurs, is very interesting, for bipolar rudiments are said to be lacking altogether, the endoderm in these species being solely represented by the median strip of the inner layer (homologous to the 'Blutzellenlamellae' of *C. morosus*, and the median and subsomitic mesoderm of *T. molitor*).

Problems relating to the various methods of midgut formation which involve the homology of the germ layers and the validity of the gastrulation theory have long been debated (*vide* Hirschler 1924; Eastham 1930*a*; Tiegs & Murray 1938; Johannsen & Butt 1941). Eastham regards the ventral groove of insects as a secondary feature, but Mansour (1927) and Paterson (1935) consider it a gastrulation phenomenon. The latter states that although the process 'does not fulfil all the conditions of a normal embolic gastrula... nevertheless, as a result of the invagination of the middle plate cells, the three germinal layers of the embryo are established'. While this may hold good for the chrysomelids investigated by this author, it cannot be universally applied to insects for, as seen in *T. molitor*, invagination of the middle plate does not result in the formation of the three germ layers. The stomodaeal and proctodaeal proliferations arise subsequent to the formation of the inner layer. Brachet's (1921) definition of a gastrula (*vide* Eastham 1930*a*) seems equally inapplicable to *T. molitor*. He states that 'Gastrulation is a process whereby a two-layered larva is formed at the expense of cleavage products, the larva being so constructed that its two layers, external and internal, remain in continuity with one another at a determinate point. The larva consists, in all cases, of ectoblast and endoblast, no matter how it comes about.' In *T. molitor*, however, the inner layer is not composed of endoblast but mesoblast. As invagination and midgut formation in *T. molitor* and *C. oryzae* are essentially similar, the views propounded by Tiegs & Murray (1938) concerning the homologies of these processes apply equally well to both insects. These authors draw attention to Spemann's (1915) view that 'homologizing is only possible after the formation of "Anlagen"', and state that the 'homology of an "Anlage"...is therefore determined by its fate rather than its origin. If, then, the gastral epithelium of all metazoa is homologous, so are also their "Anlagen", whatever their mode of formation'. The term 'endoderm' however, only applies 'to that component of the gastrula which is the gut "Anlage"'. Thus, according to this concept, the midgut epithelia (and their anlagen) of pterygotes and other metazoa are homologous, but the term 'endoderm', *sensu stricta*, is only applicable to it when it has formed the inner layer of the gastrula.

While the germ layer theory may still serve as a useful concept, it can no longer be rigidly adhered to, as is seen from the results of vertebrate transplantation experiments which 'show that there is no profound and permanent physiological difference between the three layers' (Waddington 1956, p. 172). Thus though the different modes of origin of the insect midgut anlage retains an interest for comparative morphology no conclusions as to its evolutionary significance can be drawn. The arguments as to the origin of the endoderm

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of insects are no longer significant; although for convenience of description the term 'endoderm' as synonymous with midgut epithelium will doubtless continue to be used.

Mesodermal segmentation

The degree of segmentation exhibited by the mesoderm of *T. molitor* appears to be unique among the Coleoptera so far investigated. Moreover, the coelomic sacs are exceptionally well developed in the head region, a condition which is undoubtedly primitive. As has been seen, pairs of distinct somites, subsequently hollowed out, occur in all segments from the labral to the tenth abdominal. As usual in the Coleoptera, the mesoderm in the terminal segment remains an undivided mass.

In most insects the segmentation of the mesoderm in the cephalic region exhibits varying degrees of suppression. The most generalized condition is found in the orthopteroid insects, in which the coelomic sacs are well developed and usually divided into a number of diverticula (Roonwal 1937). In 'higher' pterygotes there is a progressive suppression of mesodermal segmentation as the orders become more specialized. Thus while in the Orthoptera successive somites typically become completely separated from each other, in the Coleoptera this condition is unusual. In *Calandra oryzae* (Tiegs & Murray 1938), however, complete separation of the somites sometimes occurs, while in *T. molitor* this is the rule, since, with the exception of the mesoderm in the 10th and 11th abdominal and labral segments, all the somites become separated intersegmentally. The labral somites remain connected to the rest of the pre-oral mesoderm by a pair of strands which pass laterally to the stomodaeum.

