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MORPHOGENESIS AND THE DIFFERENTIATION
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THE DIGESTIVE SYSTEM IN THE EMBRYO OF
THE SOUTHERN GREEN STINK BUG, NEZARA
VIRIDULA (L.).

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MORPHOGENESIS AND THE DIFFERENTIATION OF MESODERM, THE NERVOUS SYSTEM, AND THE DIGESTIVE SYSTEM IN THE EMBRYO OF THE SOUTHERN GREEN STINK BUG, NEZARA VIRIDULA (L.)

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN ENTOMOLOGY SEPTEMBER 1969

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ABSTRACT

The external changes in the embryo prior to blastokinesis have been followed very closely. The labrum arises as a pair of appendages in the anterior germ band simultaneously with the appearance of the stomodaeum. The fusion of the two appendages occurs at a much later stage in development. The antennae arise post-orally but become pre-oral secondarily on the dorsal side of the developing head lobes.

The later development of the inner layer (mesoderm) and the formation of the coelomic sacs have been described. The coelomic sacs are of two types; closed and open. The open type is found in the thoracic and labial segments, whereas the closed type is present in the abdominal segments.

The development of the ventral neural ganglia and brain has also been described. The ventral ganglia develop similarly to that of Oncopeltus. The median cord cells are neurogenic and arise earlier than lateral cord neuroblasts. These cells contribute to the formation of cross commissures as well as the ganglia. The tritocerebral ganglia do not arise from an intercalary segment, but develop adjacent to the labral appendages in the anterior germ band and are pushed posteriorly by the protocerebral lobes of the brain.

The mesenteron rudiments are differentiated earlier than the appearance of the stomodaeum and proctodaeum and are endodermal in origin. The midgut is formed by cell proliferation from these mesenteron rudiments.
INTRODUCTION

The early embryological development of the southern green stink bug, *Nezara viridula* (L.), was described previously in a M.S. thesis (Anwar, 1968). The embryonic rudiment consists of four ventral thickenings which invaginate at the posterior pole of the egg to give rise to the germ band. The multiplication and proliferation of the cells of these embryonic thickenings as well as their invagination result in the differentiation of 3 embryonic layers. The ectoderm and amnion are differentiated earlier in development whereas the inner layer is differentiated later. During this developmental period, the early invaginated part of the embryo is the caudal end which proceeds along the periphery towards the anterior pole. The superficial head lobes of the embryo become immersed in the yolk after the posterior median thickening has invaginated to form the inner layer.

The present study was undertaken to investigate organogenesis in the southern green stink bug with primary emphasis upon the contribution and mode of participation of the differentiated ectoderm and inner layer in the development of organs such as the nervous system and digestive system, and the differentiation of mesoderm. At the same time, the external morphology of the embryo as well as its movement in relation to the yolk has been followed so as to correlate the external and internal developmental changes of the embryo.
MATERIALS AND METHODS

Many of the eggs used in this study were incubated, fixed, and processed for sectioning according to the procedures described in a previous paper by the author (Anwar, 1968). Each egg was pricked with a minuten nadeln before the egg mass was immersed in Carnoy's solution (6:3:1). After 18-24 hours in the fixative, the chorion of each egg was removed in 95% alcohol.

Embryos were also dissected out from the yolk before fixation in order to get better sections of the regions of the embryo flexed around the poles, and to prevent distortion of the sections of embryos undergoing segmental development. The dissection was carried out in Ringer's invertebrate solution under a dissecting microscope. The egg mass was immersed in the solution and the yolk mass, with the embryo, from each egg was carefully removed from the chorion. The removal of the embryo was difficult because of its delicate transparent nature. If the embryo was removed from the Ringer's solution, surface tension caused shearing and complete disintegration of the embryo. To prevent this, each yolk mass, with the embryo, was transferred by pipetting to another container with Ringer's solution. The yolk mass was then removed from the embryo by means of minuten nadeln. The freshly dissected embryo was straightened although it tended to stick to the glass surface of the container. A small amount of another fixative, Ammerman's solution, was added slowly from the side
of the container. The embryos hardened immediately after the addition of the fixative. Embryos were detached from the glass surface by means of minuten nadeln and pipetted to another container of fixative. They were left in the fixative for 1-1 1/2 hr before processing further for embedding in the usual manner. This technique could be employed only before blastokinesis, when the embryo occupied a localized superficial position on the ventral side of the yolk mass, and was not applicable when the yolk became almost enclosed by the developing embryos after blastokinesis.

Later in the study, it was found that the addition of Piccolyte to the Tissuemat hardened the wax and facilitated sectioning at thicknesses less than 10 μ. Addition of Piccolyte (about 12 1/2%) to Paraplast (Van Waters and Rogers, Inc.) improved the penetration of Paraplast into the tissue, provided better embedding of these yolk filled eggs, and made possible sectioning at 5 μ. Embryos of blastokinesis and post-blastokinesis stages were sectioned at 5 μ whereas the dissected embryos were sectioned at 7 μ.

