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THE DEVELOPMENT OF THE NERVOUS SYSTEM AND OTHER ECTODERMAL DERIVATIVES IN *TENEBRIO MOLITOR* L. (INSECTA, COLEOPTERA)

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[Plates 1 to 3]

CONTENTS

Previous work has shown *Tenebrio molitor* to be a relatively unspecialized beetle, and therefore more suitable for head segmentation studies than those hitherto investigated. Such studies must, however, be preceded by a description of the nervous system and other segmental ectodermal structures, and this forms the subject of the present paper.

The development of the nervous system is typical of insects.

The ventral nerve cord develops from neuroblasts situated in a pair of lateral cords and in a median cell strand, which develop from the intercalary to the tenth abdominal segment. Teloblasts bud off preganglionic cells which, by division, give rise to the ganglion cells. The median strand contributes to the transverse commissures and to the dorsal parts of the ganglia. The definitive cord comprises the suboesophageal ganglion (fused mandibular, maxillary and labial ganglia), three thoracic and nine abdominal pairs of ganglia.

The brain is the product of fusion of the proto-, deuto- and trito-cerebral gangliomeres, which develop in series with the ventral ganglia.

The stomodaeal nervous system arises from three invaginations in the roof of the stomodaeum, and consists of the frontal and ventricular ganglia; and the recurrent nerve.

The neurilemma forms from flattened peripheral ganglion cells.

Pairs of ectodermal invaginations develop in the antennary, gnathal meso- and metathoracic, and the first eight abdominal segments. Those of the antennary and maxillary segments form the tentorium; the mandibular, the flexor apodeme of that segment and the labial diverticula arise from the posterior gnathal segment.

The small apodeme of the mandibular extensor muscle is considered to have been derived from the transient intercalary segment. Post-gnathal invaginations give rise to trachea.

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Groups of oenocytes arise from the ectoderm above the spiracles. They are situated laterally in the haemocoel of the first eight abdominal segments.

The segmental affinities of the brain, stomatogastric system and cephalic invaginations are discussed. The conclusions indicate a six segmented head in *Tenebrio*.

Introduction

The origin and structure of the mesoderm and the formation of the coelomic sacs in this beetle have already been described in a previous communication (Ullmann 1964). Attention was there drawn to a number of features, to be regarded as primitive, which rendered *Tenebrio molitor* more suitable for head segmentation studies than the beetles, e.g. *Silpha* (Smreczyński 1932) and *Leptinotarsa* (Haget 1955) hitherto investigated. An adequate discussion of this topic, however, must be preceded by a description of the development of the nervous system.

In addition the formation of the ectodermal derivatives, the apodemes, labial diverticula, trachea and oenocytes, will also be described since, as pointed out by Eastham (1930), the cephalic invaginations are also important in evaluating the segmental constitution of the head.

A further objective was to describe the normal development of the nervous system as a preliminary step to a future study of neurogenesis at the ultra-structural level. A combination of histochemical, electron microscopical and tissue culture techniques could yield valuable results in the elucidation of certain morphogenetic questions. What, for instance, determines that of a group of seemingly similar cells one becomes a ganglion cell, another a neurilemma cell, while yet another degenerates altogether? It is intended that in the near future such an investigation will be undertaken.

Materials and methods

The methods of culture maintenance, egg collection and the preparation of serial paraffin sections have already been described (Ullmann 1964). Aqueous Bouin was most commonly used as a fixative; and Heidenhain's iron haematoxylin, counterstained with light green or Masson's ponceau–acid–fuchsin and light green, was used for staining. Prior to staining the slides were dipped into a 0·05 % solution of celloidin, to aid adherence of the sections to the slides.

Times refer to development at 27 °C.

Observations

(1) *Development of the nervous system*

(*a*) *The ventral nerve cord*

The origin of the germ rudiment and the formation of the germ band by the invagination of the middle plate have been described elsewhere (Ullmann 1964). Between the 35th and 38th hours a pair of ectodermal thickenings appears on the ventral side of the germ band and extends on either side of the midline, from the post-oral region to the tenth abdominal segment. These structures constitute the neural ridges and are the first external

signs of the developing ventral nerve cords. The shallow furrow between them constitutes the neural groove, and from its floor the median nerve strand is formed. As the intersegmental ectodermal furrows are already present at the time the neural ridges appear, the latter are secondarily segmented. The first internal indications of the differentiation of the neural tissue appear somewhat earlier, generally between the 30th and 33rd hours when mesodermal segmentation has been completed.

Examination of transverse sections at this time, at approximately 30 h, shows that the general ectoderm is composed of a strongly columnar, multilayered epithelium with oval nuclei. Histological differentiation of some of these cells now begins, first in the future prothoracic segment and from thence anteriorly and posteriorly in the protocorm. Segmentally, the nuclei in three of four of the cells on either side of the midline become enlarged. The basophil particles within them become more dispersed, while the nucleoplasm fails to stain. At the same time, the amount of cytoplasm associated with each of these enlarged nuclei increases.

These large cells constitute the neuroblasts (or nerve mother cells). During ontogeny the neuroblasts undergo mitosis and thus give rise to columns of daughter cells, the nerve cells proper, which later concentrate segmentally to form the ganglia of the ventral nerve cord. The neuroblasts also appear in the regions adjacent to the intersegments (figure 1).

In the next phase of development the neuroblasts become segregated from the unmodified ectodermal cells, those near the yolk side of the germ band withdrawing to a more superficial position. By the middle of the second day, the neuroblasts of a ridge have become rearranged to form a single layer internal to the surface layer of ectodermal cells. In a segment each ridge comprises three to four rows of neuroblasts, each five or six cells long (figure 2; and figures 3, 5 and 7, plate 1).

The single layer of ectoderm external to the neuroblasts loses its columnar form and becomes transformed into a cubical epithelium which constitutes the dermatogene layer. The change from columnar to cubical epithelium is probably a mechanical process due to the enlargement of the ridges. This process subjects the superficial ectodermal cells to tension as a consequence of which a change in shape takes place. The developing neuroblasts, adjacent to the dermatogene, constitute the neurogene layer. The dermatogene and neurogene layers first become differentiated at about the 35th hour, when they are closely applied to each other everywhere.

Soon after this spatial alinement of the nervous tissue, between the 35th and 40th hours, the neuroblasts undergo unequal division (i.e. they are teloblasts) and give rise to small daughter cells. These are produced on the inner side of the neuroblasts, that is, on the side away from the dermatogene layer. The neuroblasts now lose their tall, columnar form (figures 5 and 7) and become more compact, generally rounded or oval in shape. The large pale nuclei, measuring about 10 μm in diameter, render the neuroblasts conspicuous. The daughter cells, by contrast, are small and their spherical nuclei (5 to 6 μm in diameter) are surrounded by only a thin investment of cytoplasm. In their general staining properties these cells resemble the ectodermal cells.

By repeated division of the neuroblasts in the same plane, columns of primary ganglion cells are produced. Each column rests upon a neuroblast and projects towards the yolk. Due to the transverse and longitudinal curvature of the neural ridges in each segment

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(figures 3 to 5 and 7) the columns of ganglion cells, at right angles to the body surface, are caused to converge somewhat. The longitudinal curvature of the neural ridges is a direct consequence of the segmentation of the ectoderm, which is inflected towards the yolk intersegmentally. The convergence of the columns of ganglion cells is an indication of the segmental concentration of the nervous tissue in each ridge; from these elements the segmental ganglia are eventually formed.

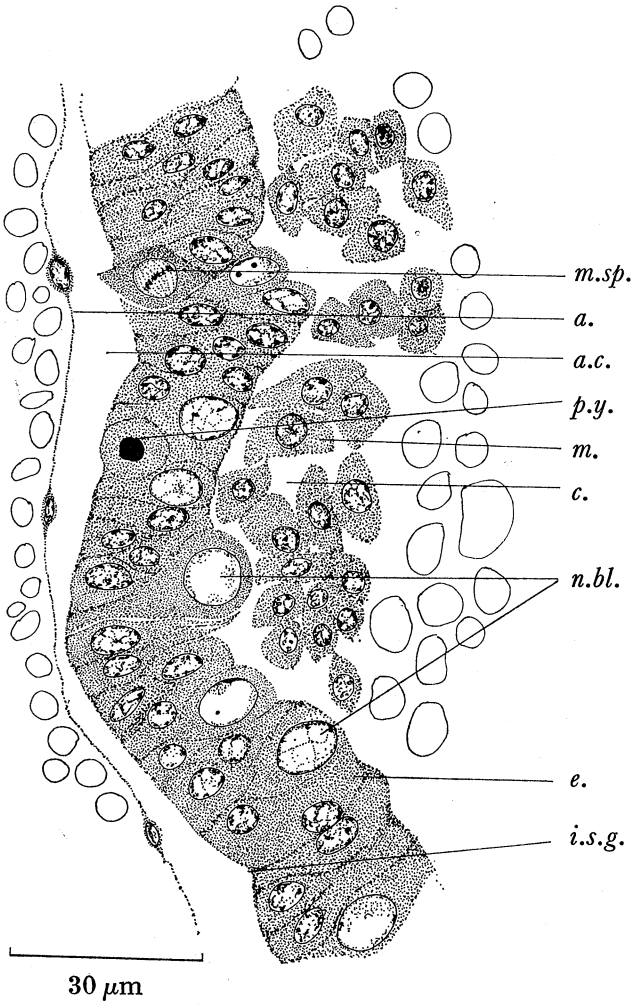

FIGURE 1. Longitudinal section through the developing lateral nerve cord of a 30-h-old embryo. Note the large neuroblasts, which are still in contact with the surface.

By the 40th hour the neural ridges have developed into prominent lateral nerve cords. These are separated by a median strand of cells, three to six cells wide, which forms the floor of the neural groove. Histologically the median cord is at first composed of columnar ectodermal cells, but these subsequently change into small, tightly packed cells with spherical nuclei. Between the 40th and 50th hours the middle cord also becomes neurogenic (figures 4 and 6) and the segmental ganglionic swellings of the lateral cords become well defined (figures 3, 5 and 7). Simultaneously the separation of the dermatogene and neurogene layers begins and the development of the nerve fibres is initiated.

Pycnotic nuclei and 'chromatic droplets' form a conspicuous feature of the developing nervous tissue, and they are most numerous during the third day. These darkly staining,

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strongly basophilic particles are most commonly found where cells are rapidly dividing (figures 4, 7 to 9, plate 1; and all figures in plate 2).

As the number of ganglion cells increases, the neuroblasts giving rise to them become farther thrust away from the yolk and consequently the segmental swellings of the lateral

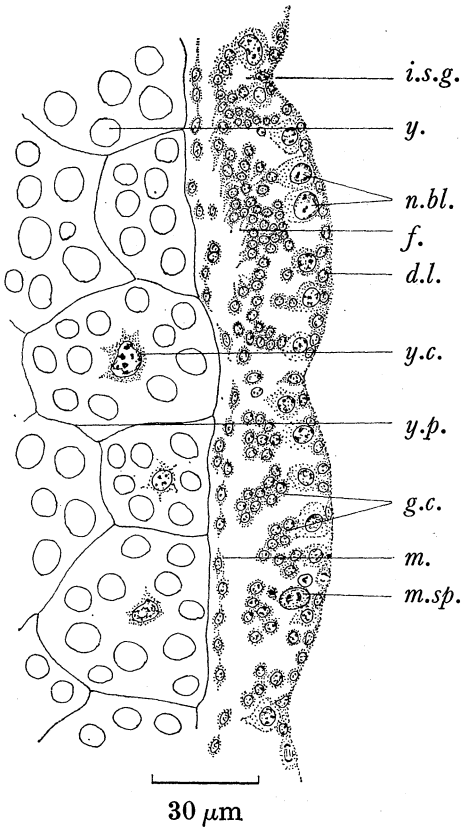

FIGURE 2. Longitudinal section through the lateral nerve cord of a 45-h-old embryo. Note the separation of the dermatogene and neurogene layers and the fibrils beginning to develop from the small ganglion cells.

ridges project farther from the ventral body surface. These paired segmental swellings are the developing ganglia. In *T. molitor* the ganglion cells themselves subsequently undergo division, so that the orderly arrangement of the columns of primary ganglion cells (pre-ganglionic cells of Poulson 1950) gradually becomes disorganized, and two or three rows of cells may surmount each neuroblast (figures 5 and 7).

The median strand is not of the same width throughout its length. In the posterior region of each segment it expands; while in the middle, where the ganglia attain their maximum development, it is correspondingly reduced in width. In transverse section the median strand is seen to be wedge-shaped (figure 4), the broader side being directed towards the yolk: the neuroblasts of the middle cord are localized nearly intersegmentally, i.e. in the broadest region of the cord and resemble the neuroblasts of the lateral cords. Those of the median strand, however, have not been observed to divide. The rest of the median strand cells resemble the ganglion cells.

The median strand is not at this stage covered by a dermatogene layer. During the third day, when the ganglia separate from the dermatogene layer, the latter grows over

the surface of the median strand, and so comes to line the neural groove. Later, when the haemocoel extends between the median strand and the dermatogene layer, the groove becomes obliterated (figure 10, plate 1).

The separation of the dermatogene and neurogene layers begins at about the 43rd hour, in the anterior trunk region. It first occurs on the inner side of the ganglionic swellings, i.e. adjacent to the median strand. The separation of the two layers appears to be largely a mechanical process and, as suggested by Heider (1889), is probably the result of the curvature of the ganglionic mass.

Between the 44th and 50th hours the nerve fibres begin to develop. These arise from cytoplasmic processes which are produced from the first formed, i.e. from the most deeply seated of the ganglion cells. These processes are directed away from the neuroblasts, towards the centre of the ganglion, and they quickly elongate and become filamentous. Owing to the columnar arrangement of the nerve cells, the filaments produced by the apical cells converge in the dorsal parts of the ganglia. With an increase in length of the filaments a mass of intertwined fibrils forming the neuropile ('Punktsubstanz' of German authors) is produced. At first the neuropile is confined to the dorso-median regions of the ganglia and the fibres do not communicate with those of adjacent ganglia.

Description of plate 1

Figure 3. Transverse section through the centre of the mandibular segment of a 43-h-old embryo, showing the developing lateral and median nerve cords. In the lateral nerve cords note the large nuclei of the neuroblasts, which are beginning to migrate below the surface; the conspicuous neural groove, the floor of which is formed by the wedge-shaped median strand and the numerous pycnotic nuclei.

Figure 4. Transverse section through the mandibular-maxillary intersegment of a 43-h-old embryo. Note the inconspicuous neural groove and the greater width of the median strand which, in this region, contains a few neuroblasts.

Figure 5. Longitudinal section through the lateral cord of a 44-h-old embryo in the thoracic region. Note the columns of ganglion cells supported by the large neuroblasts.

Figure 6. Longitudinal section through the median strand of a 44-h-old embryo. Note the neuroblasts in the intersegmental regions.

Figure 7. Longitudinal section through the lateral cord of a 45-h-old embryo in the abdominal region. Note the neuroblasts and the convergence of the columns of ganglion cells. The dermatogene layer covers the neuroblasts externally.

Figure 8. Horizontal section through the abdomen of a 64-h-old embryo above the spiracles, to show the oenocytes. The segmental masses have spread posteriorly, so that the oenocytes now form a strand on either side.

Figure 9. Horizontal section through the stomodaeum and protocerebral lobes of a 44-h-old embryo. Note the invaginating optic lobes and the numerous pycnotic nuclei. The dermatogene and neurogene layers have not yet separated.

Figure 10. Transverse section through the abdominal ganglion of a 94-h-old embryo. Note the segmental nerve, invested by the neurilemma.

Figure 11. Transverse section through the intersegmental region of a 94-h-old embryo. Note the fibrils in the longitudinal connectives, which are surrounded by the neurilemma; and the spacious haemocoel which separates the midgut epithelium from the epidermis.