In the Coleoptera, mandibular, maxillary and intercalary somites are generally wanting and in some cases, e.g. *Silpha obscura* (Smreczyński 1932), the segmentation in the pre-gnathal region may be totally suppressed. In this beetle the mesoderm in the protocephalic region is said to be uniform in structure, though not in distribution, and in it there are no indications of metamerism.

In *C. oryzae* (Tiegs & Murray 1938) somites in the mandibular and maxillary segments 'do not occur, the lateral zones of mesoderm being not even epithelial in character'; while in 'the region of the intercalary segment it is impossible to distinguish a somite'. Heider (1889) denies the existence of the intercalary segment in *Hydrophilus piceus*, but Paterson (1935) states that in *Corynodes pusis* the somites of the intercalary segment are fairly conspicuous.

The typical coleopteran condition foreshadows that of the more specialized orders, e.g. the Lepidoptera and Hymenoptera, where the somites do not separate at the intersegments. Eastham (1930*b*) states that in *Pieris rapae* mesoblastic somites are only readily definable in the gnathal segments; anterior to this region mesodermal masses are differentiated, rather than somites. In *Apis* (Nelson 1915) also, the mesoderm exhibits an ill-defined segmentation; while in some Diptera, e.g. *Dacus tryoni* (Anderson 1962), it is altogether suppressed. Thus the degree of mesodermal segmentation in *Tenebrio molitor*, especially in the pre-gnathal region, is atypical of the Coleoptera and is not encountered in the higher insects. In this respect *T. molitor* shows undoubted affinities with the lower pterygotes.

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The protocephalic coelomic sacs

As has been seen, there are nineteen pairs of coelomic sacs in *T. molitor*, three of which are pre-gnathal in position. This is remarkable in view of the fact that the cephalic mesoderm in other Coleoptera is but poorly segmented, if at all. Even in cases where labral mesodermal masses can be identified, e.g. *Corynodes pusis* (Paterson, 1935), these are not transformed into sacs, but generally form a clump of cells which migrate from a post-oral position into the labral anlagen where, after a short time, they disaggregate into loosely associated cells which apply themselves to the developing stomodaeum.

The chief importance of coelomic sacs lies in the fact that they, or the unexcavated somites, are amongst the most reliable criteria for the identification of a segment (cf. Manton 1960). Thus the presence of coelomic sacs in the pre-oral region has a direct bearing on the assessment of the number of segments comprising the insect head. As long ago as 1901 Heymons postulated the probable existence of a pre-antennary segment in insects, following his discovery of pre-antennary coelomic sacs in the myriapod *Scolopendra cingulata*. His prediction was fulfilled a quarter of a century later when pre-oral sacs were discovered in an insect, *Carausius morosus*, by Wiesmann (1926). This author describes two pairs of sacs, labral and pre-antennary, lying diagonally the one behind the other, the labral being median and anterior to the pre-antennary.

The occurrence of labral sacs has since been reported in *Diacrisia virginica* (Lepid.) by Johannsen (1929); *Rhodnius prolixus* (Hem.) by Mellanby (1936); *Locusta migratoria* (Orth.) by Roonwal (1937); *Pteronarcys proteus* (Plec.) by Miller (1940) and more recently in *Pyrilla perpusilla* Walker (Hem.) by Sander (1956). As far as the writer is aware, these are the only recorded instances of the occurrence of labral coelomic sacs in insects. The retention of unspecialized and primitive features is to be sought in the phylogenetically older orders; it is, therefore, not altogether surprising to find that all but one of the above-mentioned insects, possessing labral sacs, are exopterygotes. The occurrence of these structures in *T. molitor* is therefore most significant.