The hematoxylin and eosin stains employed in the previous work (Anwar, 1968) were replaced by Feulgen (Pearse, 1965) because the latter stain was found superior for the identification of differentiated tissue of the embryo.

In the preparation of the whole mounts, the embryos with and without yolk mass were stained with borax carmine and mounted on microscopic slides in Canada balsam. The embryos
with the yolk mass were mounted in a Turtox plastic ring and were oriented and supported with small pieces of capillary glass tubes while the embryos without a yolk mass were mounted directly on the slide.

Line drawings representing the plane of sectioning of the embryos for clarification of the photomicrographs are given in the appendix.
The external segmentation of the embryo starts soon after the head lobes become immersed in the yolk and are overgrown by the amnion lobes. According to Seidel (1924), the amniotic cavity in Pyrrhocoris apterus L. is closed on the dorsal side of the head lobes by the fusion of the amniotic folds while the germ band in the meantime has reached its maximum length and some of the appendages have already appeared. Closure of the amniotic cavity in N. viridula is similar, but segmentation of the embryo occurs after the amniotic cavity has closed anteriorly. At this stage of development (50-51 hr), the major portion of the embryo is ventral and the embryo is flexed at both poles of the egg; the cephalic end around the posterior pole and the abdominal region around the anterior pole. Thus, both anterior and posterior extremities of the embryo lie opposite one another on the dorsal side of the egg (Fig. 1). A similar position has been reported for the embryos of Cimex (Heymons, 1899), P. apterus (Seidel, 1924), Belostoma flumineum Say (Hussey, 1926), and Oncopeltus fasciatus (Dallas) (Butt, 1949). The appearance of transverse grooves on the ventral side of the embryo results in the differentiation of thoracic and cephalic segments by this stage but the abdominal region remains unsegmented (Fig. 2). There are 3 thoracic segments and the cephalic segments consist of 1 antennal and 3 gnathal segments and a pair of
FIG. 1 *Nezara viridula* (L.) (50-51 hr)

Lateral view of the embryo and the yolk mass. X 123.

<table>
<thead>
<tr>
<th>abbr</th>
<th>label</th>
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<tbody>
<tr>
<td>an</td>
<td>anterior pole</td>
</tr>
<tr>
<td>dr</td>
<td>dorsal side</td>
</tr>
<tr>
<td>hd. 1b.</td>
<td>head lobe</td>
</tr>
<tr>
<td>pt</td>
<td>posterior pole</td>
</tr>
<tr>
<td>vt</td>
<td>ventral side</td>
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FIG. 2  *Nezara viridula* (L.) (50-51 hr)

Ventro-lateral view of the embryo and the yolk mass.

X 130.

<table>
<thead>
<tr>
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<th>Description</th>
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<tbody>
<tr>
<td>hd. lb.</td>
<td>head lobe</td>
</tr>
<tr>
<td>I, II, III</td>
<td>thoracic segments</td>
</tr>
<tr>
<td>1m</td>
<td>labial segment</td>
</tr>
<tr>
<td>mx</td>
<td>maxillary segment</td>
</tr>
</tbody>
</table>
head lobes. The segments are separated from each other by conspicuous intersegmental clefts. The thoracic segments are prominent and broader than the gnathal segments and occupy most of the ventral side of the egg (Fig. 3). The lateral sides of each thoracic and antennal segment stain deeply due to greater cell numbers in these areas. The gnathal segments occupy the posterior pole and constitute the anterior flexed part of the embryo (Fig. 4). The head lobes and the caudal tip face each other and are located directly above thoracic segments I & III respectively on the dorsal side of the egg. The stomodaeum and the labral segment are not evident at this stage. The abdominal region is narrow and unsegmented.

In the next 2-3 hr (total 53-54 hr), the total length of the embryo remains the same. The abdominal region, during this time, appears to elongate at the caudal tip because it extends now almost to the middle of the dorsal side of the egg (Fig. 5). The deep staining of this area indicates a higher number of cells at the caudal tip at this stage. At the same time, the lateral cell proliferation in the cephalic and thoracic segments results in the broadening and contraction of the embryo in these areas. While the caudal end pushes posteriorly, the head lobes recede from their position and cover only the posterior pole of the egg (Figs. 5 and 6). The rudiments of antennae and thoracic appendages appear as evaginations on the lateral sides of the respective segments.
FIG. 3 *Nezara viridula* (L.) (50-51 hr)

Ventral view of the embryo and the yolk mass. X 140.

an ........................................... anterior pole
I, II, III ................................. thoracic segments
pt ................................. posterior pole
FIG. 4 *Nezara viridula* (L.) (50-51 hr)

Apical view of the posterior pole of the egg. X 136.

| ant |.............................| antennal segment |
| lm  |.............................| labial segment   |
| mb  |.............................| mandibular segment |
| mx  |.............................| maxillary segment |
FIG. 5 *Nezara viridula* (L.) (53-54 hr)

Dorsal view of the egg. X 136.

cau. end ............................ caudal end
hd. lb. ............................... head lobe
FIG. 6  *Nezara viridula* (L.) (53-54 hr)

Lateral view of the embryo and the yolk mass. X 136.

cau. end  ......................... caudal end
hd. lb.  ......................... head lobe
I, II, III  ......................... thoracic appendages
(Fig. 7) and each appendage appears as a small protuberance when viewed laterally (Fig. 6). The evaginations have not appeared in the gnathal segments but the deep staining areas in the lateral sides of mandibular, maxillary and labial segments mark the sites of development of future appendages. An examination of the posterior pole of the egg (Fig. 8) reveals that the antennae have appeared as lateral evaginations from the antennal segment. A crescentic deep-staining area in the mid-anterior of the antennal segment indicates the shallow stomodeal invagination at this stage. The labral appendages mark their place of origin as two deep-staining areas in the antero-medial ventral surface of the germ band. The external segmentation of the abdominal region is initiated at this stage.