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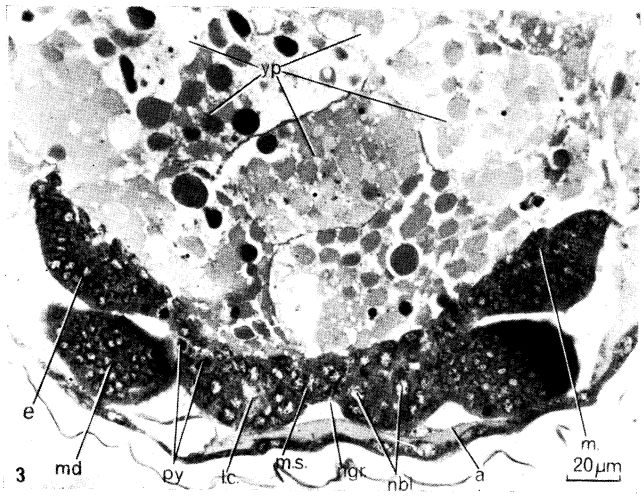

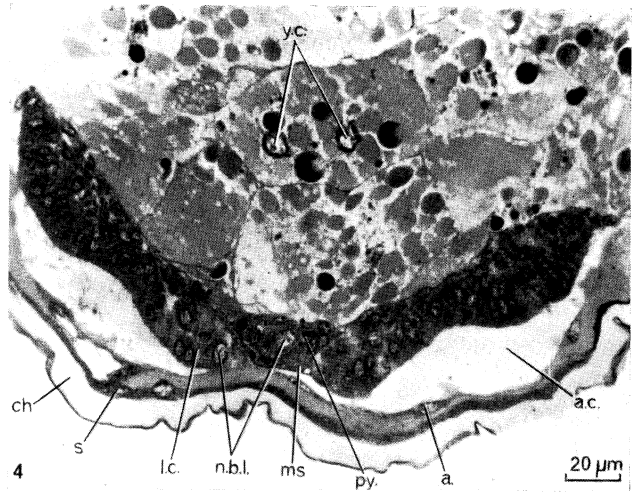

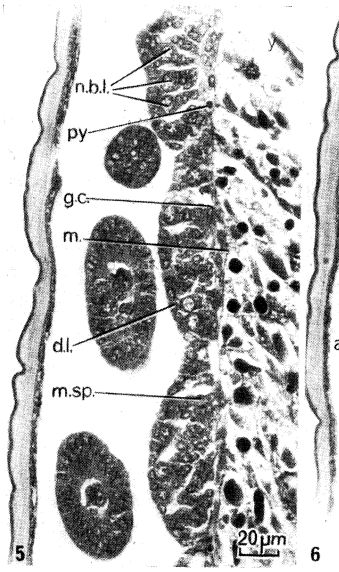

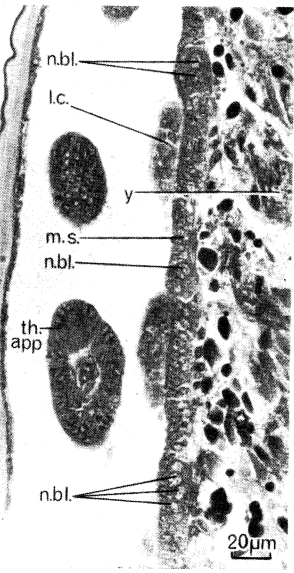

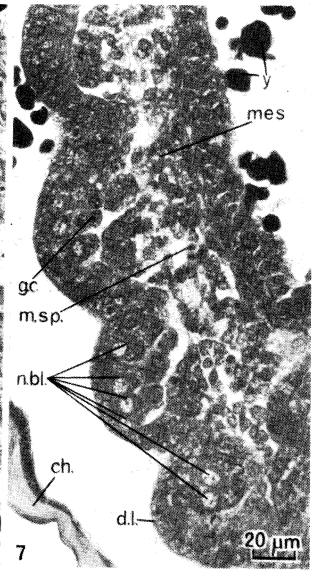

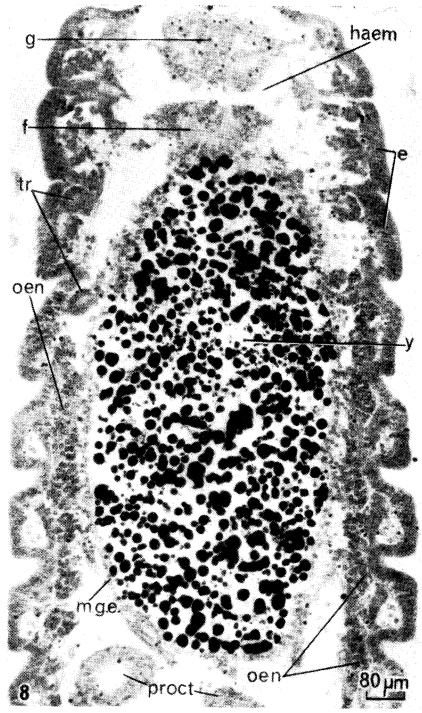

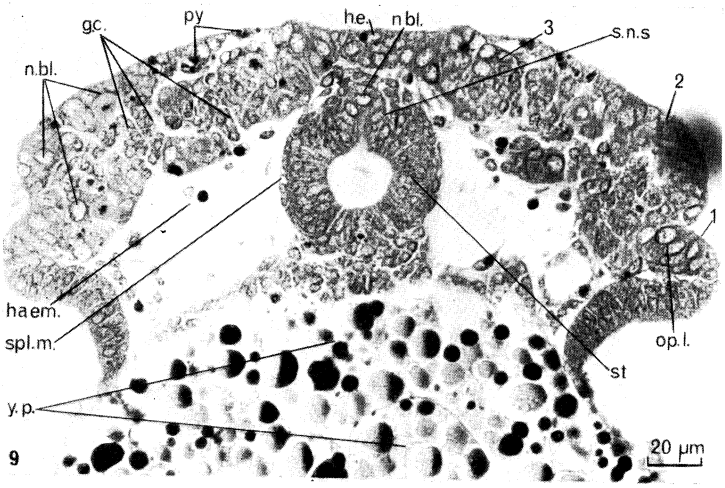

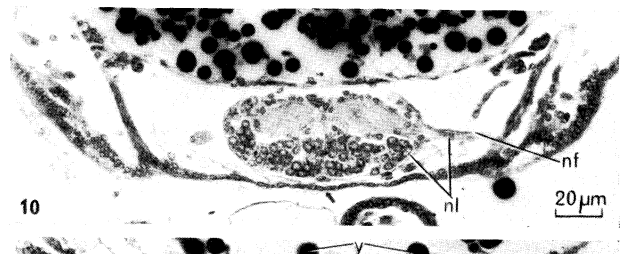

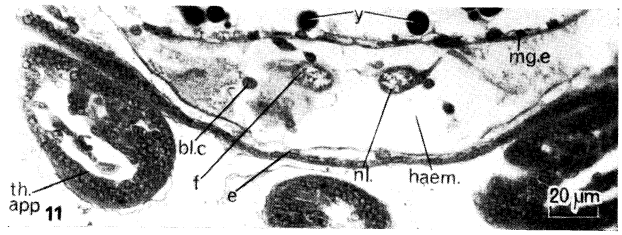

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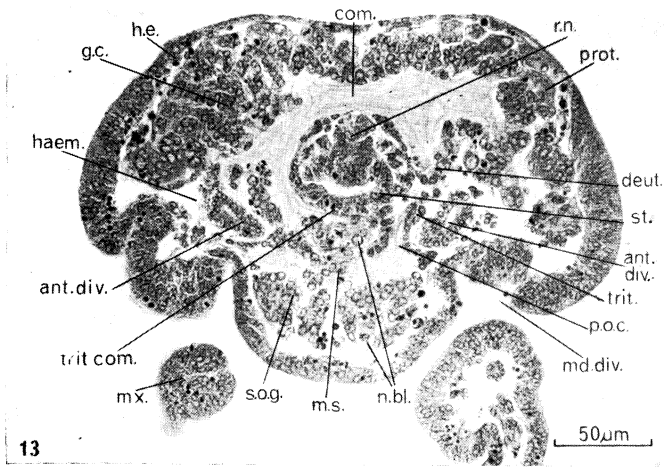

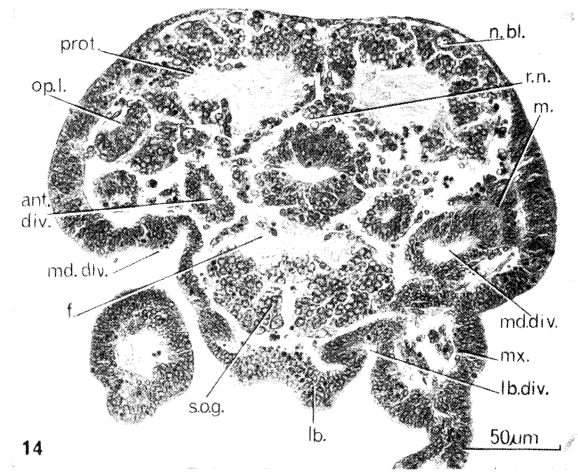

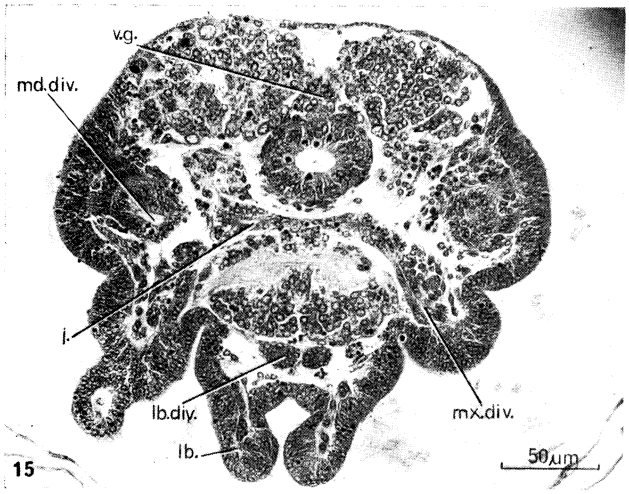

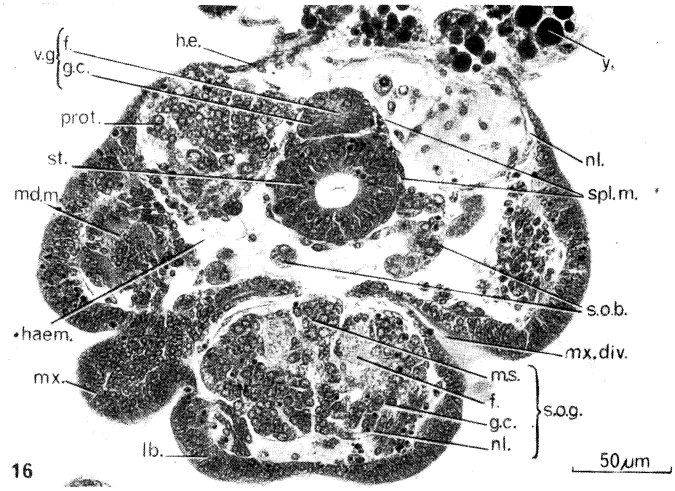

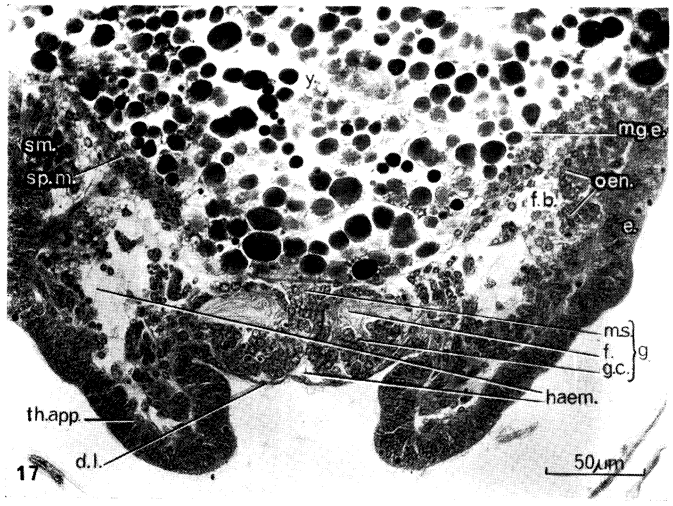

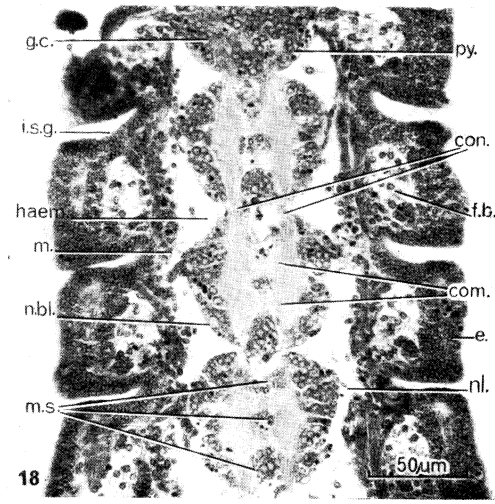

As the ganglia enlarge and the convexity they produce on the body surface increases, the neuropile tends to be pushed more centrally within them. During the third day the filaments in each ganglion grow out longitudinally, in an anterior and posterior direction. As they elongate and intertwine it becomes impossible to trace out individual 'fibrils'. In this manner communication is established between successive ganglia in each lateral cord, and thus the longitudinal fibre tracts of the ventral nerve cord arise.

Between the 55th and 60th hours, soon after the longitudinal connectives have made their appearance, the development of the transverse fibre tracts is initiated. These are the commissures which connect the pairs of segmental ganglia. In each segment two commissures arise, which appear to develop both from the median strand and the lateral cord ganglion cells. The commissures divide the ganglia and median strand into zones—anterior, middle and posterior—as in other insects, and this is clearly seen in horizontal sections (figure 18, plate 2). Thus the ladder-like nerve cord, typical of insects, is developed.

With the formation of the commissures the segmental ganglia increase in size and, as a consequence, the median strand situated between them becomes reduced in width. The ventral portions of the ganglia on either side bulge towards each other below the median strand (figure 17, plate 2). Simultaneously the dermatogene layer separates from the lateral cords (p. 6) and extends medially to form a continuous sheet of cells over the surface of the median strand. Thus the two ganglia of a segment gradually begin to lose their identity as separate structures and eventually fuse together, with the median strand between them (figure 10). The resulting compound structure constitutes the definitive segmental ganglion of the larva.

The nerve cells on the dorso-lateral aspects of the ganglia undergo mitotic divisions and the single layer of cells produced extends over the dorsal part of the ganglia, above the

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Description of plate 2

Figure 13. Transverse section through the mandibular-maxillary intersegment of a 64-h-old embryo (slightly oblique). Note the proto-, deuto- and trito-cerebral lobes; the massive supra-stomodaeal commisure, with fibres from the protocerebral and deutocerebral neuromeres; the commissure connecting the tritocerebral neuromeres below the stomodaeum; the para-oesophageal connectives; the recurrent nerve; the median strand incorporated within the suboesophageal ganglion; the antennary and mandibular diverticula and the numerous pycnotic nuclei.

Figure 14. Transverse section through the maxillary segment of a 64-h-old embryo. Note the invaginated optic lobe which lies below the head ectoderm and still retains a cavity; and the developing labial diverticula between the apposed maxillary and labial appendages.

Figure 15. Transverse section through the labium of a 64-h-old embryo. Note the lateral position of the mandibular apodeme; the maxillary diverticula approximating medially; the labial diverticula and the labial appendages.

Figure 16. Transverse section through the posterior head of a 64-h-old embryo. The large ventricular ganglion lies above the stomodaeum from which it is not, as yet, separated by the splanchnic mesoderm. Note the incomplete dorsal closure; and the numerous pycnotic nuclei.

Figure 17. Transverse section through the prothoracic segment of a 64-h-old embryo, to show the structure of a pair of ventral chain ganglia in the process of fusion. Note the oenocytes.

Figure 18. Horizontal section through the abdominal ganglia of a 64-h-old embryo, showing the ladder-like structure. The pycnotic nuclei are numerous.

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neuropile. The innermost cells of the median strand in the segmental regions also appear to contribute to the dorsal portions of the ganglia (figures 10, 13 to 16, and 17, plates 1 and 2). The median strand neuroblasts form the posterior median portions of the fused ganglia, becoming withdrawn from the intersegmental regions and attached to the ganglia in the preceding segment.

At the same time as the commissures are developing, a pair of lateral segmental nerves grow out from each fused pair of ganglia (figure 10).

Between the 55th and 65th hours, as the ventral portions of the ganglia extend between the median strand and the dermatogene layer, the neural groove becomes obliterated. Simultaneously the dermatogene layer changes from a cubical to a pavement epithelium. The neurogene and dermatogene layers of the lateral cords become completely separated as a haemocoelic space extends between them, and the ventral nervous system no longer protrudes from the body surface, as it did formerly (compare figures 3, plate 1 and 17, plate 2).

The nervous system of insects is invested by a nucleated sheath, the neurilemma. In *T. molitor* it is initiated in the middle of the third day by the differentiation of certain of the peripheral ganglion cells which lose their spherical form and become flattened. Eventually an exceedingly delicate sheath is produced which in many cases is only detectable by its sparsely distributed nuclei (figures 10, plate 1 and 16 and 18, plate 2). The longitudinal connectives consist of a central core of fibres surrounded by a single layer of cells, the whole being invested by the neurilemma (figure 11, plate 1).

An inner neurilemma is also developed between the nerve cells and the neuropile by the ganglion cells adjacent to the fibres, forming a sheath around them. The cells of this sheath become only very slightly flattened. Such a condition has been described for *Agelastica alni* by Mazur (1960).

With the production of the required number of ganglion cells, the neuroblasts begin to disappear. They diminish in number throughout the third day, at the end of which they are rarely found. The neuroblasts in all probability disintegrate and also contribute to the formation of the numerous 'chromatic droplets' (p. 4) which are such a conspicuous feature at this stage (figure 8, plate 1; and figures 13 to 18, plate 2).

During the third and fourth days the three pairs of gnathal ganglia fuse to form the large suboesophageal ganglion (figures 13 to 16). At the end of the third day the suboesophageal ganglion is still trilobed, thus betraying its compound origin. The ganglia of the ninth and tenth abdominal segments also unite simultaneously with the fusion of these segments. This compound terminal ganglion, situated in the ninth abdominal segment, is thus larger than those preceding it.

By the fourth day the major feature of the ventral nervous system have been established and during the following days, until eclosion on the sixth day, only minor histological changes occur.