Although Imms (1957) states that 'coelomic sacs are certainly present in the labral region' of *Pieris rapae*, according to Eastham (1930*b*) mesodermal masses, rather than somites, differentiate in front of the gnathal segments in this insect. Cavities of doubtful significance occur in the pre-oral mesoderm which migrates into the labral rudiments, but no regular radial arrangement is exhibited by the cells of the labral mesoderm. Eastham thus considers the spaces as due to the looseness of the pre-oral mesoderm, and does not think them to be true somitic cavities. Accordingly, Imms appears to have misinterpreted Eastham's observations.

The labral sacs of *T. molitor*, situated in the hollow, rounded labral rudiments, merge posteriorly with the stomodaeal mesoderm, as in other species. In *Locusta migratoria* when the labral sacs have fully differentiated they form two distinct structures in the labrum, losing connexion with each other medially and with the mesoderm behind. In *C. morosus* the labral sacs are medially connected by a single-layered sheet of cells which is homologous with the 'Blutzellenlamellae' of posterior segments and like the latter, give rise to blood cells. This and other features listed by Wiesmann (1926) indicate the serial homology of the labral with succeeding coelomic sacs.

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Among other arthropods labral sacs or their homologues (pre-antennary, pre-cheliceral) are found in the Symphyla, e.g. *Hanseniella agilis* (Tiegs 1940); Chilopoda, e.g. *Scolopendra cingulata* (Heymons, 1901); Crustacea, e.g. *Hemimysis lamornae* (Manton 1928) and Arachnida (Kishinouye 1894). No 'pre-antennary' sacs in Wiesmann's sense have been described in any other myriapod or insect. The pre-antennary sacs in *S. cingulata* and *H. agilis* are long, and in the former extend into the acron. It has been suggested by Manton (1928) that if two pre-oral sacs indeed exist in *C. morosus*, these have arisen by the division of a single large pre-antennary somite. Such a division occurs in the spider (Kishinouye 1894) and in *Eoperipatus weldonii* (Evans 1902). Events in *P. rapae* (Eastham 1930*b*) also suggest that this is taking place. Roonwal (1937), however, is not convinced by this argument. Nevertheless, until further relevant information is available Manton's explanation of the facts appears to be the most plausible. Thus the presence of pre-antennary sacs in *C. morosus* does not necessarily imply a seven-segmented insect head.

The fate of the labral mesoderm in insects and chilopods is similar, i.e. it gives rise to stomodaeal musculature. It is interesting to note that in *H. agilis* the stomodaeal dilator muscles are derived from this mesoderm; in *T. molitor*, similarly, these muscles appear to arise from the labral mesoderm. This would mean that the labral sacs, though not histologically differentiated into splanchnic and somatic portions, yet give rise to both splanchnic and somatic muscles. The fate of the labral sacs thus, to a considerable extent, resembles that of the typical sacs, although they do not form cardioblasts or fat body.

The coelomic sacs most commonly present in the protocephalon of insects are the antennary. The post-oral origin, structure, development and fate of these sacs in *T. molitor* is fairly typical. In the Orthoptera, however, the extra-appendicular portion of each antennary coelom extends into dorso-rostral and dorso-anal pouches, which form the cephalic aorta (Wiesmann 1926; Roonwal 1937). The dorsal, posteriorly extending portion of the antennary sacs in *T. molitor* is comparable with the dorso-anal pouch of Orthoptera, the dorso-rostral portion, as in the endopterygotes generally, being suppressed. The antennary sacs in *C. oryzae* closely resemble those in *T. molitor*.

In the other Coleoptera investigated antennary coelomic sacs are of sporadic occurrence. Thus in *Silpha obscura* (Smreczyñski 1932) they form as rather ill-defined cavities within the antennary mesoderm, and are bounded by only a few centripetal cells. They eventually separate from the solid core of cells which fills the cavity of the antenna. Nothing equivalent to the appendicular or dorso-anal pouches, found in *T. molitor*, occur so that in the latter an apparently primitive condition is retained. Heider (1889) did not find antennary sacs in *Hydrophilus piceus*; nor did Hirschler (1909) in *Donacia crassipes*. In the latter instance, as already pointed out by several authors, Hirschler has probably mistaken the antennary for the intercalary coelom, since he states that the cephalic aorta arises from it.