As development continues, the whole abdominal region becomes segmented although the intersegmental clefts are not very deep. The abdominal region consists of 11 segments. A narrow terminal protuberance, the proctodaeum, appears during this period and becomes flexed over the abdominal segment XI. The labral and the 3 pairs of gnathal appendages make their appearance. The 2 labral appendages which arise as small evaginations on the antero-medial side of the head lobes become prominent and guard the anterior end of the stomodaeum. Examination of embryos at 61-62 hr (Fig. 9) reveals that the segmentation of the abdominal region has become very prominent. The thoracic appendages have considerably elongated.
FIG. 7  *Nezara viridula* (L.) (53-54 hr)

Ventral view of the embryo and the yolk mass. X 132.

ant  .........................  antenna
I, II, III  .....................  thoracic appendages
FIG. 8 *Nezara viridula* (L.) (53-54 hr)

Ventro-apical view of the posterior pole of the egg.

X 124.

ant .................................................. antenna
hd. lb. ............................................. head lobe
st ................................................... stomodaeum
FIG. 9 Nezara viridula (L.) (61-62 hr)

Embryo without last abdominal segment. X 60.

ant ................................................. antennae
hd. lb. ........................................... head lobe
lm ................................................... labial appendage
lr ................................................... labral appendage
I, II, III .......................................... thoracic appendages
mb ................................................... mandible
mx ................................................... maxilla
st ................................................... stomodaeum
While the bases of thoracic appendages cover the lateral side of the respective thoracic segments, the appendages, obliquely placed to the central axis of the embryo, appear to localize in the posterior half of the segments. The thoracic appendages are bisegmented, whereas, the gnathal appendages show no segmentation. The labial appendages are longer than the other two gnathal appendages. The head lobes have broadened (Fig. 10) and show a deep staining of the margin. The labral appendages have shifted posteriorly and the bases form a roof over the anterior end of the stomodeaeum. A deep-staining horse-shoe shaped area around the stomodeal invagination indicates a greater cellular concentration in this region. The stomodeaeum and the cells around it extend up to the posterior portion of the antennal segment. The antennae have become closer to the head lobes.

In the next 4-5 hr (total 66-67 hr), the head lobes broaden and recede to the ventral half of the posterior pole (Fig. 11) indicating further shortening of the embryo. The stomodeaeum loses its original shape and appears as a narrow blind sac, the oral opening being guarded by the labrum (Fig. 12). The labral appendages show an initiation of union in the middle and move further posteriorly. The antennae become more closely associated with the head lobes (protocephalic lobes). The thoracic and gnathal appendages increase in size and their bases become thickened (Fig. 13). The labium is twice the length of the mandibles and maxillae,
FIG. 10 Nezara viridula (L.) (61-62 hr)

Cephalic region of the embryo. X 262.

ant  ................................... antenna
hd. lb.  ................................... head lobe
lr  ................................... labral appendage
st  ................................... stomodaeum
FIG. 11  *Nezara viridula* (L.) (66-67 hr)

An apical view of the posterior pole of the egg. X 123.

ant ..........................  antenna
hd. lb. ..........................  head lobe
lr ..............................  labrum
FIG. 12 *Nezara viridula* (L.) (66-67 hr)

Cephalic end of the embryo. X 261.

ant ................................. antenna
hd. lb. ................................. head lobe
lr ................................. labrum
st ................................. stomodaeum
FIG. 13  *Nezara viridula* (L.) (66-67 hr)

Cephalic and thoracic regions of the embryo. X 129.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ant</td>
<td>antenna</td>
</tr>
<tr>
<td>hd. lb.</td>
<td>head lobe</td>
</tr>
<tr>
<td>lm</td>
<td>labium</td>
</tr>
<tr>
<td>lr</td>
<td>labrum</td>
</tr>
<tr>
<td>mb</td>
<td>mandible</td>
</tr>
<tr>
<td>mx</td>
<td>maxilla</td>
</tr>
<tr>
<td>I, II, III</td>
<td>thoracic appendages</td>
</tr>
<tr>
<td>st</td>
<td>stomodaeum</td>
</tr>
</tbody>
</table>
which are almost equal in length. Abdominal segments I to VI broaden and the lateral margins in these segments become thickened (Fig. 14). The proctodaeum covers the last 2 abdominal segments.