As the haemocoel develops, it surrounds the ventral nerve cord. The fat body, lying in the ventro-lateral region of the body, gradually extends medially and insinuates itself between the ganglia and the dermatogene layer, the latter now forming the ectodermal body wall in the sternal region. By the fifth day the ganglia form compact oval structures, smooth in outline. The slight median dent, formerly present, has disappeared so that there is no obvious indication of the paired origin of each segmental 'ganglion'.

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(*b*) *The brain*

The cerebral ganglion or brain, as in other insects, is a composite structure formed by the fusion of the first three pairs of neuromeres. These are the protocerebral, deutocerebral and tritocerebral neuromeres which belong respectively to the embryonic labral or protocephalic, antennary and intercalary segments. The tripartite origin of the cerebral ganglion is indicated in the larva by the three lobes of the brain, the protocerebrum, the deutocerebrum and the tritocerebrum.

The three pairs of brain ganglia arise from the protocephalon, the first two being preoral, the third postoral in origin. The protocephalic neuromeres arise more or less simultaneously with the neuromeres of the lateral cords. The processes of neuroblast and ganglion cell formation are similar to those in the lateral nerve cords (pp. 3 and 4). The greater portion of the protocephalic ectoderm gives rise to the protocerebral neuromeres, each of which is trilobed. Antero-medially they are separated by the labral rudiments. The deutocerebral neuromeres are separated by the stomodaeal aperture (figure 12).

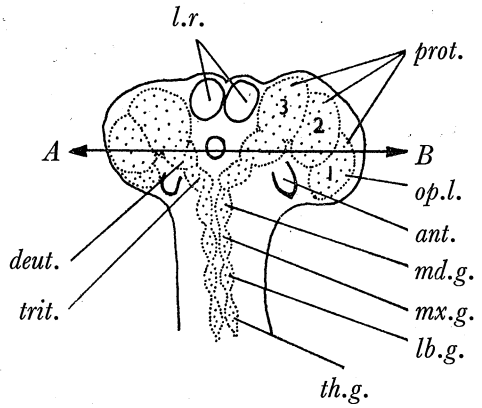

FIGURE 12. Reconstruction of the protocerebrum of a 2-day-old embryo, to illustrate the relationships of the cephalic ganglia. Note the trilobed protocerebral ganglia; the postero-dorsal orientation of the optic lobes. A section passing through *AB* would transect the 1st and 2nd protocerebral lobes, the deutocerebral neuromeres and the stomodaeum simultaneously.

The first or optic lobes of the protocerebrum do not produce neuroblasts, as do the second and third lobes, but invaginate to form the optic ganglia. The second and third lobes are indistinctly demarcated from each other, as seen from figure 9, plate 1.

The protocephalic lobes represent the widest region of the germ band (Ullmann 1964, p. 252) and even before segmentation begins, the sides of the protocephalon are dorsally inclined. This tendency for dorsal growth continues throughout the second day. Between the 40th and 45th hours, due to katatrepsis, the germ band migrates forward so that the protocephalon comes to be situated at the anterior end of the egg. Thus, as the protocephalic lobes grow dorsally and approach each other medially, the protocerebral rudiments are folded in towards the cephalic haemocoel (Ullmann 1964, figures 13, 14, 18 and 22 to 24).

The oval neuroblast nuclei are about twice the size of those of the ectoderm and measure approximately 12 μm in length. When the neuroblasts divide, the axes of the spindles are

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always at right angles to the body surface. Thus columns of ganglion cells, as in the lateral cords, are produced (figure 9, plate 1). Subsequent division of the ganglion cells then causes the disorganization of the orderly arrangement of these columns. Due to the 'folding in' of the protocephalic lobes, these columns of cells increasingly converge towards each other.

The invagination of the optic lobes takes place simultaneously with the formation of the protocephalic dorsal closure, between the 40th and 50th hours. The optic lobe anlagen are composed of large pale cells which resemble the neuroblasts of the second and third protocerebral lobes. As, however, they do not produce ganglion cells, they are not true neuroblasts (figure 9). At the beginning of invagination, a narrow dorso-ventrally running

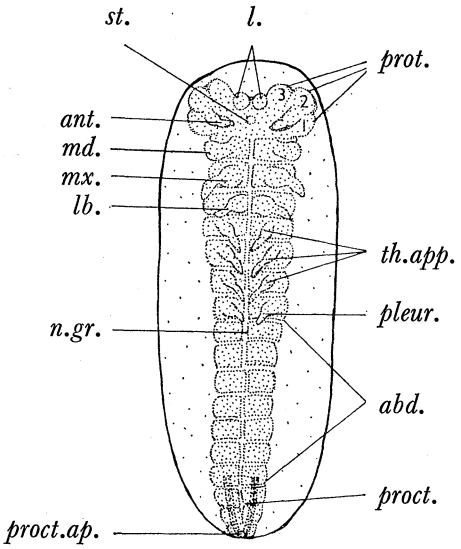

FIGURE 21. External features of a 48-h-old embryo. The appendange rudiments are undergoing differentiation and the antennae are in the process of forward migration; the gnathal appendages still point laterally. Note the tripartite division of the head lobes, indicating the protocerebral neuromeres; and the conspicuous labral rudiments above the stomodaeal aperture.

cleft appears in the optic region. As it deepens, the optic lobe anlage is pushed into the cephalic haemocoel, the invagination being completed between the 50th and 55th hours.

The deutocerebral ganglia arise from the ectoderm lateral to the stomodaeal aperture. Each deutocerebral neuromere is continuous anteriorly with the third protocerebral lobe, from which it is but indistinctly demarcated. Posteriorly, each merges into the tritocerebral neuromere of its own side. When they arise, between the 28th and 35th hours, the deutocerebral neuromeres are situated just in front of the antennary rudiments which, at this time, are still postoral in position (figures 12 and 21).

When the tritocerebral neuromeres first become distinguishable behind the stomodaeal aperture, they lie medially to the antennary bases. Since the antennary rudiments grow out from the postero-lateral aspects of the protocephalic lobes (Ullmann 1964, figure 1) the position of the tritocerebral neuromeres at their bases indicates that the intercalary segment is an integral part of the protocephalon. The tritocerebral neuromeres resemble those of the lateral nerve cords; the median strand, however, is broader in the intercalary segment.

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The ganglion cells of the cerebral neuromeres develop nerve fibre anlagen between the 44th and 50th hours and the process is similar to that described for the lateral cord neuromeres on p. 6. No fibres arise from the optic lobes. The turning-in of the head lobes, which precedes the protocephalic dorsal closure, causes the neuropile (produced by the innermost of the ganglion cells) to acquire a more central position within the neuromere.

Although at the end of the second day neurogene and dermatogene elements are distinguishable in the cerebral neuromeres, these are not yet arranged in separate layers. Groups of neuroblasts abut on to the ventral surface of the germ band, the smaller undifferentiated ectodermal cells scattered between them indicating the rudiments of the dermatogene layer (figure 9). During the third day the neurogene and dermatogene layers separate and the ganglion cells grow over the neuropile. The latter is eventually surrounded by several layers of ganglion cells (figures 13 to 16, plate 2).

The protocerebral lobes on each side extend postero-dorsally in such a way that the optic lobes become the most posteriorly orientated (figure 12). Thus by the 45th hour the optic ganglia are seen in transverse section to be situated at the level of the anterior end of the mandibular segment.

The dorsal closure, at the anterior end, results in the median approximation of the protocerebral lobes above the stomodaeum (figures 13 to 16). With the development of the suprastomodaeal commissures (see below), the first pair of neuromeres fuse to form the protocerebrum.

Following the separation of the neurogene and dermatogene layers, the deutocerebral neuromeres move dorso-laterally on either side of the stomodaeum and gradually fuse with the protocerebral rudiments. The smaller tritocerebral rudiments also assume a more lateral position to the stomodaeum and partially fuse with the deutocerebral rudiments. The brain ganglia thus form an 'arch' over the stomodaeum (figure 13).

Simultaneously with the initiation of the process of fusion of the cerebral ganglia, commissures and connectives are formed between them. The nerve fibres, hitherto confined to the inner portions of the neuromeres, grow out towards each other. A thick mass of intertwined fibres develops which communicates between the neuropiles of the protocerebral neuromeres, giving rise to the massive suprastomodaeal commissure (figure 13).

At the same time the fibres from the three pairs of fusing cerebral neuromeres grow out towards each other longitudinally and thus the neuropiles of successive ganglia become connected. Fibres also connect the tritocerebral with the mandibular ganglia and in this manner two longitudinal fibre tracts are developed which are continuous from the protocerebral to the terminal abdominal ganglia.

Some fibres from the deutocerebral grow anteriorly into the protocerebral neuromeres; others extend medially, towards each other, immediately above the stomodaeum. These fibres give rise to the preorally situated deutocerebral commissure; the latter increases in size during development and eventually approximates to and fuses with that of the protocerebrum. The suprastomodaeal commissure is thus a composite structure formed by fibres derived from the first and second protocerebral lobes and the deutocerebral commissure.

The tritocerebral ganglia are joined below the stomodaeum by the suboesophageal commissure, formed by fibres from the ganglion cells. The median strand also appears to make a contribution to this commissure (figure 13).

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During the latter half of the third day the neurilemma of the brain ganglia makes its appearance. Its development is similar to that surrounding the ventral nerve cord, being the product of flattened peripheral ganglion cells which form a thin investing layer. The brain is now completely delimited from the ectoderm, but lies close below it. The cerebral ganglia become completely fused, the tritocerebrum forming small lateral lobes on either side of the stomodaeal aperture. The contours of the brain are now smooth and rounded.

In the protocerebrum large spherical cells with granular cytoplasm and large spherical vesicular nuclei are scattered around the periphery. These cells, which persist throughout the embryonic period, measure about 20 μm in diameter. Occasionally they are also found in the deutocerebrum. They are most probably neurosecretory and not persistent neuroblasts, as the latter disappear from the nervous system when the definitive number of ganglion cells has been produced, during the course of the fourth day (p. 8).

No further changes in the brain have been observed until eclosion between the 125th and 130th hours.

(*c*) *The stomodaeal nervous system*

The development of this system in *T. molitor* occurs towards the end of the second day, at a time when the labral mesoderm begins to spread over the roof of the stomodaeum (Ullmann 1964, p. 261). Before this mesoderm becomes organized into the splanchnic sheath, three small, median ectodermal invaginations make their appearance in the roof of the short stomodaeal invagination.

At these points the stomodaeum is sharply indented on the side adjacent to the lumen. Some of the cells bordering these invaginations enlarge and their nuclei become somewhat paler and spherical in the process. Thus, in general appearance, they come to resemble the neuroblasts of the central nervous system (p. 3). Unlike the latter, however, these cells are not teloblasts but divide by equal divisions. Tiegs & Murray (1938) describe a similar condition in *Calandra oryzae*.

The anterior invagination gives rise to the frontal ganglion. In its definitive position the latter is situated above the stomodaeal musculature, in front of the brain (figure 20 *B*, plate 3). The second invagination gives rise to a very ill-defined swelling which appears to be homologous to the hypocerebral ganglion of *C. oryzae*. The latter is stated to be an inconspicuous swelling on the short 'recurrent' nerve. From the posterior invagination the ventricular ganglion, situated behind the brain, originates (figures 13 to 16, plate 2; 19 and 20 *B*, plate 3).

While the frontal and hypocerebral invaginations are short-lived and disappear at the end of the second day, the ventricular persists throughout the third day and cells continue to proliferate from this region until about the 65th hour. The cell groups derived from the anterior two invaginations are indistinctly demarcated from each other, and soon fuse to form an elongate mass (figures 19 and 20, plate 3).

From the frontal invagination cells extend forwards, along the roof of the stomodaeum, towards the labrum. At the same time the labral mesoderm is extending posteriorly, so that in this region ectodermal and mesodermal cells are migrating in opposite directions (figures 19 *A*, *B*).

Cells from the ventricular invagination also extend forwards and merge with the anterior

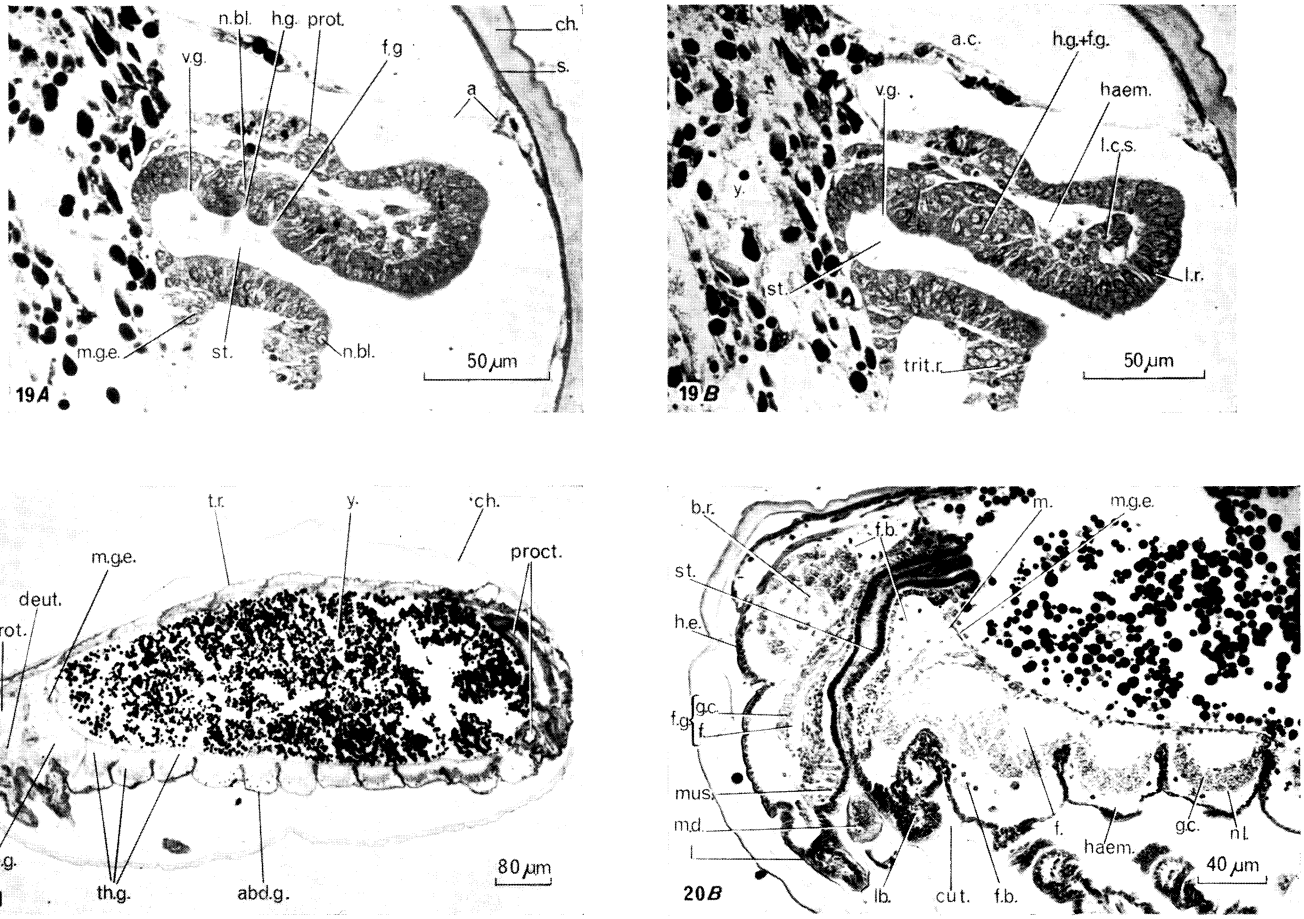

FIGURE 19. Serial longitudinal section through the stomodaeum and labral rudiments of a 45-h-old embryo, to show the origin of the stomodaeal nervous system. *A*. Invagination of the frontal, hypocerebral (occipital) and ventricular rudiments from the roof of the stomodaeum. Note the large neuroblasts. *B*. Fused mass of the frontal and hypocerebral rudiments.

FIGURE 20. Serial longitudinal sections through a 5-day-old embryo, nearing eclosion. *A*. General appearance of the central nervous system, showing the brain and the ventral chain of ganglia. *B*. Enlarged head region, to show the frontal ganglion and the recurrent nerve above the stomodaeum. Note also the neurilemma and the embryonic cuticle.

(*Facing p.* 12)

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clump of nerve cells. Thus, early on the third day, the stomodaeal nerve rudiment is represented by a discrete and elongate cell mass above the stomodaeum.

As the stomodaeum increases in length and its distal end is carried farther posteriorly, the nerve rudiment becomes drawn out longitudinally along the dorsal surface of the organ. A slight swelling anteriorly marks the position of the frontal ganglion while at the posterior end of the strand the ventricular ganglion forms a conspicuous mass (figure 16). The nerve cells connecting these two ganglia form the stomatogastric or 'recurrent' nerve.

Transverse sections through the stomatogastric nerve and the ventricular ganglion during the third day show the former to be circular, the latter oval in outline (figures 13 to 16). The nerve is composed of about six or eight cells, with spherical nuclei surrounded by a thin investment of cytoplasm; they thus resemble the ganglion cells of the central nervous system (p. 3). The large, transversely oval ventricular ganglion is composed of similar cells.