Intercalary coelomic sacs are found in the majority of apterygotes (Hoffmann 1911) and in numerous exopterygotes, e.g. *Xiphidium* (Wheeler 1893), *Forficula* (Heymons 1895), *Rhodnius prolixus* (Mellanby 1936). In all they are small, evanescent structures. Within the Coleoptera they are recorded for *Euryope terminalis* and *Corynodes pusis* (Paterson 1932, 1935) and for *Calandra callosa* by Wray (1937). In *C. oryzae*, however, Tiegs & Murray (1938) found no trace of them. In *T. molitor* the intercalary sacs are small but distinct. They are among the most highly specialized of the coelomic sacs and deviate most from the

basic plan in topography and fate. Nevertheless, they originate in a pair of mesodermal cell masses which are undoubtedly true somites and which are, characteristically, united medially by a strand of cells. The tritocerebral anlagen are all that remain of the intercalary ectodermal tissue. Finally, the fate of the intercalary sacs is noteworthy. This diverges radically from that of all the other sacs which, without exception, contribute to the somatic and splanchnic musculature. The intercalary mesoderm, in *T. molitor* as in most other insects, gives rise to the suboesophageal body.

In conclusion a difference in the nature of the mesoderm lining the cavities of the head (except labial) and trunk appendages must be pointed out. In the head these are lined by the coelomic sacs themselves, which keep pace with the expansion of the appendage rudiments. In the trunk, on the other hand, the sacs lie dorso-laterally to the rudiments, which are lined by the subsomitic mesoderm, as in *Pieris rapae* (Eastham 1930*b*). The statement by Selys-Longchamps (1904) that in *T. molitor* the coelomic sacs penetrate the trunk appendage rudiments is thus incorrect.

To summarize, the *in situ* origin of the pre-oral mesoderm, the complete intersegmental separation of all but the terminal somites, the occurrence of three pairs of coelomic sacs in the protocephalon, the relatively small germ rudiment and the initially posteriorly located differentiation centre are to be regarded as primitive features in a holometabolan, indicating exopterygote affinities.

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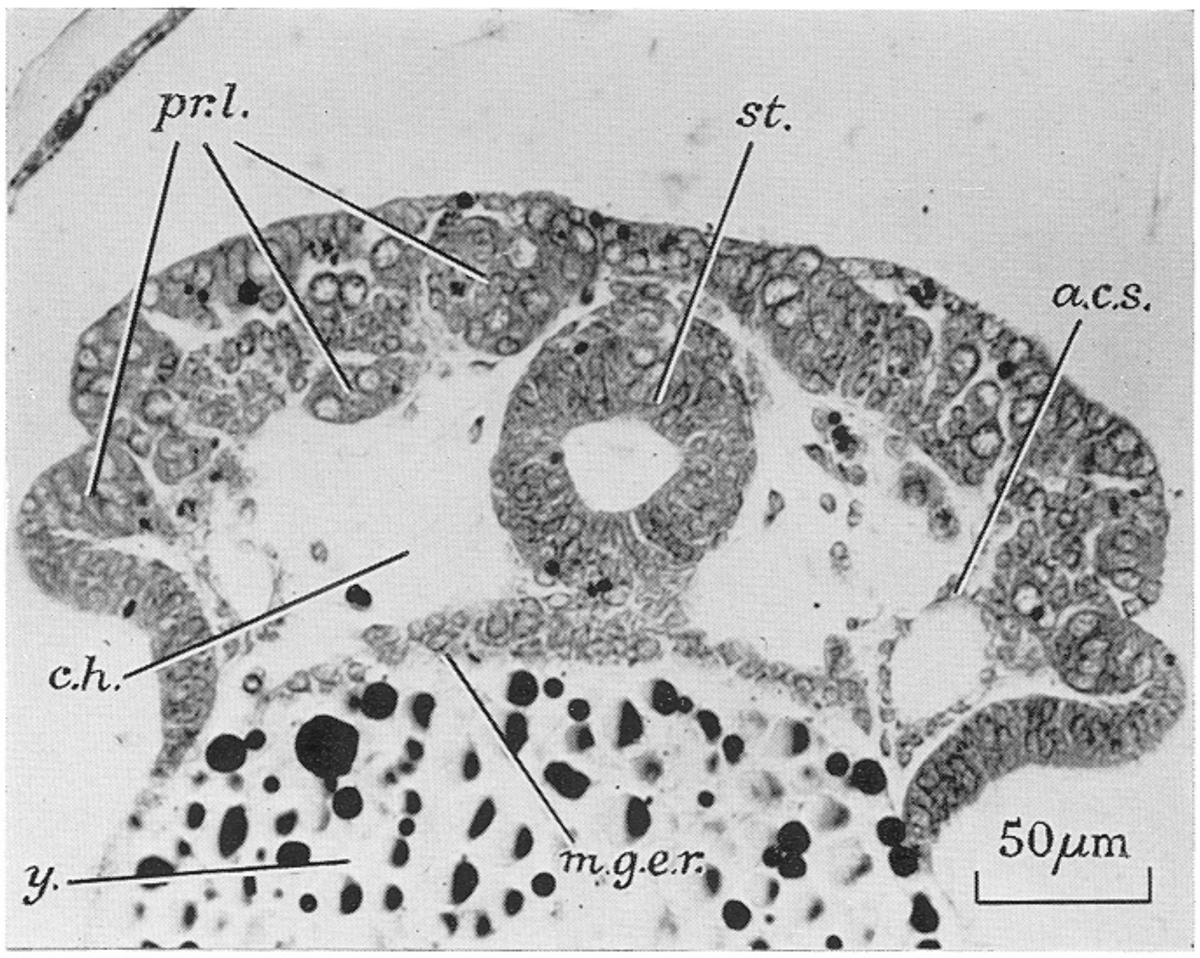