During the next 4-5 hr (total 71-72 hr), broadening and reduction in size of the abdominal region indicate further shortening of the embryo. The caudal end recedes from its original position so that the posterior abdominal region occupies the anterior pole and does not cross over to the dorsal side of the egg. The lateral margins of the abdominal region become very thickened and produce pronounced lateral outgrowths in abdominal segments I through VIII. The proctodaeum is still flexed on the dorsal side (Fig. 15) and reaches to the abdominal segment IX. The malpighian tubules are developed at this time. The labral appendages, though of the same size, show a complete union of their basal regions and shift posteriorly (Fig. 16). The bases of the antennae shift anteriorly and become attached to the postero-dorsal side of the head lobes near the median line. The antennae become elongated and show signs of segmentation. The oral opening is surrounded now by the labrum and the bases of the antennae indicating that the antennae are in the process of becoming preoral in position.

At about 75-76 hr after egg deposition, the folding of appendages as well as lateral sides of abdominal segments on the ventral side of the embryo, is initiated and continues
FIG. 14 *Nezara viridula* (L.) (66-67 hr)

Abdominal region of the embryo. X 131.

ab. I ......................... abdominal segment I
ab. IX ......................... abdominal segment IX
pr  .................. proctodaeum
FIG. 15  *Nezara viridula* (L.) (71-72 hr)
Caudal end of the embryo. X 201.

mp. tub. .............................. malpighian tubule
pr ................................. proctodaeum
FIG. 16 Nezara viridula (L.) (71-72 hr)
Cephalic end of the embryo. X 264.

ant .......................... antenna
hd. lb. .......................... head lobe
lr .......................... labrum
mb .......................... mandible
prior to blastokinesis. The thoracic appendages begin to fold on the ventral side of the embryo (Fig. 17). This folding of the embryo is also accompanied by shifting of the cephalic appendages. The head lobes, still separated from each other, show greater cell concentration in the middle (Fig. 18), and also indicate a dorso-ventral shift of their ectodermal covering. The bases of the antennae shift anteriorly and occupy the mid-dorsal position on each head lobe. The antennae encircle the head lobes and their distal ends lie on the ventral side of the germ band. The labrum, with almost completely united appendages except for the terminal end, shifts further posteriorly by this stage and completely covers the oral opening. The stomodaeum has become a continuous canal. The gnathal appendages shift towards the median line and come to lie close to each other. By 79-80 hr, the bases of the antennae occupy the antero-dorsal position on the respective head lobes, and the head lobes have begun to unite along the median line (Fig. 19). The antennae encircle the head lobes and their distal swollen ends lie attached to the ventral side of the respective head lobes. The antennae thus become pre-oral in position by this stage. The labrum shifts still more posteriorly and lies immediately anterior to the gnathal appendages. As a result of rearrangement of the gnathal and labral appendages, the embryo loses its segmented appearance in the cephalic region while the segmentation in the thoracic and abdominal
FIG. 17  *Nezara viridula* (L.) (75-76 hr)

Ventral view of the embryo. X 134.

ant .............................. antenna
hd. 1b. .......................... head lobe
I, II, III ......................... thoracic appendages
1r ................................. labrum
FIG. 18 *Nezara viridula* (L.) (75-76 hr)

Cephalic end of the embryo. X 263.

ant ................................. antenna
hd. lb. ................................. head lobe
lr ................................. labrum
st ................................. stomodaeum
FIG. 19  *Nezara viridula* (L.) (79-80 hr)

Cephalic end of the embryo. X 276.

ant ................................. antenna
hd. lb. ................................. head lobe
lr ................................. labrum
regions is still maintained. The terminal anal opening of the embryo becomes prominent by this stage (Fig. 20). The shortening and folding of the embryo continues until it becomes restricted mostly to the ventral side of the egg by 84-85 hr and only cephalic and caudal ends extend to the posterior and anterior poles respectively (Fig. 21). The 2 head lobes become fused with each other and the appendages become considerably elongated. The bases of the antennae are shifted still more anteriorly and become antero-ventral in position. The folded thoracic appendages occupy the mid-ventral portion of the germ band and are covered by its folded lateral margins. It is difficult to distinguish ventrally any of the appendages (Fig. 22) because of their complete folding. The embryo is now ready for blastokinesis.
FIG. 20 Nezara viridula (L.) (79-80 hr)

Abdominal region of the embryo. X 259.

as ............................................ anus
pr ............................................. proctodaeum
FIG. 21  *Nezara viridula* (L.) (84-85 hr)

Ventro-lateral view of the embryo and the yolk mass.

X 129.

an ........................................... anterior pole
pt ............................................... posterior pole
FIG. 22  Nezara viridula (L.) (84-85 hr)

Ventral view of the embryo and the yolk mass. X 130.

an   ..........................................  anterior pole
pt   ..........................................  posterior pole
MESODERM

Formation of the inner layer during invagination of the embryonic rudiment has been described earlier (Anwar, 1968). The inner layer differentiates as a ridge of cells occupying the middle of the gastral groove and extends almost the entire length of the embryo.