Between the 50th and 60th hours the neuropile in the stomodaeal system develops, in a manner similar to that of the central nervous system (p. 6). This development is best observed in vertical longitudinal and horizontal sections through the system. From the frontal ganglion a pair of lateral nerves runs towards the brain.

Between the 55th and 60th hours the system becomes separated from the underlying stomodaeal wall, except at the posterior end, where this is delayed until the end of the third day. As the system becomes severed from the stomodaeum the splanchnic mesodermal cells insinuate themselves between the two structures and thus the splanchnic sheath around the stomodaeum is gradually completed. By the fourth day the rudiment of the ventricular ganglion has also become severed from the stomodaeal wall.

The neurilemma investing the stomodaeal system develops in a manner similar to that described on p. 8.

(2) *Ectodermal derivatives*

(*a*) *In the head*

During the third day, ectodermal invaginations develop in association with the antennae and gnathal appendages. Some of these give rise to the cephalic endoskeleton; others to gland-like tubules. These invaginations retain the ectodermal property of cuticle secretion. In the apodemes (skeletal struts), the cuticularization of the inner walls of the diverticula eventually obliterates their lumina. The hardened struts which remain act as surfaces of attachment for the cephalic muscles. The inner surfaces of the glandular invaginations likewise secrete a cuticle, but this is very delicate (Srivastava 1959).

(i) *The tentorium*

The tentorium is the endoskeleton of the head. In *T. molitor* it arises during the third day from the fusion of two pairs of apodemes. The anterior arms of the tentorium arise from a pair of diverticula which invaginates behind and lateral to the antennary rudiments. These antennary diverticula extend medially and then take a horizontal posterior course, ventro-lateral to the stomodaeum (figures 13 and 14, plate 2).

The posterior arms of the tentorium arise from invaginations postero-lateral to the maxillary bases. The diverticula extend horizontally and medially, above the suboesophageal ganglion and below the stomodaeum. When the diverticula meet medially, their

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distal ends fuse, so that an ectodermal 'bridge' is formed at the back of the head. The antennary diverticula grow back on either side and by the 65th hour have fused with the posterior tentorial bridge (figure 15).

(ii) *The apodemes*

The mandibular flexor apodemes. The apodemes of the large mandibular flexor muscles invaginate postero-laterally to the mandibular bases (figures 13 and 14). In their definitive position, however, they are located on the median aspect of the mandibles; taking a diagonal, postero-lateral course, they terminate blindly in the dorso-lateral region of the

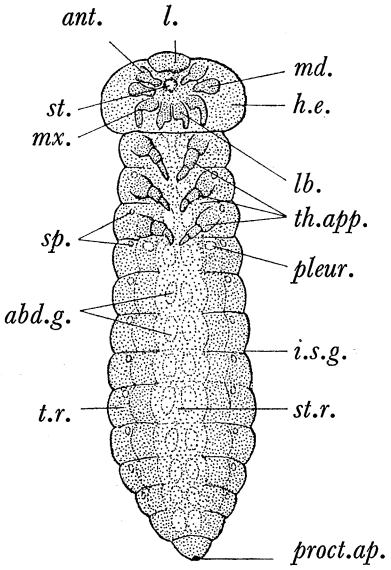

FIGURE 22. Ventral view of a 66-h-old embryo. Head and trunk are now distinctly demarcated; the labral rudiments have undergone fusion and the gnathal segments cephalization. The head appendages are now radially arranged around the stomodaeal aperture. Note the orientation of the gnathal appendages; the spiracles and the segmental ganglia.

labial segment, just below the level of the brain (figures 14 and 15). These diverticula are the largest in the head and measure approximately 60 μm in diameter.

The change in the relative position of apodeme and mandible is due to the rotation of the latter during development. The mandibles, which arise in the second day, are flattened, platelike structures at this time (figure 21, p. 10). During the third day, when cephalization is in progress, the mandibles migrate forwards from their postoral point of origin and, as they do so, rotate through 180° in a postero-median direction. This rotation may be followed in serial transverse sections of the mandibles, using the flexor mandibularis apodeme as an indicator, as has been done for *Hydrophilus piceus* by Heider (1889). With the conclusion of migration and rotation, the mandibles come to lie on either side of the mouth, towards which their distal ends point (figure 22).

Cuticularization of the apodemes occurs late on the third day. During the fourth day the distal ends of the mandibular flexor apodemes become forked. As the major portion of these apodemes lies in the maxillary segment it is very probable that the maxillary mesoderm contributes substantially to the mandibular flexor muscles, as it does in *Silpha obscura* (Smreczyński 1932).

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The great flexor muscles occupy a large part of the head. By the middle of the third day, mesodermal masses are seen associated with the distal ends of the apodemes. By the end of the third day the mesoderm has divided into four groups of myoblasts, the cells of which have elongate nuclei. The muscle blocks originate on the lateral wall of the head in the maxillary segment, covering a substantial surface area; from here they converge, to be inserted on the mandibular apodeme or tendon.

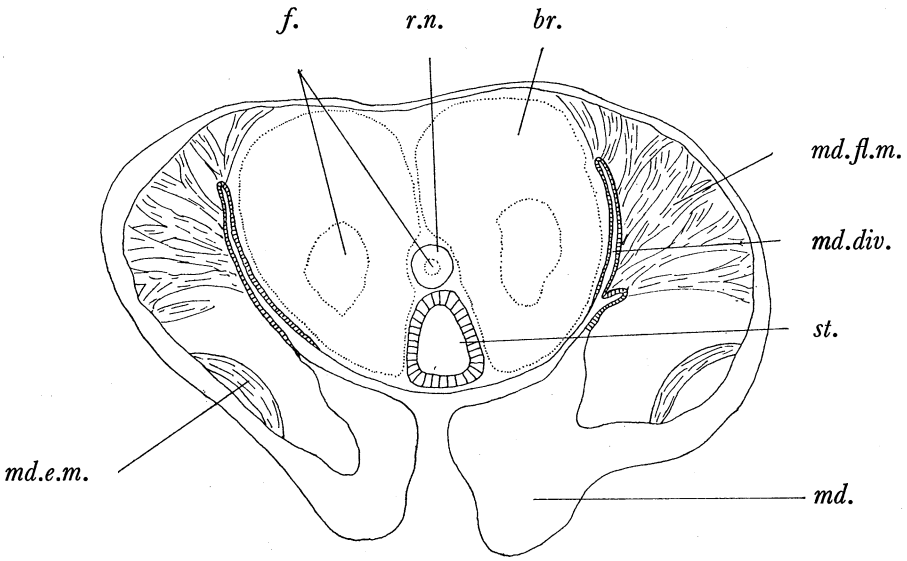

FIGURE 23. Transverse section through the mandibular segment of a 4-day-old embryo, to show the mandibular flexor apodemes and muscles.

The mandibular extensor apodemes. These arise early in the fourth day as short postero-dorsally directed invaginations of the epidermis, one lateral to each mandible, at a time when the latter has already undergone rotation towards the midline. The myotomes of the mandibular extensor muscles are attached at one end to the distal end of this apodeme and at the other to the lateral wall of the head, ventral to the mandibular flexor muscle (figure 23).

(iii) *The labial diverticula*

By the 60th hour the labial appendages have approximated in the midline and have migrated forwards, so that they come to be insinuated between the bases of the maxillae (figure 22). Simultaneously with the development of the apodemes a pair of invaginations, which originate probably in the labial segment, appear between the maxillary and labial rudiments, i.e. medially to the maxillary and laterally to the labial appendages. The invaginations approach each other horizontally, penetrating the basal region of the labium (figure 14, plate 2) until they almost touch; they then bend abruptly backwards and take a posterior course. These diverticula measure approximately 15 μm in diameter and run parallel to each other below the suboesophageal ganglion, as far as the posterior region of the labial segment. Histologically they are composed of a single layer of small cubical cells with spherical nuclei (figure 15, plate 2).

During the course of the fourth day the diverticula continue to elongate, taking a shallow postero-dorsal course. They thus pass between the suboesophageal and prothoracic ganglia which are so close together, owing to the telescoping of the head into the

prothorax, that the longitudinal connectives between them do not appear to exist. In the larva these diverticula terminate in the region of the prothoracic spiracles.

(*b*) *In the trunk*

(i) *The tracheal system*

Early on the third day the trachea arise as simple ectodermal invaginations of the body wall in the mesothorax, metathorax and the first eight abdominal segments. The spiracles are situated antero-lateral to the appendage rudiments in the limb-bearing segments; and in a corresponding position in the limbless abdominal segments. In the latter, i.e. posterior to the pleuropodia which are the modified appendages of the first abdominal segment, the spiracles open onto ventro-lateral prominences of the body wall.

By the middle of the third day the tracheae are T-shaped diverticula. During the fourth day the tracheal branches grow out from each rudiment; one anteriorly, one posteriorly, one dorso-laterally and one medio-ventrally, towards the nerve cord. The anterior and posterior diverticula of successive segments eventually join up and give rise to the longitudinal tracheal trunks of the larva. The dorso-lateral diverticulum overlies a group of proliferating oenocytes, above the spiracles (see below). The ectodermal cells of the trachea secrete a thin cuticle during the fourth day.

(ii) *The oenocytes*

The oenocytes in *T. molitor* were first observed at about the 60th hour. Four or five rows of ectodermal cells, located immediately above the spiracles and below the lateral myoblast plate, enlarge and from them columns of similar cells project into the haemocoel. Their histology renders them conspicuous: their nuclei enlarge and become spherical, the amount of cytoplasm increases and the cells become polyhedral, the homogenous cytoplasm staining deeply with light green or eosin (figures 8 and 17, plates 1 and 2).

The oenocytes form rounded masses in the first eight abdominal segments and for a time maintain contact with the ectoderm. During the latter part of the third day they extend posteriorly from their point of origin and form elongate segmental masses (figure 8, plate 1). By the 70th hour the oenocytes have completely separated from the ectoderm and come to be wedged between the midgut medially, the somatic mesoderm laterally and the fat body ventrally.

The oenocytes retain the same relative position throughout development. As, however, the lateral edges of the body wall grow up around the yolk the oenocytes, too, are carried dorsad. Thus at eclosion they are situated laterally in the body.

The oenocytes persist throughout larval development and metamorphosis, and Roth (1942) has drawn attention to their large size in the adult.

Discussion

(1) *The nervous system*

(*a*) *The ventral nerve cord*

The development of the nervous system in *Tenebrio molitor* conforms in essential features to that of many insects. Preganglionic cells divide to give rise to the definitive ganglion cells as in *Calandra oryzae* (Tiegs & Murray 1938), *Doryphora decemlineata* (Wheeler 1889),

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Apis mellifera (Nelson 1915), *Drosophila melanogaster* (Poulson 1950), *Pyrilla perpusilla* (Sander 1956), and *Agelastica alni* (Mazur 1960). In yet other insects subsequent division of the daughter cells does not occur. The latter include *Forficula auricularia* (Heymons 1895), *Lucilia* (Escherich 1902), *Eutermes* (Strindberg 1913), *Pieris rapae* (Eastham 1930), *Locusta migratoria* (Roonwal 1937), *Pteronidea ribesii* (Shafiq 1954) and *Chilo suppressalis* (Okada 1960).

These two methods of ganglion cell formation do not appear to have phylogenetic importance, although cytogenetic and ultrastructural studies may subsequently reveal the significance of this variation in development.

Whereas the development of the lateral nerve cords varies little, the fate of the median strand differs in different insects. In *Lucilia* Escherich (1902) describes a median nerve with paired intersegmental extensions derived from the median strand cells. Poulson's (1950) work on *Drosophila*, however, throws doubt on Escherich's interpretations; here the median strand cells spread over the dorsal parts of the ganglia and eventually give rise to the neurilemma of the ventral nerve cord and segmental nerves. The suggestion (Johannsen & Butt 1941; Mazur 1960) that the condition as described for *Lucilia* is primitive would thus appear to be erroneous. It seems more likely that primitive features would occur in the phylogenetically older orders than in such highly specialized insects as the Diptera Cyclorrapha.

The intra-ganglionic portion of the median strand contributes to the definitive ganglia in *Tenebrio* as in the majority of insects investigated (Johannsen & Butt 1941). It has not, however, been possible to distinguish between the ganglion cells originating in the lateral cords and those from the median strand. In *Tenebrio* some of these median strand cells spread over the dorsal surface of the ganglia and contribute to the neurilemma, as in *Xiphidium* (Wheeler 1893) *Corynodes* (Paterson 1935) and *Drosophila* (Poulson 1950).

The fate of the inter-segmental portion of the median strand is more variable. In some cases a single neuroblast develops, as in *Xiphidium* (Wheeler 1893) and *Locusta* (Roonwal 1937); while in others several may occur, as in *Forficula* (Heymons 1895) *Calandra* (Tiegs & Murray 1938) and *Tenebrio*. These neuroblasts may contribute to the definitive ganglia, as in *Calandra* and *Tenebrio*; or take no part in their formation, as in *Pieris* (Eastham 1930) and *Calandra callosa* (Wray 1937). Or the strand may degenerate altogether and take no part in the development of the nervous system, as in *Malanoplus differentialis* (Baden 1938). The significance of these differences in the development of the median strand is obscure. It is evident, however, that they do not constitute an evolutionary series, and there does not appear to be any other obvious explanation for them.

Similarly, the origin of the neurilemma is variable. It may arise from the median strand cells, as already mentioned (e.g. *Xiphidium*, *Corynodes* and *Drosophila*); or from flattened ganglion cells, as in *Formica*, *Chrysomela*, *Eutermes* (Strindberg 1913), *Pieris* (Eastham 1930), *Locusta* (Roonwal 1937) and *Agelastica* (Mazur 1960). Or the neurilemma may have a dual origin, as in *Calandra* (Tiegs & Murray 1938) and *Tenebrio*. Here again, no evolutionary series is discernible and in my opinion the origin of the neurilemma does not appear to be a matter of any phylogenetic significance.

In *Tenebrio*, as in the majority of insects investigated, the ectoderm—and consequently the neuroblasts—becomes segmented later than the mesoderm. Thus the latter is the first region to manifest segmentation in the embryo. This fact should be borne in mind when discussing the relative reliability of the nervous system and somites as segment indicators.

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(*b*) *The brain*

The development of the brain in *Tenebrio* is similar to that of other Coleoptera (Tiegs & Murray 1938; Mazur 1960). In these, as in the Hymenoptera (Nelson 1915), the optic lobes form by invagination and not by delamination as in the Orthoptera (Viallanes 1891; Roonwal 1937), Dermaptera (Heymons 1895), Isoptera (Strindberg 1913) and Hemiptera (Sander 1956). In *Tenebrio*, as in the great majority of insects investigated, the cells of the optic lobes have not been observed to give rise to ganglion cells as do the second and third protocerebral lobes. The invaginated optic lobes soon fuse with the posterior portion of the second lobes, and their cells become indistinguishable from neighbouring cells.

The trilobed protocerebral ganglia appear to be a constant feature of insect brains, and homologous structures have been described also in the 'myriapods' *Scolopendra* (Heymons 1901), *Hanseniella* (Tiegs 1940) and *Pauropus* (Tiegs 1947). As has been shown in *Tenebrio*, these ganglia differ in structure and orientation from the succeeding ones. Heymons (1901), from his study of *Scolopendra*, concluded that the protocerebral ganglia are, in fact, presegmental, developing in association with the archicerebrum. The implications of these observations are discussed below.

In a number of insects the suprastomodaeal commissure is said to arise from the epidermis between the protocerebral ganglia, e.g. *Forficula* (Heymons 1895), *Apis* (Nelson 1915), *Pieris* (Eastham 1930) and the beetles *Hydrophilus* (Heider 1889) and *Corynodes* (Paterson 1935), but this is not the case in *Tenebrio* or *Calandra* (Tiegs & Murray 1938). The possible significance of this is discussed by Heymons (1901), who suggested that the participation of the mid-dorsal protocephalic ectoderm in the formation of this commissure in insects may be homologized with the archicerebrum of annelids and *Scolopendra*. Tiegs, however, was unable to demonstrate an archicerebrum in either *Hanseniella* or *Pauropus* though he was able to confirm Heymons's contention that the protocerebral ganglia are not 'members of the ventral series, for despite their great size, they do not develop in association with ventral organs'. More recently Anderson (1959), working on the annelid *Scolops armiger*, has concluded that in the polychaetes 'the head has evolved its own specializations, and cannot be directly compared with the onychophoran-arthropod condition'.

It appears then, that at present it is difficult to homologize the arthropod with the annelid brain because development in the two phyla is so different and the ancestry of the Arthropoda still so obscure (Tiegs & Manton 1958). Moreover, the possibility that such similarities as do occur have arisen as a result of parallel evolution of the two groups cannot be precluded. On the other hand, a comparison between the brains of insects and myriapods seems more justifiable, in view of their closer phylogenetic relationships.

In the 'myriapods' mentioned above a pair of diminutive pre-antennary ganglia occur which represent the first of the ventral segmental series. In *Hanseniella* (Tiegs 1940) the pre-antennary ganglia move from their place of origin and merge into the frontal lobes of the protocerebrum during development. Though pre-antennary ganglia are not known in insects, the condition in *Scolopendra* and *Hanseniella* gives reason to suspect that the insect protocerebrum is, in fact, a composite structure incorporating presegmental elements and the suppressed pre-antennary ganglia. In structures other than the nervous system, there is convincing evidence for a pre-antennary segment in a number of insects; for example, in

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Tenebrio it has been shown that well-developed (labral) coelomic sacs occur in front of the antennary sacs (Ullmann 1964).