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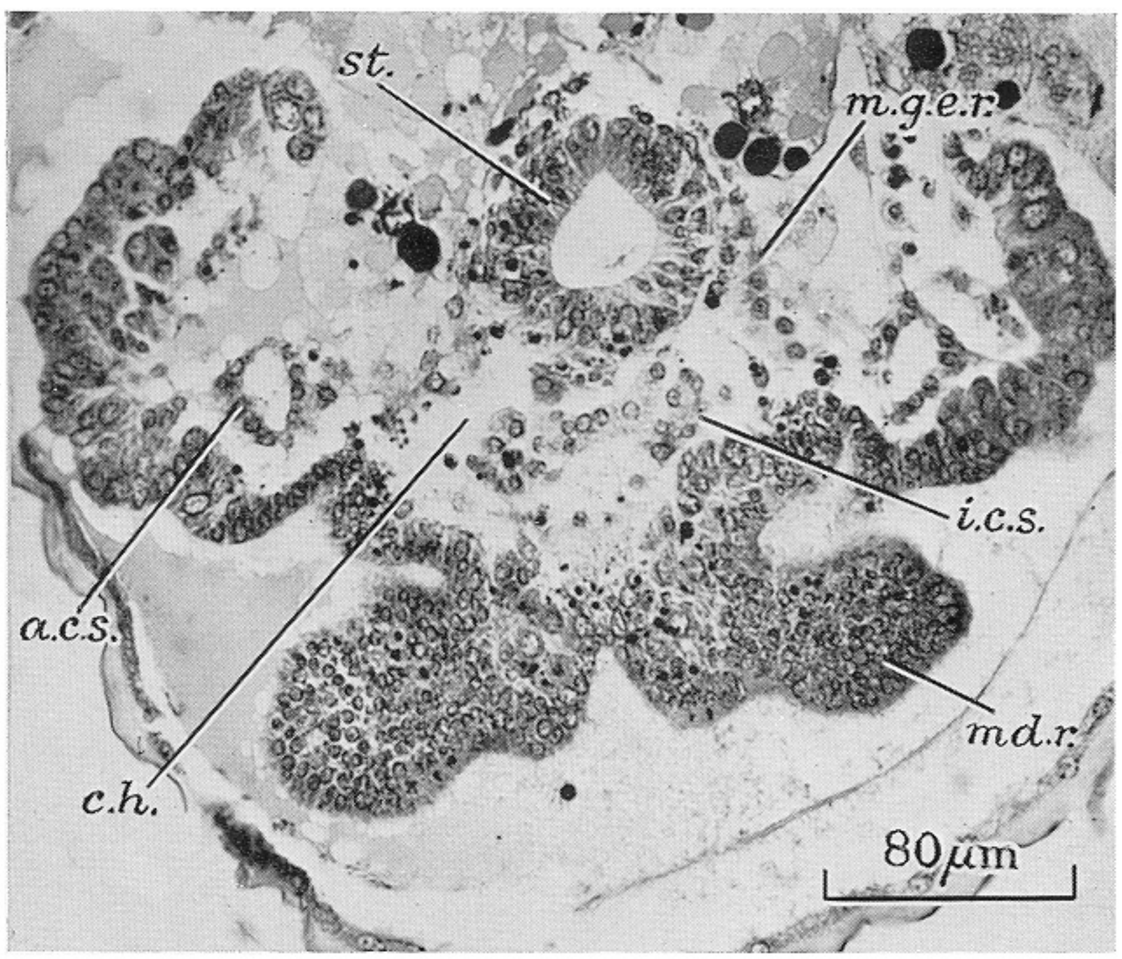