The flattening of the inner layer ridge starts at 44-45 hr of development, before the head lobes become internal, and continues after the amniotic cavity is closed anteriorly by 48 hr. The inner layer begins to spread laterally on the dorsal side of the germ band. The process is initiated in the thoracic region and continues anteriorly and posteriorly, though slower in the abdominal region. In fact, the thoracic region appears to be the center of differentiation because all the developmental changes take place earlier here than in other regions of the embryo. The inner layer then splits in the middle and assumes the form of two lateral bands which completely cover the dorsal surface of the ectoderm from the middle to the lateral width of the germ band on either side.

By 50-51 hr, the inner layer has split to form 2 lateral bands in the thoracic segments which along with the ectoderm, in a transverse section, give a trough-like appearance at the lateral margins of the embryo (Fig. 23). The inner layer has also spread laterally over the ectoderm of the germ band in the gnathal and anterior abdominal segments but is still connected medially (Figs. 24 and 25). The intersegmental
FIG. 23 *Nezara viridula* (L.) (50-51 hr)

Cross section of the embryo through the thoracic region.

X 552.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>am</td>
<td>amnion</td>
</tr>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>inn. lyr.</td>
<td>inner layer</td>
</tr>
<tr>
<td>pc</td>
<td>paracyte cells</td>
</tr>
<tr>
<td>yk</td>
<td>yolk</td>
</tr>
</tbody>
</table>
FIG. 24 Nezara viridula (L.) (50-51 hr)

Cross section of the embryo through the gnathal region.

X 654.

am .................................................. amnion
ect .................................................. ectoderm
inn. lyr. .......................................... inner layer
yk. cl. .......................................... yolk cell
FIG. 25  *Nezara viridula* (L.) (50-51 hr)

Cross section of the embryo through the anterior abdominal region. X 654.

- **am** ........................................ amnion
- **ect** ....................................... ectoderm
- **inn. lyr.** ................................ inner layer
- **sr** ......................................... serosa
- **yk** ......................................... yolk
inner layer occupies the middle of the germ band as a unicellular layer (Fig. 26). The inner layer ridge of cells and its lateral extensions are present in the mid-abdominal region (Fig. 27) while only the ridge occupying the gastral groove is present in the posterior abdominal region (Fig. 28). Two clusters of cells, 1 posterior to the antennal segment (Fig. 29) and 1 near the posterior extremity of the embryo (Fig. 30) are also present at this stage. These are the anterior and posterior mesenteron rudiments. The rudiments, although similar in appearance and staining to the cells of the inner layer, lie dorsally to the inner layer at this stage of development. The inner layer in the cephalic region, anterior to the anterior mesenteron rudiment, is not a unicellular layer but consists of loose cells which show their connection to a smaller clump of cells in the anterior half of the antennal segment. The inner layer hereafter will be called mesoderm.

The formation of coelomic sacs in the thoracic and gnathal segments takes place in the following manner: As the rudiments of the thoracic appendages appear as sac-like evaginations at 53-54 hr, the mesoderm also follows, lining the inner surface of the ectodermal layer. The cavity thus enclosed in the mesoderm of the rudimentary appendages, opening dorsally between the germ band and the yolk, is the beginning of the coelomic cavity (Fig. 31). By 56-57 hr, the coelomic sacs become enlarged (Fig. 32) and, due to the
FIG. 26 *Nezara viridula* (L.) (50-51 hr)

Cross section of the embryo through the intersegmental region. X 688.

<table>
<thead>
<tr>
<th>am</th>
<th>amnion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>inn. lyr.</td>
<td>inner layer</td>
</tr>
</tbody>
</table>
FIG. 27 *Nezara viridula* (L.) (50-51 hr)

Cross section of the embryo through the mid-abdominal region. X 1042.

\[\begin{array}{ll}
\text{am} & \text{.......................... amnion} \\
\text{ect} & \text{.......................... ectoderm} \\
\text{inn. lyr.} & \text{.......................... inner layer}
\end{array}\]
FIG. 28 *Nezara viridula* (L.) (50-51 hr)

Cross section of the embryo through the posterior abdominal region. X 1108.

- **am**: amnion
- **ect**: ectoderm
- **inn. lyr.**: inner layer
FIG. 29  *Nezara viridula* (L.) (50-51 hr)

Cross section of the embryo passing anterior to the mandibular segment. X 656.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>am</td>
<td>amnion</td>
</tr>
<tr>
<td>an. msn.</td>
<td>anterior mesenteron rudiment</td>
</tr>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>inn. lyr.</td>
<td>inner layer</td>
</tr>
</tbody>
</table>
FIG. 30  *Nezara viridula* (L.) (50-51 hr)

Cross section of the embryo through the caudal end.

X 656.

am ............... amnion
ect ............... ectoderm
inn. lyr. ............. inner layer
pc ............... paracyte cell
pt. msn. ............... posterior mesenteron rudiment
FIG. 31 *Nezara viridula* (L.) (53-54 hr)

Cross section of the embryo through the thoracic region.

X 577.