It is thus evident that the subject of insect brain development is intimately bound up with the difficult subject of head segmentation. This topic has been discussed for over three quarters of a century, and has been approached from both the embryological (e.g. Heymons 1901; Weismann 1926; Roonwal 1937; Tiegs 1940; Pflugfelder 1948) and the anatomical (e.g. Holmgren 1916; Hanström 1928; Snodgrass 1938; Henry 1948; Chaudonneret 1950; Ferris 1953) points of view, yet there is little agreement between the investigators and we are far from a satisfactory solution of the problem.

The controversy centres around the status of the region anterior to the intercalary segment, whose neuromeres are post-oral in origin, i.e. whether it is to be regarded as segmental or pre-segmental. There is also a lack of agreement as to the kind of criteria by which a segment may be defined. For example, Horridge (Bullock & Horridge 1965, p. 814) states that '...the segment must be defined independently of its innervation before a test can be made as to whether a nerve runs to its own segment. For this reason and because the nervous system is a structure which necessarily overrides segmental boundaries, the structure of the adult or even larval nervous system cannot be used to define segments without further qualifications, as a number of workers in this field have done (Holmgren, Hanström, Snodgrass; Ferris and his school)'. Embryological criteria have so far proved more satisfactory, a view supported by Tiegs (1940) and by Horridge, who points out that observations 'on the inter-relationship of many organs, both ectodermal and mesodermal, through many stages in the early life history are relevant before the segmental status of any particular part of the nervous system can be determined'. Further, 'the numbering of segments cannot be based on studies of single species but must be derived by an overall view' when the group as a whole is considered.

Bearing all this in mind, I am inclined to regard the brain in *Tenebrio* as being three segmented and incorporating the following elements; the protocerebral ganglia, which are largely pre-segmental but include presumably also the rudiments of the suppressed pre-antennary ganglia belonging to the labial (pre-antennary, pre-oral) segment; the deutocerebral ganglia which belong to the antennary (first post-oral) segment; and the tritocerebral ganglia belonging to the intercalary segment.

(*c*) *The stomodaeal nervous system*

Relatively few references to the development of the stomatogastric system in the Coleoptera are to be found (Johannsen & Butt 1941). The origin of this system in *Tenebrio* is typical for the insects whose development has so far been described, though variations in structural detail do occur among the species investigated. For instance in *Agelastica alni* (Mazur 1960), *Pieris rapae* (Eastham 1930) and *Chilo suppressalis* (Okada 1960) it is formed by the transformation of cells in the roof of the stomodaeum rather than by invagination.

It is of interest to note that in *Chilo* the stomodaeal nervous system develops precociously in relation to the stomodaeal invagination; the first neuroblast arises in the unpaired epipharyngeal lobe, prior to its incorporation into the stomodaeal roof. Later the neuroblasts in the dorsal wall of the stomodaeum arrange themselves in a column which divides into three, to become the anlagen for the three parts of the system.

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Okada (1960) suggests 'that the neuroblasts in the median cord and in (the) epipharynx are homologous'. While his theory that the antennary segment—to which he ascribes the neuroblast in the epipharynx—is pre-oral is incorrect, his interpretation of the stomodaeal nervous rudiment as being the protocephalic homologue of the median nerve strand is both feasible and attractive. Though it is tempting to go further and to suggest that the stomatogastric ganglia are segmental structures, I do not think that this would be justified. As has been shown (p. 17) the fate of the median strand in insects is far more variable than that of the lateral cords. This variation in the development of the median strand indicates that it is an unreliable structure from which to infer segmentation. The stomatogastric system may, however, represent a remnant of the median strand of the antennary segment.

The development of the stomodaeal system in other Coleoptera is described by Wheeler (1889) for *Doryphora*, Heider (1889) for *Hydrophilus*, Smreczyński (1932) for *Silpha*, Paterson (1935) for *Corynodes*, Wray (1937) for *Calandra callosa*, Tiegs & Murray (1938) for *C. oryzae* and Mazur (1960) for *Agelastica*. There is considerable variation in the arrangement of this system in insects (Bickley 1942), but I regard this to have a functional and not a segmental significance.

(2) *Ectodermal derivatives*

The development of the cephalic apodemes in *T. molitor* is typical of pterygotes, and in particular resembles that in *Calandra oryzae* (Tiegs & Murray 1938) except that in the latter the 'transverse bar is largely derived from the antennary and not the maxillary ingrowth'. In *Corynodes pusis*, despite the fact that the apodemes invaginate not behind but anterior to the appendages, Paterson (1935) states that they belong to the segment behind. According to her the displacement is due to the reduction of the post-oral intersegmental regions during development and I find this a feasible explanation of the events in view of the evidence for cell migrations at this time (see below). In some insects, e.g. *Hydrophilus* (Heider 1889) and *Silpha* (Smreczyński 1932) a pair of dorsal diverticula arise from the antennary invaginations, but these do not occur in *Tenebrio*. In *Calandra callosa* (Wray 1937) the anterior and dorsal arms of the tentorium are said to arise separately, as paired invaginations in the protocephalic ectoderm.

Eastham (1930) has drawn attention to the value of the cephalic invaginations as 'affording confirmatory evidence on which to found a concept of head segmentation' and, as was Paterson (1935), I am in agreement with his interpretations. According to this, in *Pieris* the anterior tentorial invaginations belong to the antennary segment, the small extensor mandibular tendons to the transitory premandibular (intercalary) segment, the mandibular apodeme to the mandibular segment, the posterior tentorial invagination to the maxillary segment, and the labial glands to the labial segment. It might be pointed out that a discrepancy occurs between Easthams's (1930) statement on p. 40 of his paper and the tabular summary on p. 41, as to the segment to which the flexor mandibular tendon belongs: the statement on p. 40 is obviously a misprint. This segmental interpretation in *Pieris* may equally well be applied to *Tenebrio*, where invaginations arise segmentally behind the cephalic appendages. Associated glands such as are found in *Pieris* have not, however, been located in the head of *Tenebrio*; nor have the corpora allata as yet been found.

Okada (1960) describes and illustrates extensive cell migrations in the protocephalic

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and gnathal regions of *Chilo*, and much of this can be inferred from the displacement of the appendages during cephalization (labral rudiments, antennae, mandibular apodemes and labial rudiments). He also describes an anterior surging of the ventral ectoderm while the lateral portions of a segment lag somewhat behind this movement.

Okada (1960) further describes ectodermal invaginations in *Chilo* which arise in the sequence and in similar positions to those of *Pieris* (Eastham 1930) and *Tenebrio*. He adopts a different segmental interpretation of the events with which, however, I am not convinced. The reasons for this are given below.

Okada attributes the anterior tentorial invagination in *Chilo* not to the antennary but to the intercalary segment, although he is not sure whether the latter still exists at the time of invagination. Ten hours later, when the mandibles have migrated forwards to a position formerly occupied by the intercalary segment, the apodemes of the mandibular extensor muscles invaginate at the anterior bases of the mandibles, 'though very near to the antennal region' (p. 271). Okada assigns these apodemes to the mandibular segment though in my opinion, they could as well be regarded as belonging to the suppressed, intercalary segment, especially when the ectodermal cell movements already referred to are borne in mind. The mandibular extensor apodemes generally have a more lateral position than the other cephalic invaginations, so it may well be that the anteriorly displaced mandibular segment has portions of originally intercalary ectoderm incorporated into its lateral regions. According to Okada the anterior tentorial invaginations belong to the intercalary segment; the posterior tentorial invaginations to the maxillary segment; the apodemes of the mandibular flexor and extensor muscles to the mandibular segment and the silk and prothoracic glands to the labial segment.

It seems highly probable that the discrepancies between the Eastham interpretation and that of Okada have arisen because the surging movements of the ectoderm in *Chilo* have made the precise determination of the segments in which the invaginations occur extremely difficult for Okada.

Obviously we need much more information as regards the mechanism of cephalization before the problem of the segmental significance of the apodemes can be finally solved. In the meantime the scheme adopted by Eastham (1930) and outlined on p. 20 appears to be the least contrived and most nearly to fit the facts.

The invaginations of the labial segment in insects generally give rise to the salivary glands, but these organs are not commonly found in the Coleoptera.

Tiegs & Murray (1938) describe salivary glands in the weevil *Calandra oryzae* but say that these 'are associated not with the labium but with the base of the maxillae, on the inner aspect of which they open'. These salivary glands forming 'long tubular invaginations of the ectoderm' are similar to the labial ducts found in *Tenebrio* (p. 15).

Smreczyński (1932) in *Silpha* likewise describes a gland which arises as a tubular invagination on the anterior aspect of the maxilla on either side. It undergoes rapid development late in embryonic life and its function is obscure.

Srivastava (1959), in a study of the maxillary glands of some Coleoptera, describes the ducts of the larva of *T. molitor* as follows: '...as the two glands approach the ventral nerve cord, they pass between the paired longitudinal connectives and run forwards close to each other beneath the suboesophageal ganglion. In the head they run ventral to the

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foregut and finally diverge laterally again, when each opens separately on the membrane in the angle between the labium and maxilla of its side.' This author calls these organs 'maxillary glands' and describes them as being composed of secretory cells which rest on a basement membrane and produce a thin cuticle internally. He showed by means of the iodine test that amylase was absent from these cells and concluded that the 'maxillary glands' in *Tenebrio* do not secrete saliva.

In view of the fact that these organs in *Tenebrio* arise in the angle between the closely associated maxillae and labium and of the results of Srivastava (1959), which suggest that the diverticula have some function other than salivary, two questions pose themselves:

(i) To which segment do these invaginations belong?

(ii) What is the function of these ducts?

Embryological evidence indicates that these ducts belong to the labial segment, though the antero-median migration of the labial appendages made the determination somewhat difficult. This approximation of the posterior gnathal segments may be the reason why the salivary glands of *Calandra oryzae* are said to be associated with the maxillary and not the labial segment.

In *Tenebrio* the diverticula bear the same relationship to the labium as do the posterior tentorial invaginations to the maxillae. Moreover, the labial diverticula traverse the basal segment of the labium transversely, before they curve to run back into the prothorax (p. 15 and figures 14 and 15, plate 2). For these reasons the organs described by Srivastava as 'maxillary' glands are regarded by me as belonging to the labial segment. Since Srivastava investigated not the embryo but only the larva, in which the 'glands' were already established, it is not surprising that he should have mistaken the segment in which these ducts originate.

If these structures are regarded as belonging to the maxillary segment, then two pairs of ectodermal invaginations will have to be ascribed to the latter. While this is possible, it is more probable on theoretical expectations that these paired invaginations should arise segmentally, as in some other insects, e.g. *Pieris* (Eastham 1930), and this is borne out by the morphological evidence. Moreover, in *Calandra* as in *Tenebrio* the posterior tentorial invaginations arise from the maxillary segment and thus the salivary glands of the former and the labial invaginations of the latter are in all probability homologous.

Though Srivastava could not ascribe a salivary function to the labial diverticula, he yet describes them as 'glands' composed of 'secretory cells'. He makes no suggestion as to the nature of these 'glands' nor as to what is being secreted. It is left to conjecture as to what anatomical or functional criteria he used which led him to believe these structures to be glandular. Until the function of these tubules is better understood the non-committal, but anatomically correct, term 'labial diverticula' is preferable to the term 'maxillary glands' to describe them.

The descriptive section of this paper forms part of a Ph.D. thesis, and the work was carried out at the Chelsea College of Science and Technology between 1958 and 1961, during the tenure of a Research Assistantship.

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Abbreviations

a.	amnion
abd.	abdomen
a.c.	amniotic cavity
ant.	antenna
bl.c.	blood cell
br.	brain
c.	coelomic sac
ch.	chorion
com.	commissure
con.	connective
cut.	embryonic cuticle
deut.	deutocerebrum
d.l.	dermatogene layer
e.	ectoderm
f.	fibres
f.b.	fat body
f.g.	frontal ganglion
g.	ganglion
g.c.	ganglion cell
haem.	haemocoel
h.e.	head ectoderm
h.g.	hypocerebral ganglionic rudiment
i.s.g.	intersegmental groove
j.	junction of antennary and maxillary diverticula
l.	labrum
lb.	labium
lb.app.	labial appendages
lb.div.	labial diverticula
l.c.	lateral cord
l.c.s.	labral coelomic sac
l.r.	labral rudiment
m.	mesoderm
md.	mandible
md.div.	mandibular diverticulum
md.e.m.	mandibular extensor muscle
md.fl.m.	mandibular flexor muscle
mg.e.	midgut epithelium
md.g.	mandibular ganglion
m.s.	median strand
m.sp.	mitotic spindle
mus.	muscle
mx.	maxilla
mx.div.	maxillary diverticulum
mx.g.	maxillary ganglion
n.bl.	neuroblast
n.f.	nerve fibre
n. gr.	neural groove
nl.	neurilemma
nu.	nucleus
oen.	oenocytes
op.l.	optic lobe
pleur.	pleuropodia
p.o.c.	para-oesophageal connectives
proct.	proctodaeum
proct.ap.	proctodaeal aperture
prot.	protocerebrum
p.y.	pycnotic nucleus
r.n.	recurrent nerve
s.	serosa
s.o.b.	suboesophageal body
s.o.g.	suboesophageal ganglion
sp.	spiracle
spl.m.	splanchnic mesoderm
st.	stomodaeum
st.r.	sternal region
t.	trachea
th.app.	thoracic appendages
th.g.	thoracic ganglion
t.r.	tergal region
trit.	tritocerebrum
trit.r.	rudiment of tritocerebrum
trit.com.	tritocerebral (post-oesophageal) commissure
v.g.	ventricular ganglion
y.	yolk
y.c.	yolk cell (vitellophag)
y.p.	yolk polyhedra

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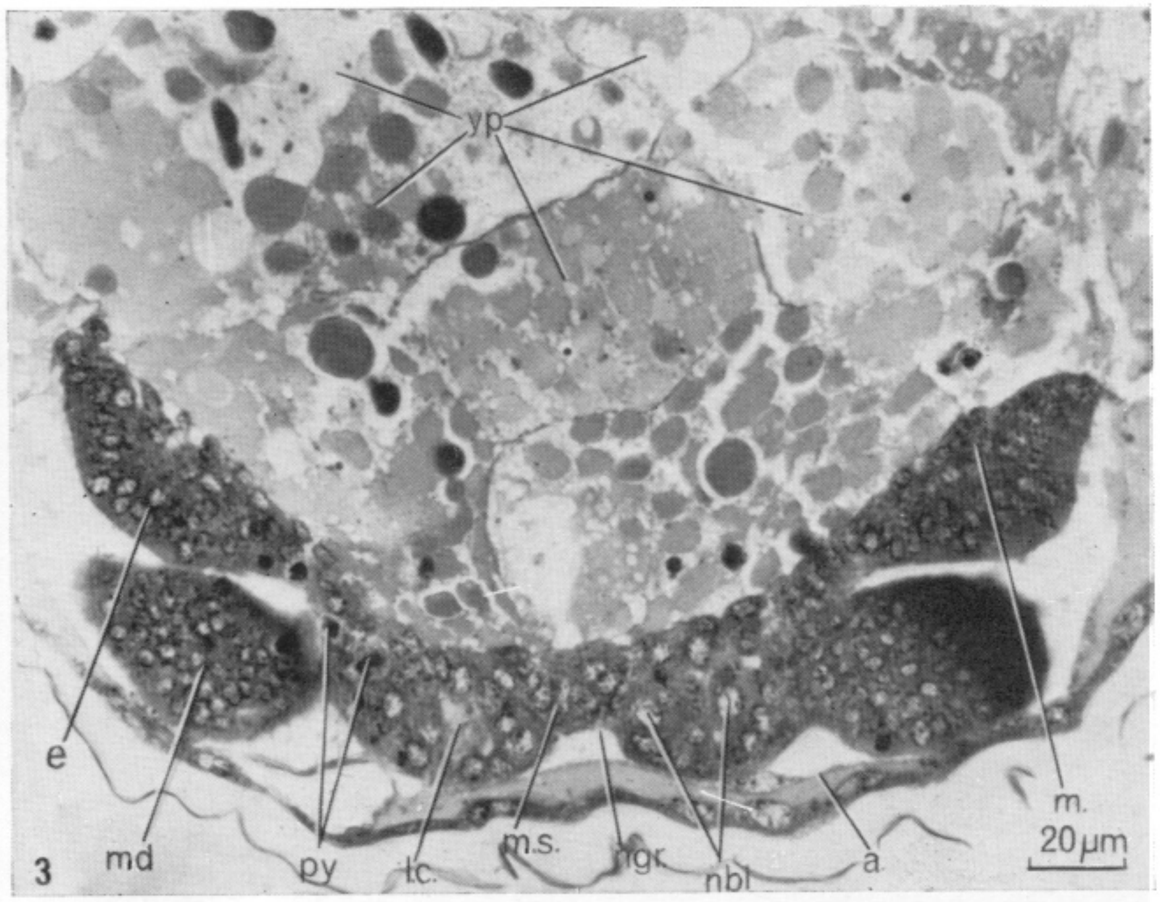