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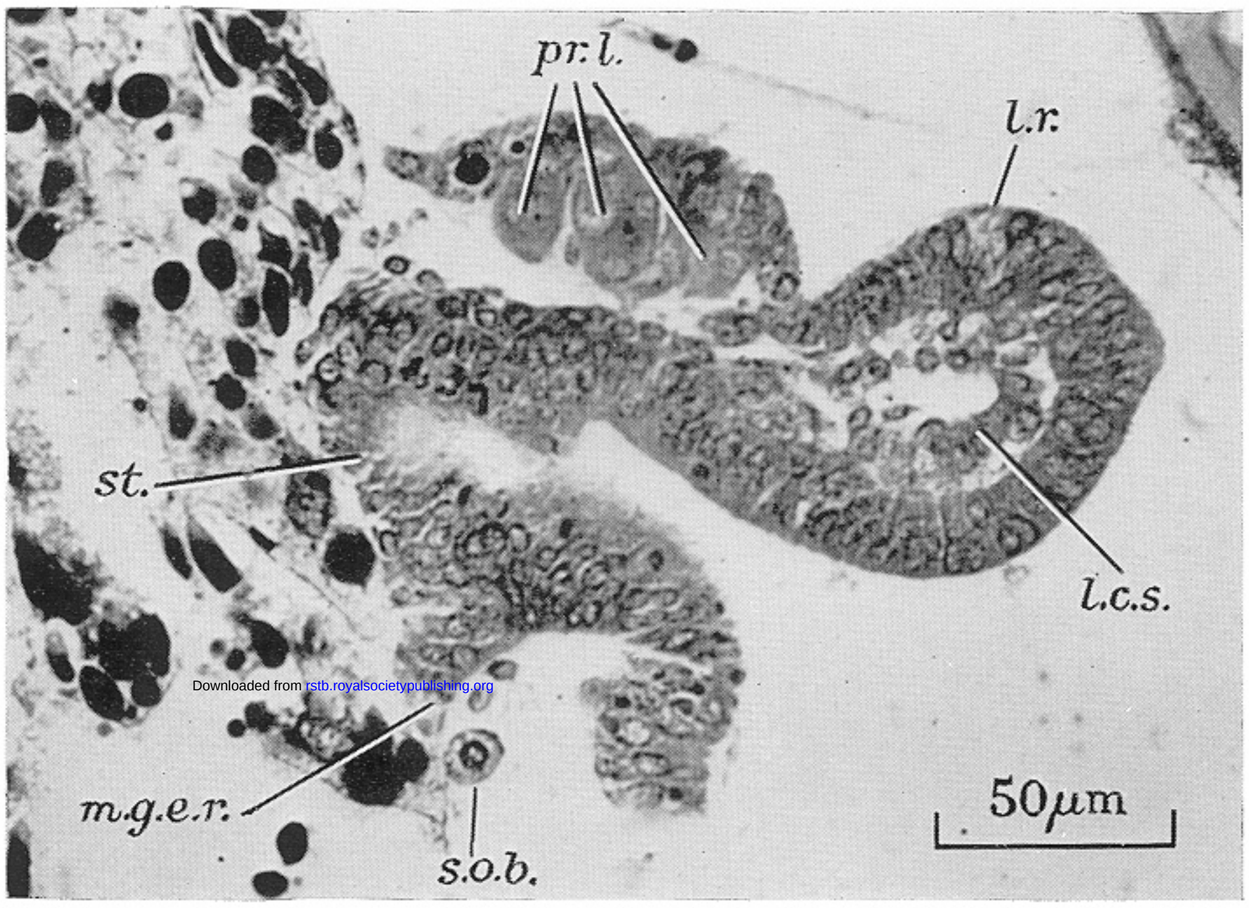