<table>
<thead>
<tr>
<th>am</th>
<th>ect</th>
<th>mes</th>
<th>nb</th>
<th>pc</th>
<th>ser</th>
</tr>
</thead>
<tbody>
<tr>
<td>amnion</td>
<td>ectoderm</td>
<td>mesoderm</td>
<td>neuroblast</td>
<td>paracyte cell</td>
<td>serosa</td>
</tr>
</tbody>
</table>

- am: amnion
- ect: ectoderm
- mes: mesoderm
- nb: neuroblast
- pc: paracyte cell
- ser: serosa
FIG. 32  Nezara viridula (L.) (56-57 hr)

Cross section of the embryo through the thoracic region.

X 521.

am ........................ amnion
ect ........................ ectoderm
ep. sin. ........................ epineural sinus
co. sac ........................ coelomic sac
mes ........................ mesoderm
position of the appendages, are obliquely placed to the longitudinal axis of the embryo. The lateral edges of the germ band start to grow towards the dorsal side by 61-62 hr and the opening of the coelomic sacs towards the epineural sinus is narrowed due to multiplication of the mesodermal cells at the bases. Transverse sections through the thoracic region at this stage often suggest a closure of the opening of the coelomic sacs (Fig. 33), but this is not so (Fig. 34). In fact, the coelomic sacs of the thoracic and labial segments always remain open in the region of the epineural sinus. The mesoderm lining the laterally curved edges of the embryo does not curl over to unite with the mesoderm lining the dorso-lateral side of the neural ganglia to form closed coelomic sacs as reported in *Locusta migratoria migratorioides* Reiche and Frm. (Roonwal, 1936) and *Pieris rapae* (L.) (Eastham, 1930). As development continues, the rapid development of the neural ganglia as well as the spiracles results in the lateral shift of the segmental mesoderm surrounding the opening of the coelomic sacs with the result that the coelomic sacs open in the epineural sinus through a tubular opening by 66-67 hr. The mesoderm which lines the lateral edges of the germ band becomes 2 to 3 cells thick. Though the coelomic sacs of the thoracic and labial segments always remain open towards the epineural sinus, the openings of the coelomic sacs of the maxillary segments into the epineural sinus are temporarily closed at
FIG. 33 *Nezara viridula* (L.) (61-62 hr)

Cross section of the embryo through the thoracic region.

X 441.

<table>
<thead>
<tr>
<th>am</th>
<th>co. sac</th>
<th>ep. sin.</th>
<th>lat. cd.</th>
<th>md. cd.</th>
<th>mes</th>
</tr>
</thead>
<tbody>
<tr>
<td>amnion</td>
<td>coelomic sac</td>
<td>epineural sinus</td>
<td>lateral cord</td>
<td>median cord cells</td>
<td>mesoderm</td>
</tr>
</tbody>
</table>
FIG. 34  *Nezara viridula* (L.) (61-62 hr)

Cross section of the embryo through the thoracic region anterior to Fig. 33. X 491.

am ..........................  amnion
co. sac ..........................  coelomic sac
ect ..........................  ectoderm
ep. sin. ..........................  epineural sinus
mes ..........................  mesoderm
yk ..........................  yolk
this stage (Fig. 35). The mesoderm which lined the lateral sides of the maxillary ganglia has united with the mesoderm which lined the lateral edges of the germ band and has given rise to a solid mass of mesodermal cells at the base of the coelomic sac on either side.

The coelomic cavities originate in the abdominal segments in the following manner: As the lateral edges of the germ band grow dorsad at 56-57 hr, the mesoderm closely follows the ectoderm (Fig. 36). The margins of the mesoderm curl over to enclose a coelomic cavity that is much smaller due to absence of appendages here (Fig. 37). The mesodermal cells then form typical somites on either side, occupying the lateral pockets of the ectoderm segmentally, and enclosing a very minute lumen. It was impossible to ascertain the origin of the coelomic sacs of the abdominal segment X because of the proctodeal covering.

So, in *N. viridula*, the coelomic sacs of thoracic and gnathal segments are formed in the same manner as in *Pyrrhocoris* and *Oncopeltus*. Butt (1949), while describing the embryology of *O. fasciatus*, maintained that the term coelomic cavity could not be applied since no lumen was formed in the mesoderm by folding over of the outer edges like that of *Locusta* or *Pieris*. I agree with Seidel (1924) in referring to these open cavities as coelomic cavities because, essentially, there is no difference in the mode of origin of these sacs in Hemiptera and other insects.
FIG. 35 *Nezara viridula* (L.) (66-67 hr)

Cross section of the embryo through the maxillary segment. X 536.

am .................................... amnion
co. sac .................................. coelomic sac
ect ....................................... ectoderm
ep. sin. .................................. epineural sinus
mes ....................................... mesoderm
neu. grv. ................................. neural groove
FIG. 36  *Nezara viridula* (L.) (56-57 hr)

Cross section of the embryo through the abdominal region.

X 461.