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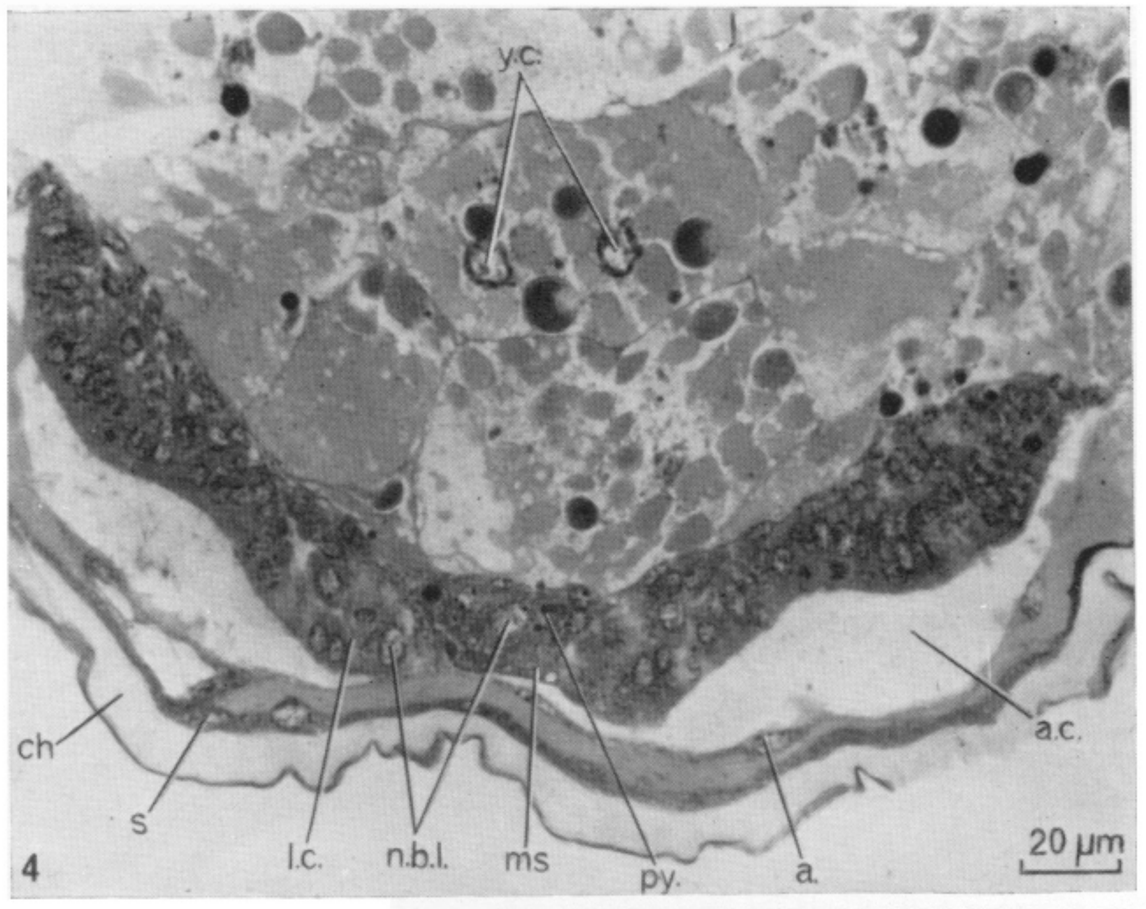

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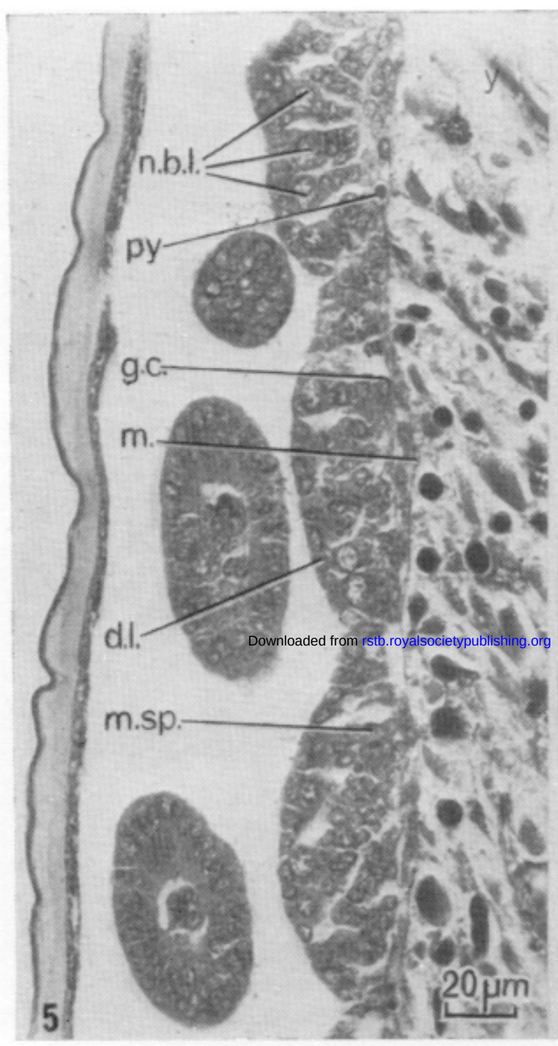

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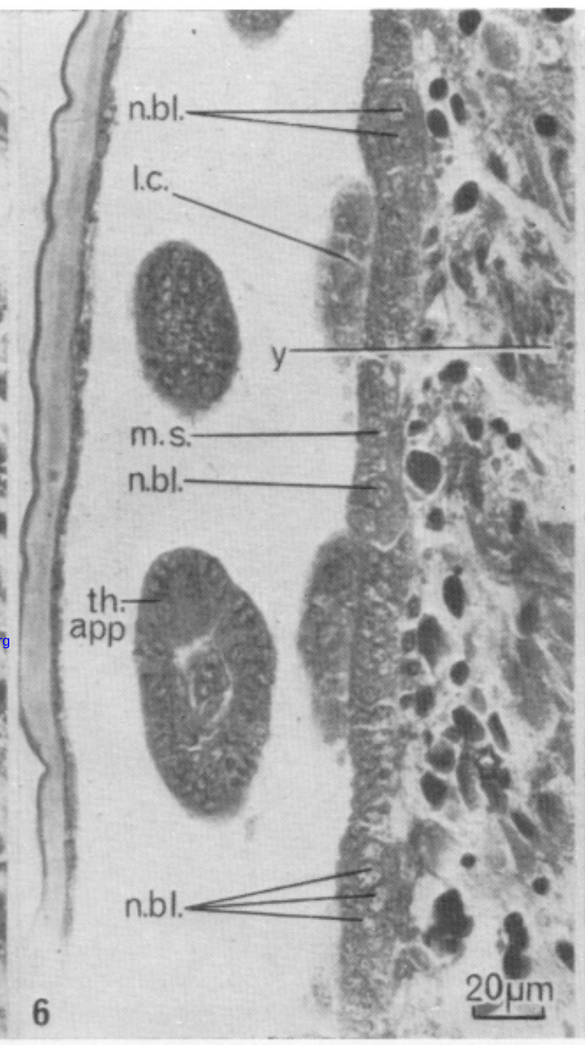

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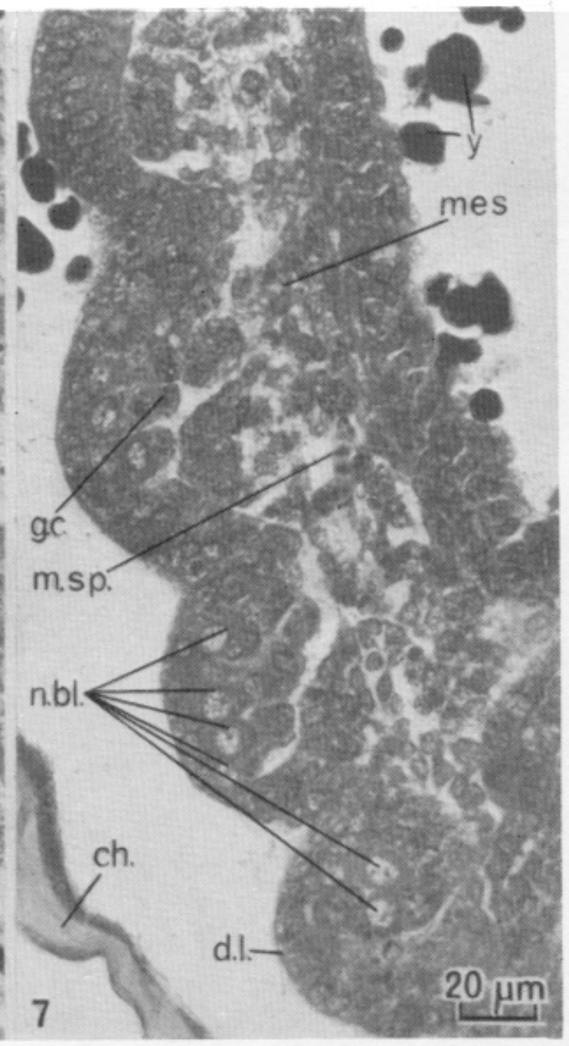

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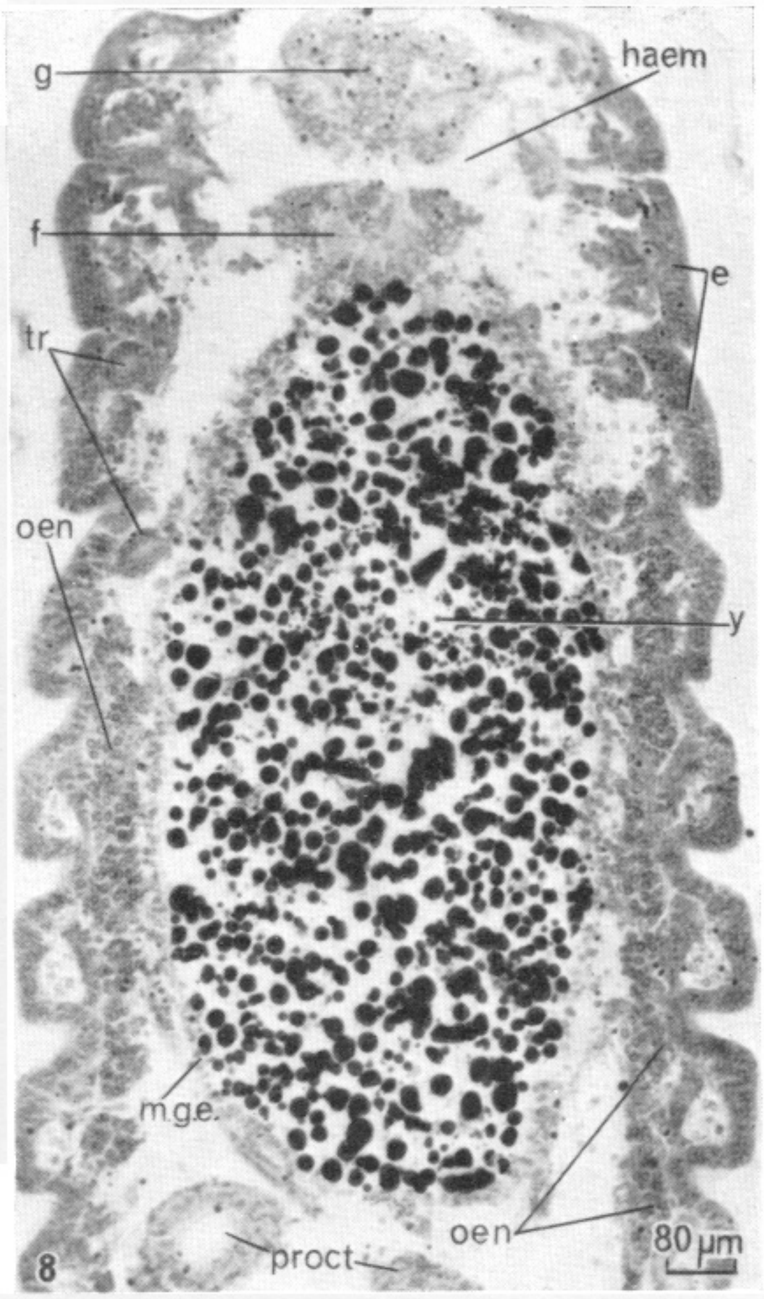

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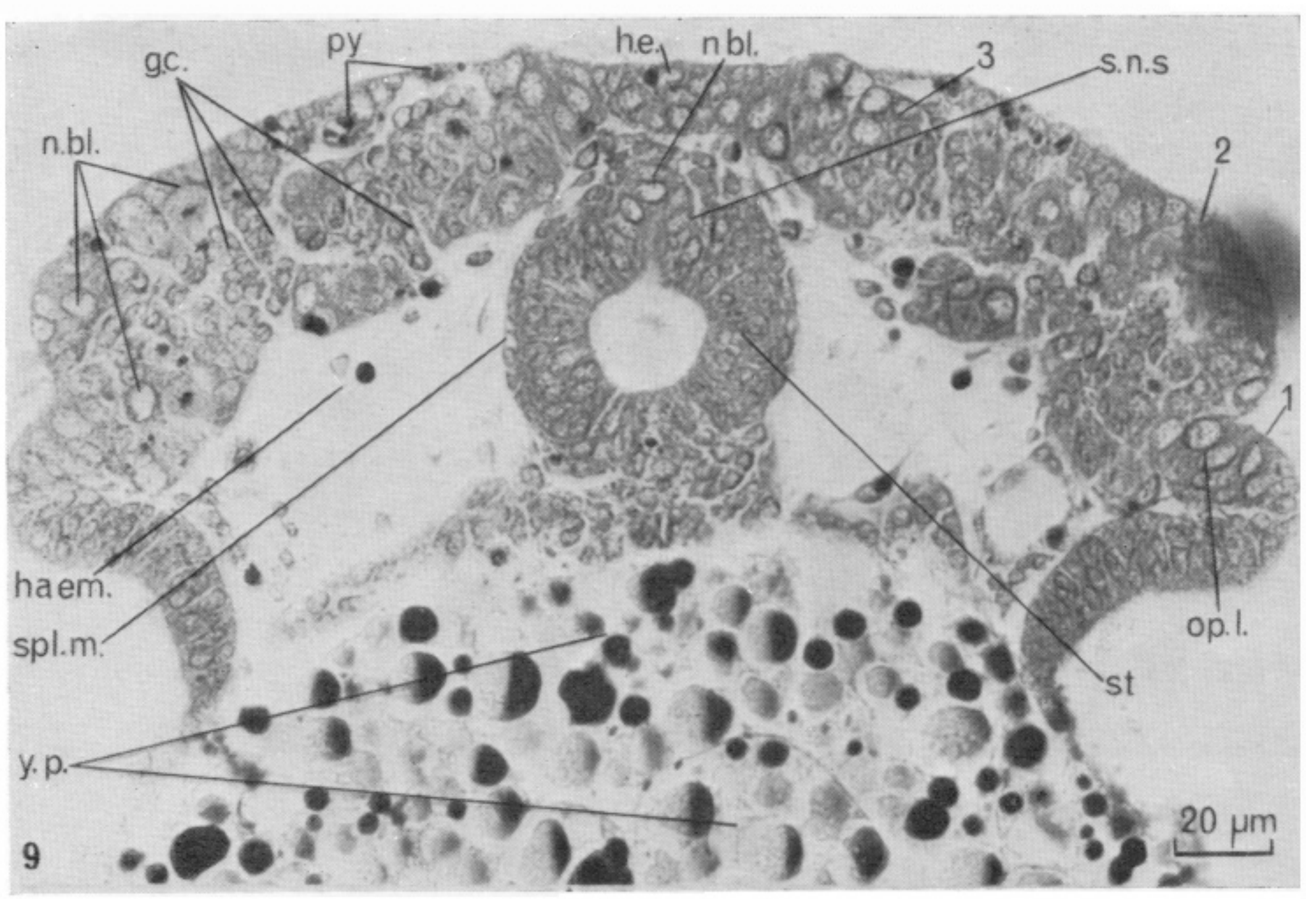

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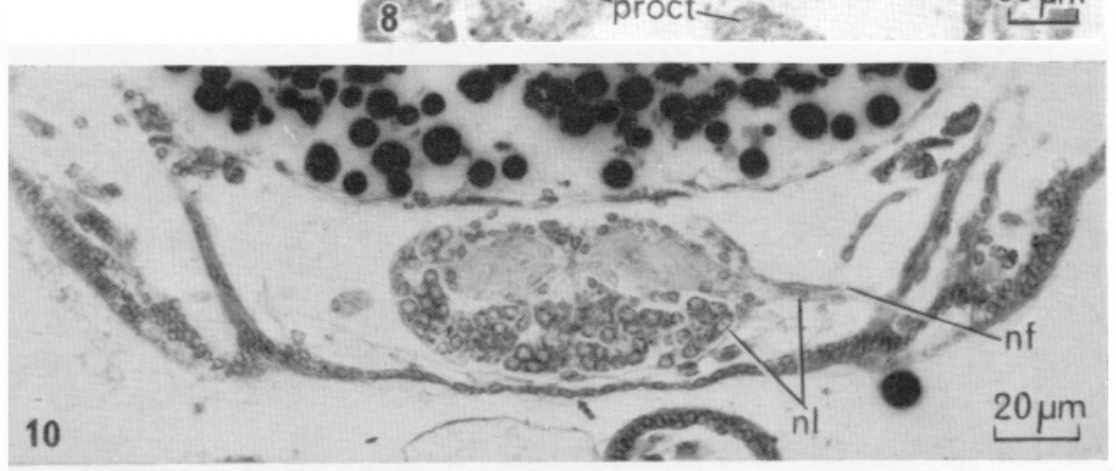