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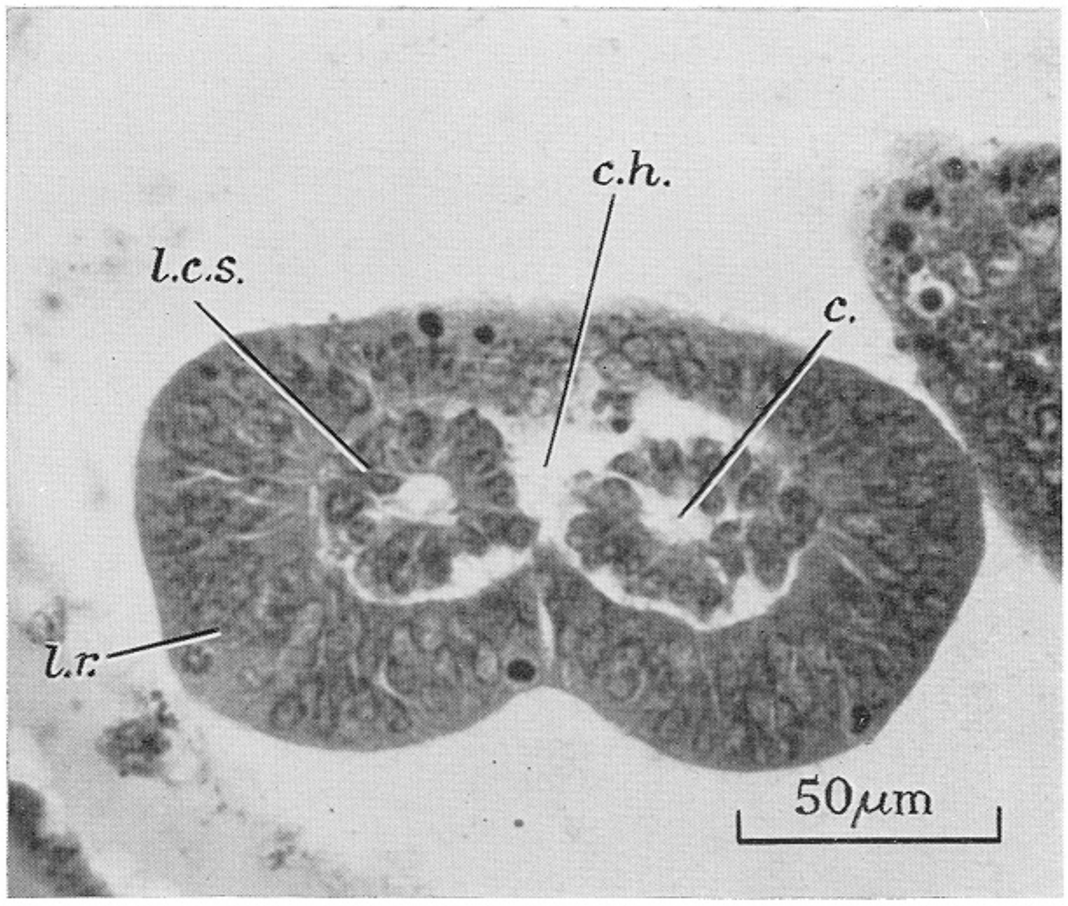