<table>
<thead>
<tr>
<th>am</th>
<th>amnion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>mes</td>
<td>mesoderm</td>
</tr>
<tr>
<td>nb</td>
<td>neuroblast</td>
</tr>
<tr>
<td>yk</td>
<td>yolk</td>
</tr>
</tbody>
</table>
FIG. 37  *Nezara viridula* (L.) (66-67 hr)

Cross section of the embryo through the abdominal region.  
X 575.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>am</td>
<td>amnion</td>
</tr>
<tr>
<td>co. sac</td>
<td>coelomic sac</td>
</tr>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>gng. cl.</td>
<td>ganglionic cells</td>
</tr>
<tr>
<td>neu. grv.</td>
<td>neural groove</td>
</tr>
</tbody>
</table>
However, the mesoderm lining the curved lateral edges of the embryo does not completely fuse with the mesoderm flanking the lateral sides of the ganglia, but a partial union of this mesoderm with the basal half of lateral mesoderm does occur. Secondly, in all the cases, the coelomic sacs lose their typical structure and the lumen becomes continuous with that of the epineural sinus. According to Mellanby (1936), the cells in the longitudinal bands, prior to appendage formation in *Rhodnius prolixus* Stål, become neatly arranged to form somites and the coelomic cavity appears in the middle of each somite. The appendages appear as hollow ectodermal outpushings and the mesoderm migrates in them thus enlarging the coelom. In *N. viridula* such mesodermal somites are not formed.

By the time appendages appear on the thoracic segments (53-54 hr), the lateral bands of mesoderm are formed in almost all the segments except for the posterior extremity of the germ band. These 2 lateral bands of mesoderm are continuous throughout the length of the embryo and show no sign of segmentation. A similar condition was reported in *Pyrrhocoris*, *Oncopeltus* and *Rhodnius* where the inner layer also formed two continuous lateral bands before dividing segmentally. Near the posterior mesenteron rudiment, the mesoderm has not split as yet but lines the still persisting gastral groove as a unicellular layer (Fig. 38).

During the developmental period between 50-55 hr, a
FIG. 38  *Nezara viridula* (L.) (53-54 hr)

Cross section of the embryo through the caudal end.

X 664.

| am     | .......... | amnion |
| ect    | .......... | ectoderm |
| mes    | .......... | mesoderm |
| pc     | .......... | paracryte cells |
| pt. msn| .......... | posterior mesenteron rudiment |
large number of deeply staining nuclei, each appearing to contain a single large nucleolus, is found on the dorsal side of the thoracic segments as well as the lateral and external sides of cephalic and caudal ends of the embryo. Their origin could not be ascertained but most probably these are the degenerating vitellophage cells (Paracyte cells) which were responsible for digestion of yolk in the vicinity of the embryo.

By 56-57 hr, the segmentation of the ectoderm of the germ band is completed. The mesoderm shows no segmentation but consists of 2 lateral bands running the length of the embryo. In the thoracic region, the mesodermal bands are far removed from the median line in the segmental and intersegmental regions (Figs. 32 and 39), but lie comparatively closer to the median line in the abdominal segments (Fig. 36). The mesodermal cells of the lateral bands still form a unicellular covering on the dorsal side of the germ band on either side of the median line and also the inner side of the rudimentary appendages where they form the coelomic sacs (Fig. 32). At this stage the lateral edges of the germ band ectoderm in the anterior abdominal segments and the anterior half of each thoracic segment curve dorsally and are closely followed by the simple mesodermal epithelium. Some of the mesodermal cells of each lateral half of thoracic segments, near the median line, lose their epithelial nature, become globular, and give rise to the blood cells. The blood cells
FIG. 39  *Nezara viridula* (L.) (56-57 hr)

Cross section of the embryo through the intersegmental region between thoracic segments II and III. X 565.

am  .................. amnion
an. md. cd.  ............... anterior median cord cells
ect  .................. ectoderm
mes  .................. mesoderm
lie freely in the epineural sinus, on the dorsal side of the future neural groove, and represent the connection of the two lateral mesodermal bands in the thoracic segments. Thus, the rudiment of the haemocoel is laid down earlier in the thoracic region. The mitotic figures in the mesoderm and ectoderm indicate that further cell proliferation in both layers is greater in the sides of the germ band.

Along with the appearance of gnathal appendages, the stomodaeum invaginates posterior to the labral appendages through the anterior mesodermal cluster. The invaginated (blind) end of the stomodaeum extends inwardly to the anterior mesenteron rudiment in the posterior half of the antennal segment (Fig. 40). The basal part of the stomodaeum becomes enveloped by the mesodermal cells through which it penetrated during invagination. Additional loose mesodermal cells are proliferated from the mesoderm in front of the stomodaeum which fill the lumina of the evaginations of the labral appendages (Fig. 41). While the mesoderm forms a simple epithelial covering over the ectoderm of each appendage-bearing segment before giving rise to coelomic sacs, the mesoderm of the labrum and antennae consists of loose strands of cells proliferated by the anterior mesodermal cluster. These cells later form a simple epithelial lining producing a lumen in the center. As there is a definite evagination corresponding to each labral appendage, supplied with loose mesodermal cells like that
FIG. 40 *Nezara viridula* (L.) (56-57 hr)

Cross section of the embryo through the posterior half of the antenanal segment. X 430.