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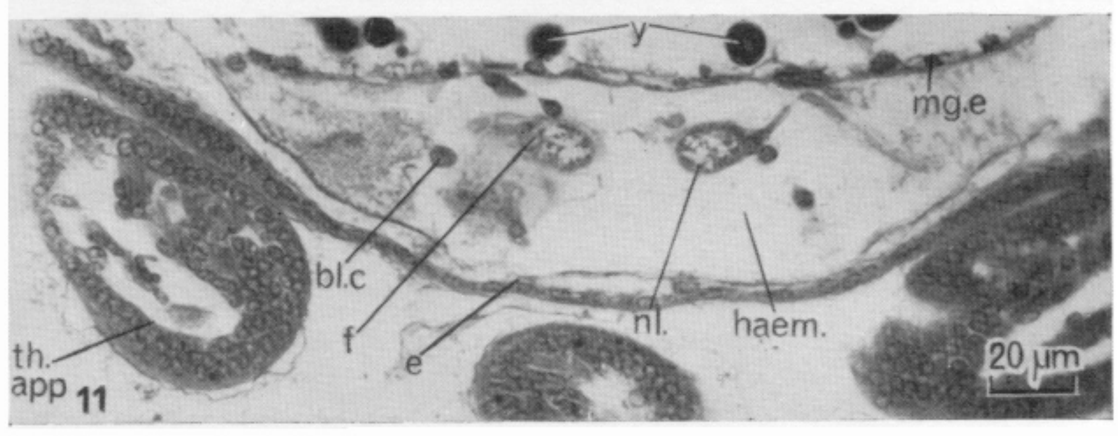

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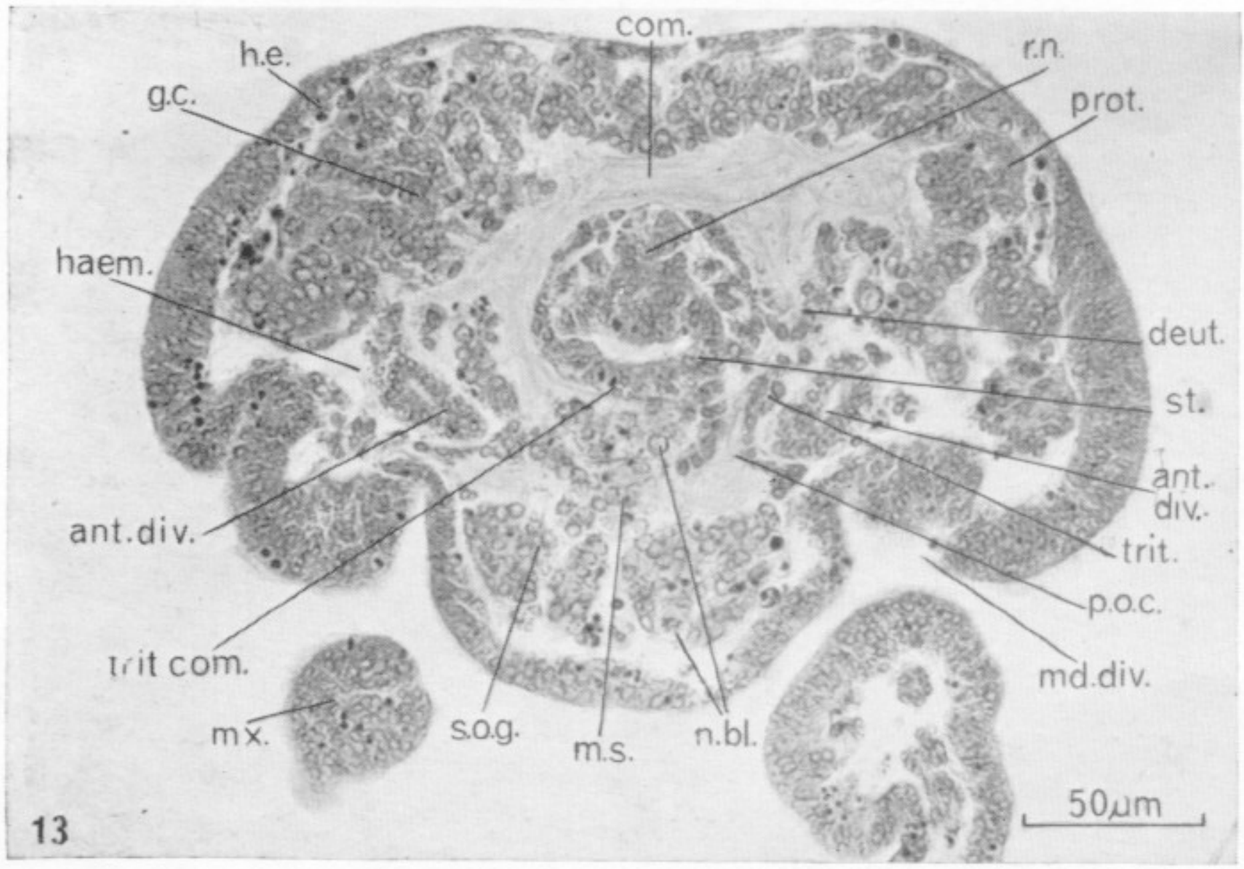