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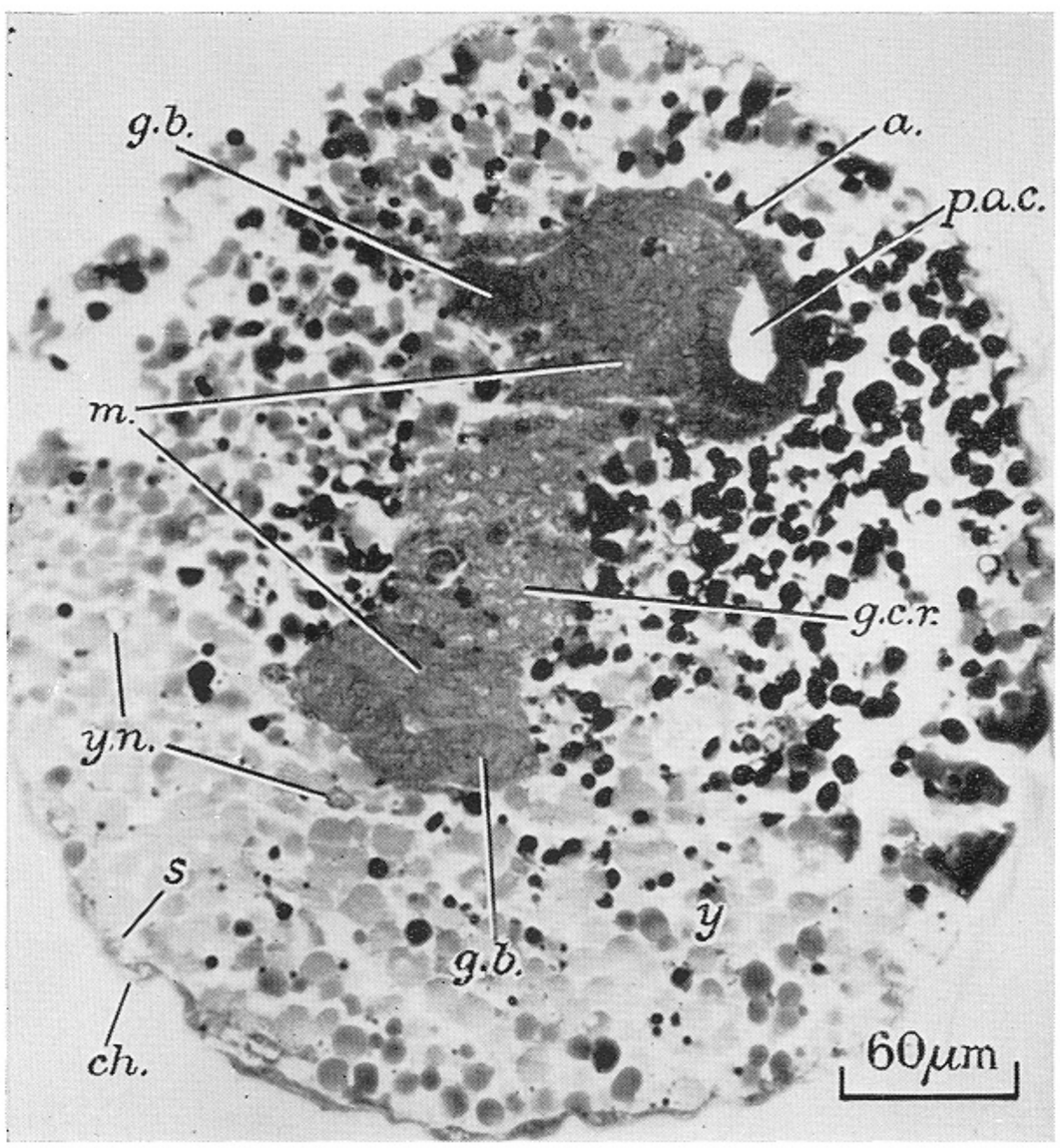

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