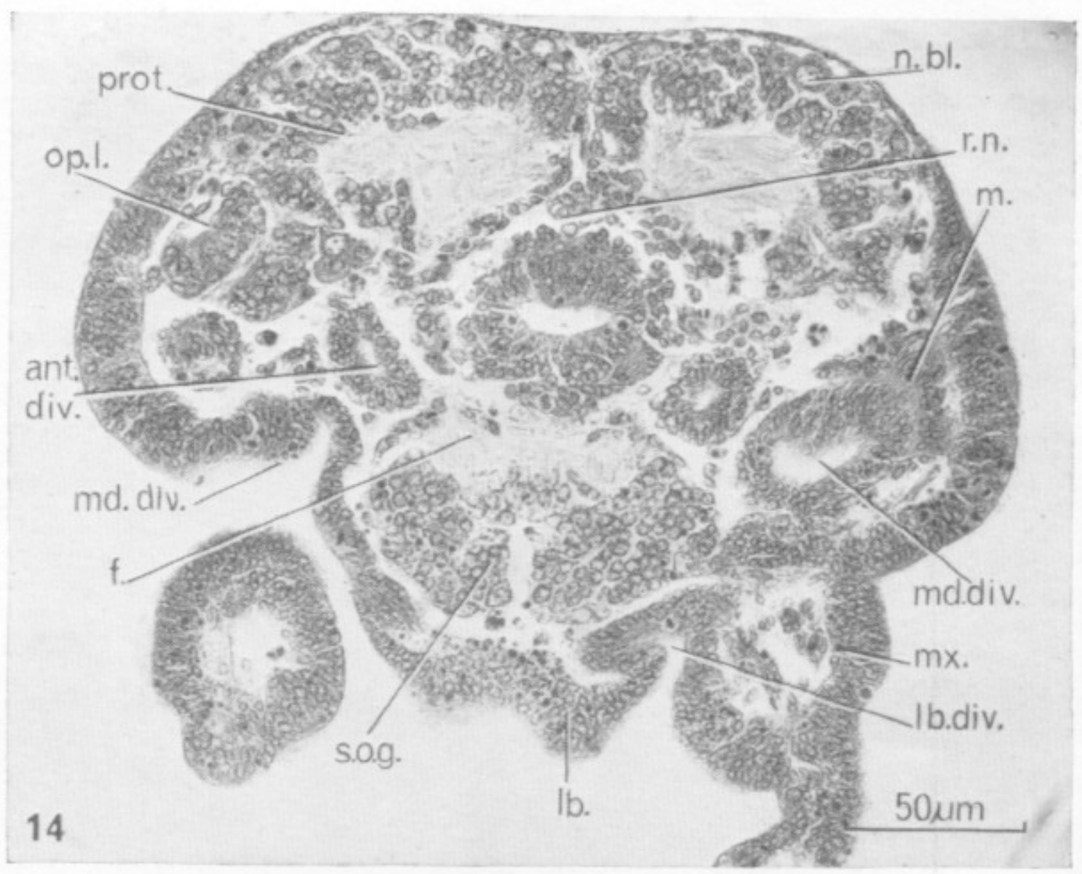

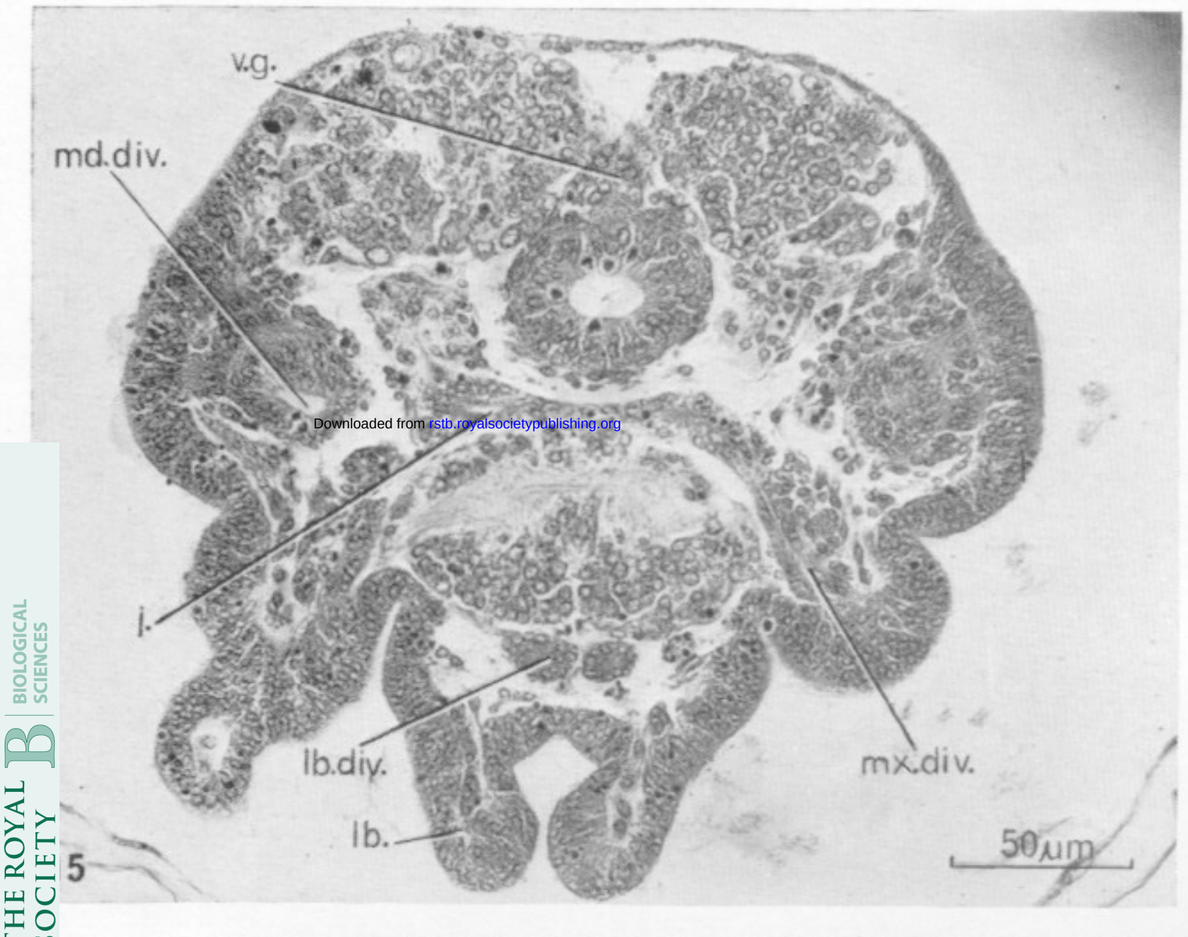

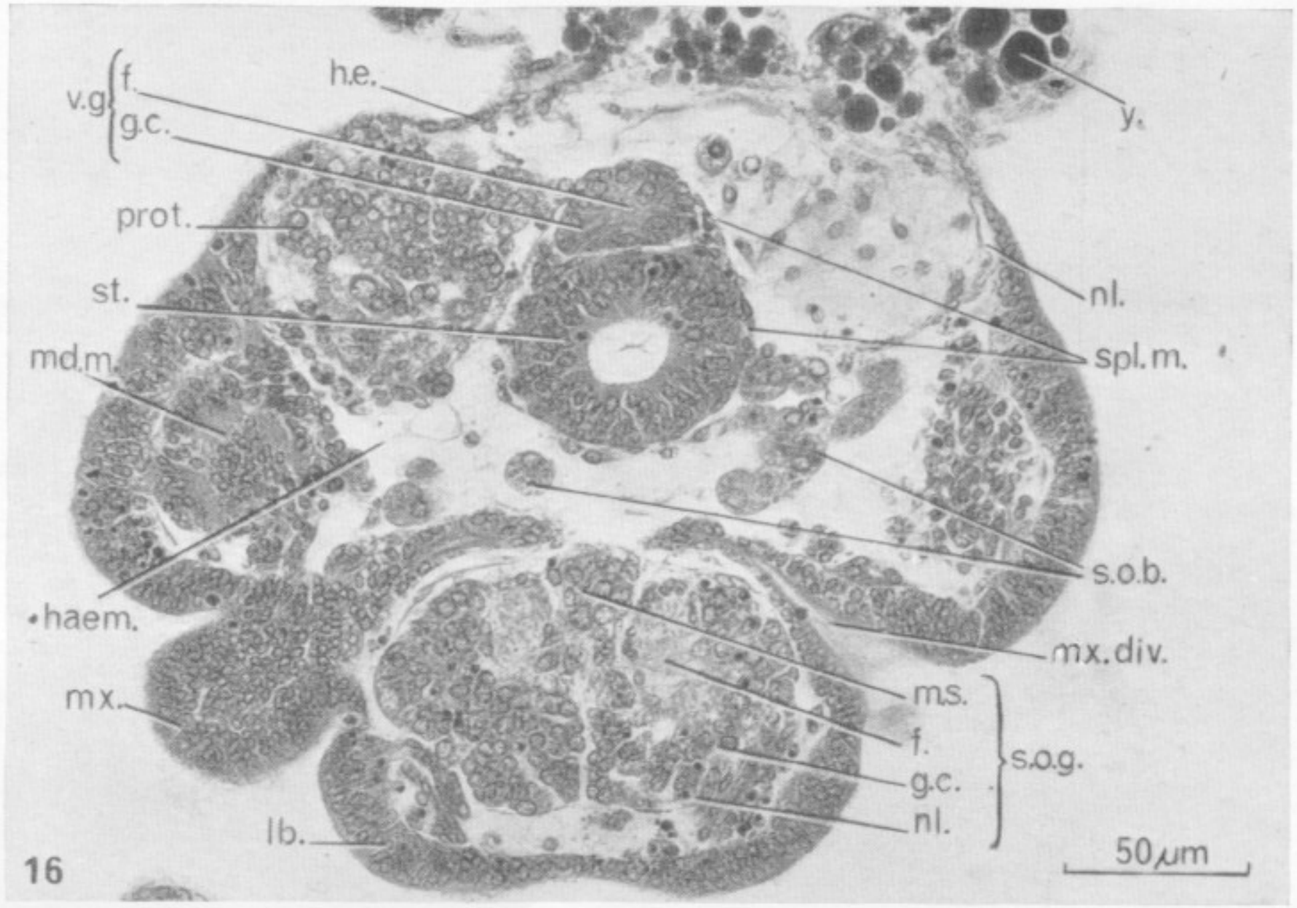

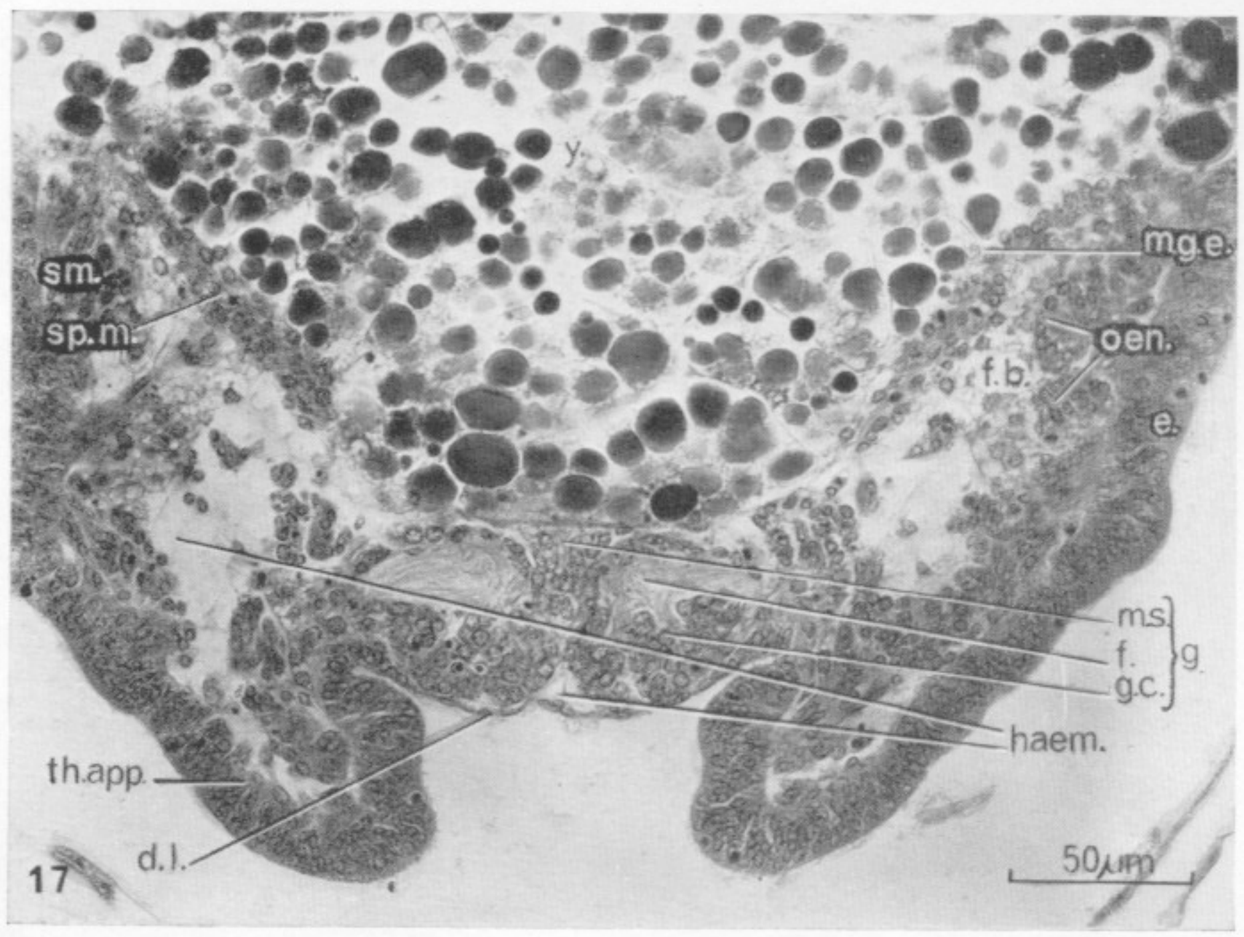

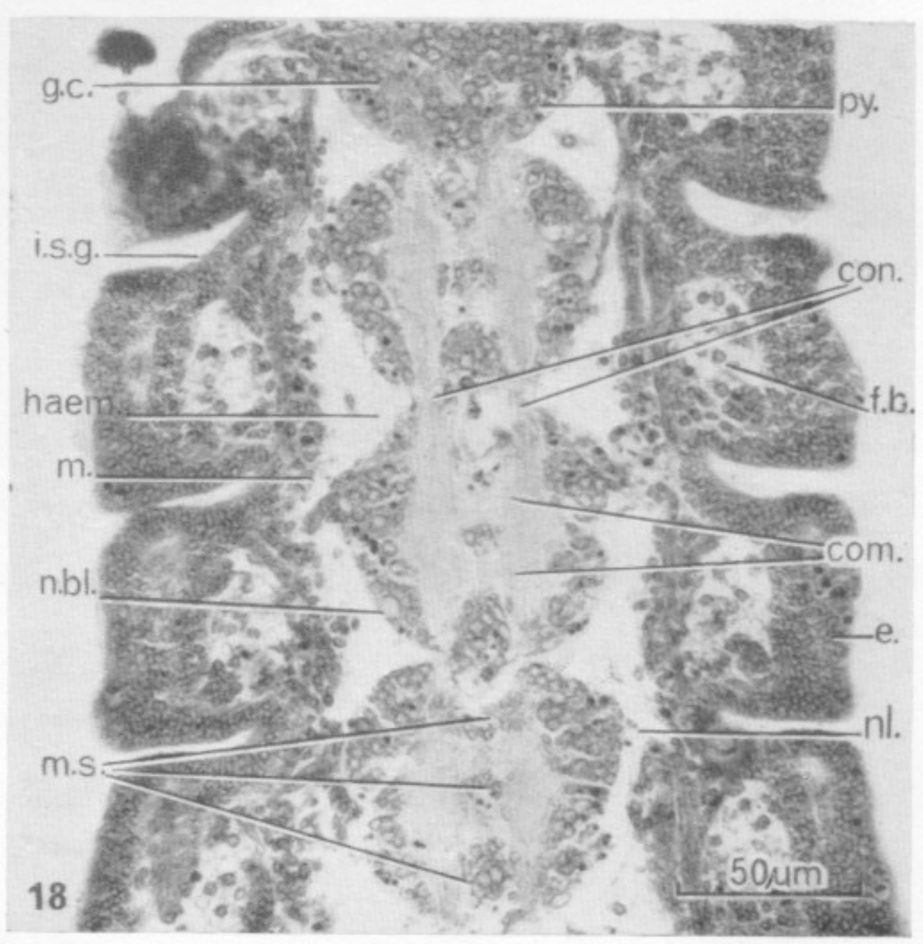

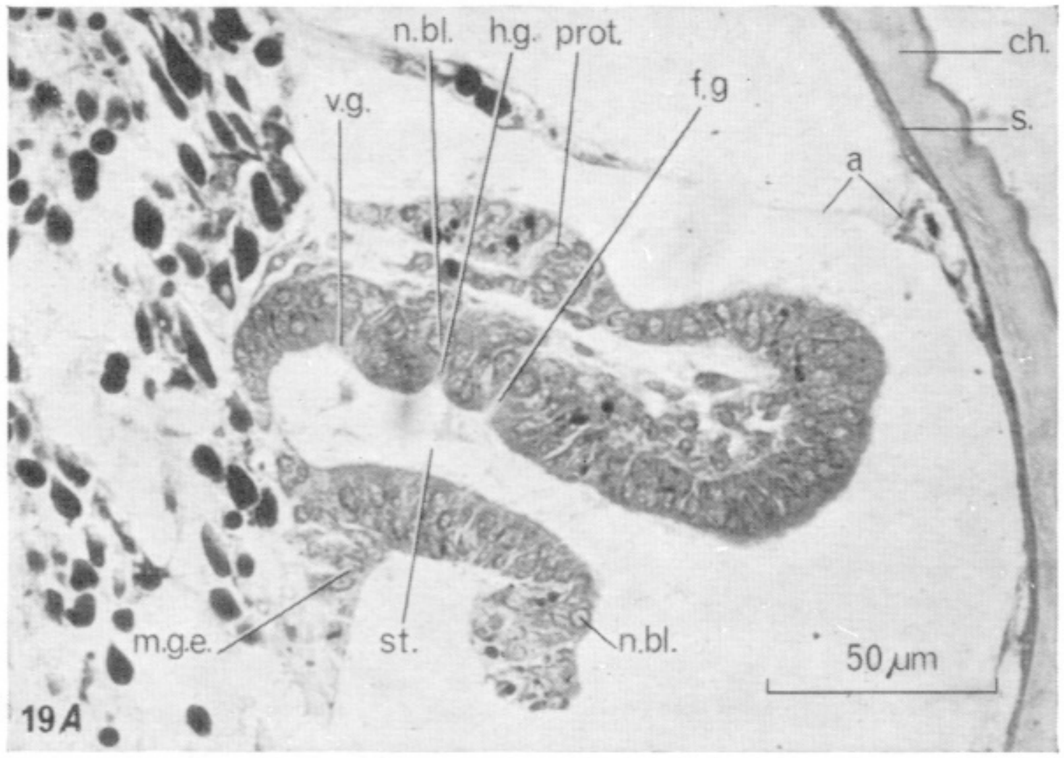

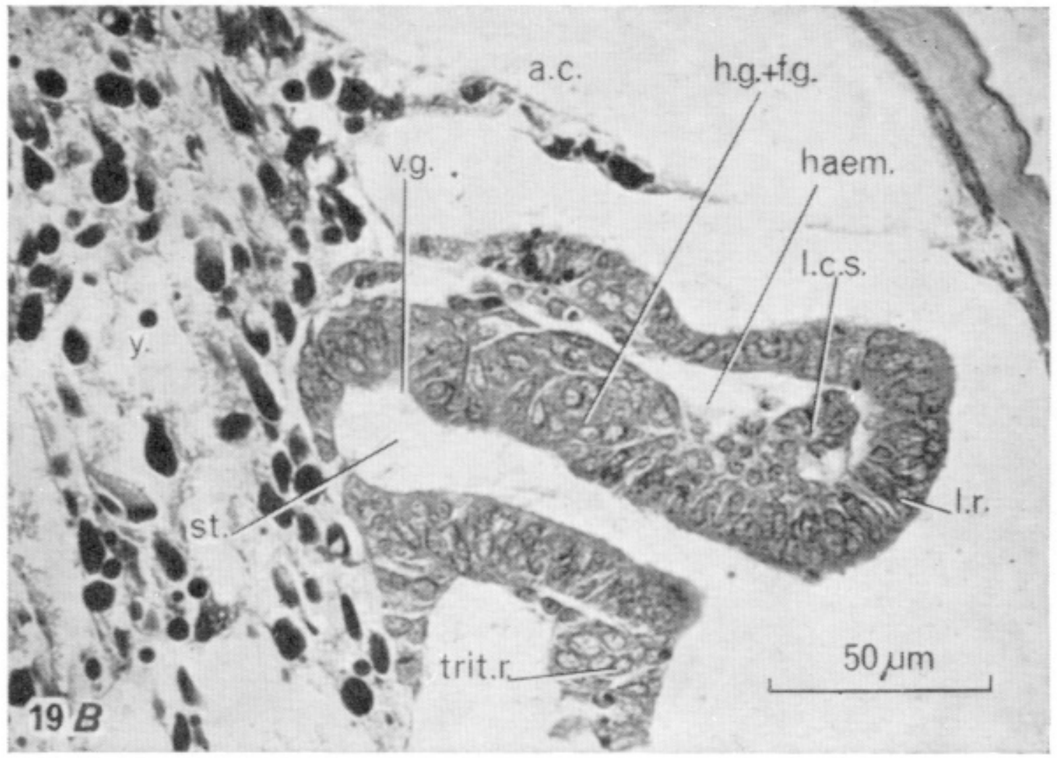

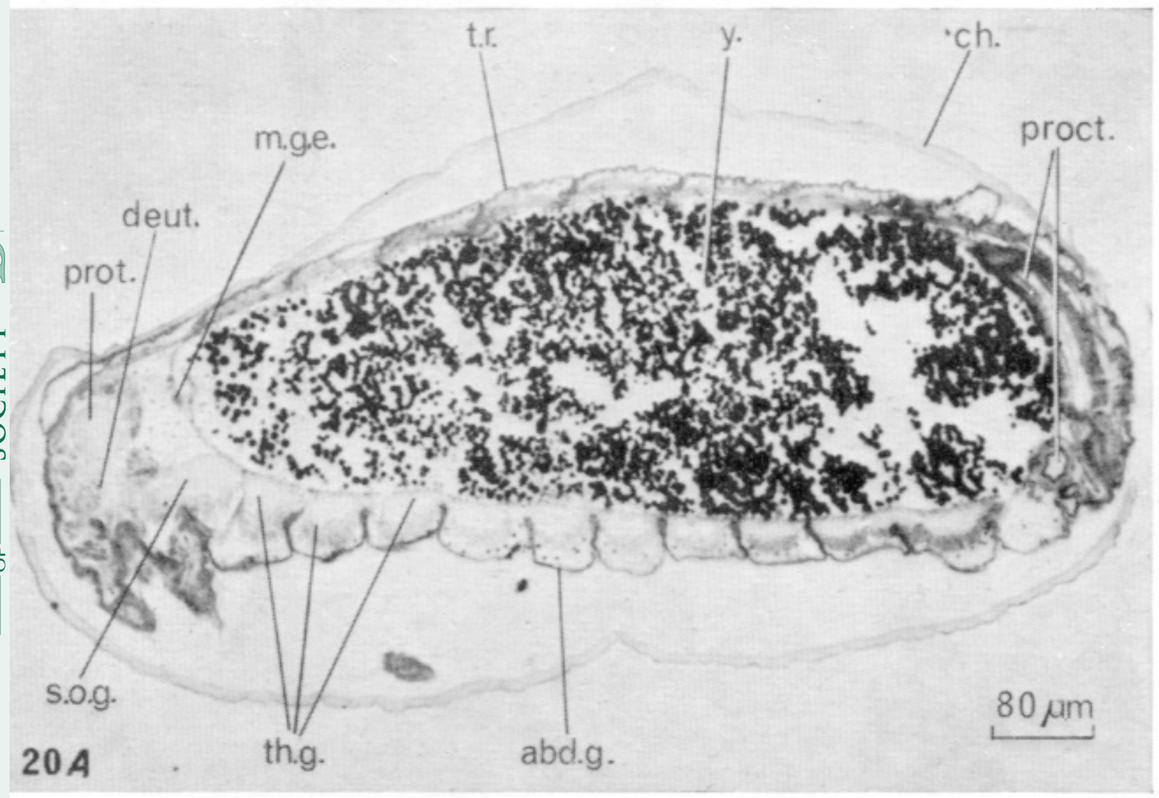

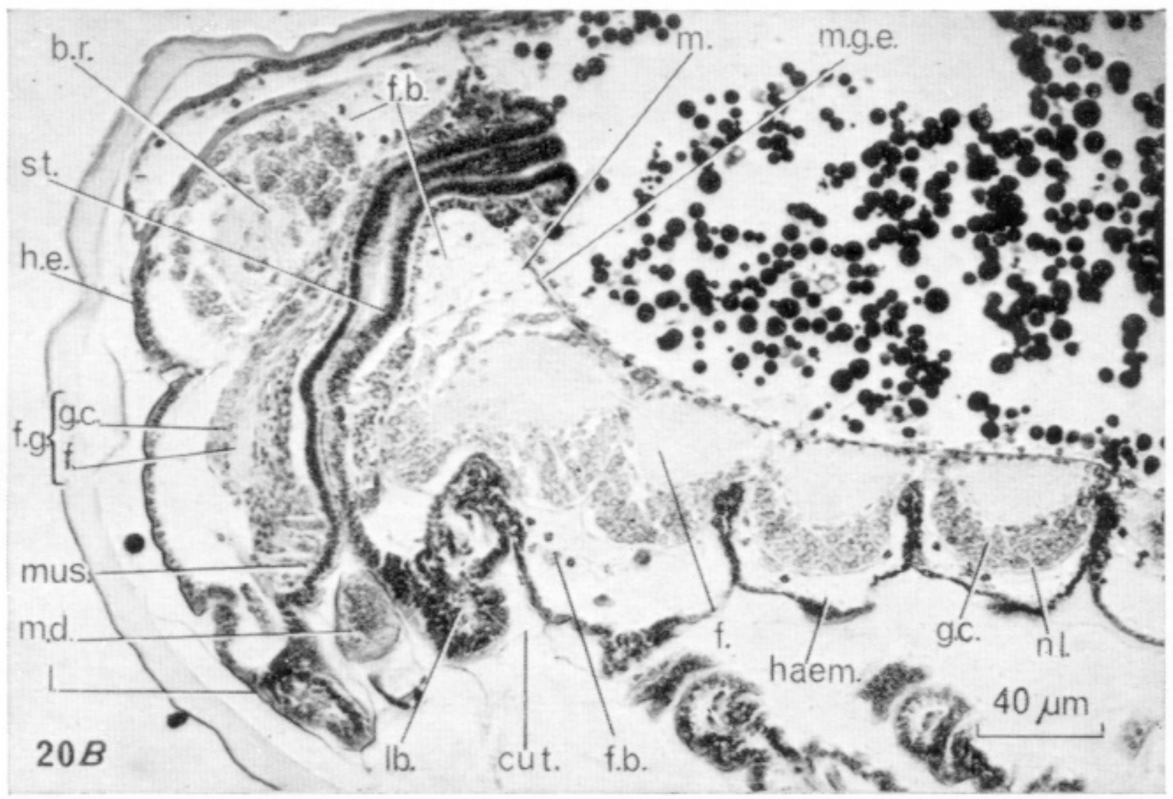

FIGURE 19. Serial longitudinal section through the stomodaeum and labral rudiments of a 45-h-old embryo, to show the origin of the stomodaeal nervous system. *A*. Invagination of the frontal, hypocerebral (occipital) and ventricular rudiments from the roof of the stomodaeum. Note the large neuroblasts. *B*. Fused mass of the frontal and hypocerebral rudiments.

FIGURE 20. Serial longitudinal sections through a 5-day-old embryo, nearing eclosion. *A*. General appearance of the central nervous system, showing the brain and the ventral chain of ganglia. *B*. Enlarged head region, to show the frontal ganglion and the recurrent nerve above the stomodaeum. Note also the neurilemma and the embryonic cuticle.