- **am**: amnion
- **an. msn.**: anterior mesenteron rudiment
- **ant**: antenna
- **ect**: ectoderm
FIG. 41  *Nezara viridula* (L.) (56-57 hr)

Cross section of the embryo through the head lobes.

X 688.

ect .............................. ectoderm
inn. lb. .............................. inner lobe
lr .............................. labrum
mes .............................. mesoderm
nb .............................. neuroblast
of antennae, the labrum thus originates in Nezara viridula as 2 separate appendages.

During subsequent development, the number of mesodermal cells increases in each segment and the segmentation of the mesoderm is completed by 61-62 hr. As the developmental changes take place in the coelomic cavities of the thoracic and abdominal segments, the antennal mesoderm shifts anteriorly on either side of the stomodaeum. Certain loose mesodermal cells are proliferated from unsegmented mesoderm of the posterior extremity of the embryo which envelop the dorsally flexed proctodaeum. The coelomic sacs of the mandibular segment are occluded from the bases of the mandibular appendages and the area becomes occupied by a solid mass of mesodermal cells (Fig. 42). Certain garland-like free cells are proliferated from the dorsal side of this mass of mesoderm which lie over the mandibular neural ganglia and form the rudiment of the sub-oesophageal body.

At 71-72 hr, soon after the completion of somite formation in the abdominal segments, the splitting of mesoderm into somatic and splanchnic layers takes place throughout the length of the embryo. The somatic mesoderm lies close to the ventral ectoderm. It fills the bases of the appendages and gives rise to the fat tissue from its dorsal side (Fig. 43). The splanchnic layer, which arose from the dorsal side of the segmental mesoderm, gets separated from the somatic mesoderm in the gnathal and thoracic segments.
FIG. 42 *Nezara viridula* (L.) (66-67 hr)

Cross section of the embryo through the mandibular segment. X 431.

- **am**: amnion
- **ant**: antenna
- **ect**: ectoderm
- **lat. gam.**: lateral ganglionic mass
- **mes**: mesoderm
- **sb. oes.**: sub-oesophageal body cells
FIG. 43  *Nezara viridula* (L.) (71-72 hr)

Cross section of the embryo through the maxillary segment.

X 685.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>am</td>
<td>amnion</td>
</tr>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>ent</td>
<td>enteron ribbon</td>
</tr>
<tr>
<td>ft</td>
<td>fat cells</td>
</tr>
<tr>
<td>np</td>
<td>neuropile</td>
</tr>
<tr>
<td>so. mes</td>
<td>somatic mesoderm</td>
</tr>
<tr>
<td>sp. mes</td>
<td>splanchnic mesoderm</td>
</tr>
</tbody>
</table>
by this stage and lies on the lateral side in the epineural sinus, between the germ band and the yolk. The splanchnic layer in the abdominal segments lies close to the somatic mesoderm (Fig. 44). At this stage, the splanchnic layer is covered on the outer side by the enteron ribbons in the gnathal segments. These ribbons are lacking in the thoracic and abdominal segments. Simultaneously with the separation of somatic and splanchnic layers, the mesodermal lining of the appendages breaks down to give rise to myoblasts.

The separation of somatic and splanchnic mesoderm is completed in the abdominal segments by 75-76 hr. The enteron ribbons become attached first to the splanchnic mesoderm in the thoracic segments (Fig. 45) and later to that of the abdominal region. The somatic mesoderm in the bases of the appendages splits into different components which will give rise to the muscles of the body, muscles of the appendages and the heart rudiment. The germ cells become arranged dorsally close to the somatic mesoderm of the abdominal segments IV to IX and thus lie embedded between the neural ganglia and the splanchnic mesoderm on either side (Fig. 46). The musculature of the gut will be formed by the splanchnic mesoderm. The intersegmental mesoderm, besides giving rise to blood cells, also forms delicate transverse muscles which pass over the ectoderm and developing nerve cord.

During subsequent development, the differentiation of mesodermal rudiments of the respective organs and tissues
FIG. 44 Nezara viridula (L.) (71-72 hr)

Cross section of the embryo through the abdominal region.

X 512.

am ........................... amnion
ect ........................... ectoderm
so. mes ........................... somatic mesoderm
sp. mes ........................... splanchnic mesoderm
FIG. 45 Nezara viridula (L.) (75-76 hr)

Cross section of the embryo through the thoracic region.

X 547.

am .......................... amnion
ect .......................... ectoderm
ent .......................... enteron ribbon
gng. cl. ....................... ganglionic cells
nb .......................... neuroblast
np .......................... neuropile
so. mes ........................ somatic mesoderm
sp. mes ........................ splanchnic mesoderm
FIG. 46  *Nezara viridula* (L.) (75-76 hr)

Cross section of the embryo through the posterior abdominal region. X 533.

- **am**: amnion
- **ect**: ectoderm
- **ent**: enteron ribbon
- **gm. cl.**: germ cells
- **sp. mes**: splanchnic mesoderm
continues prior to and after blastokinesis. The somatic mesoderm in the appendage-bearing segments gives rise to rudiments of the musculature of the appendages (Fig. 47). The splanchnic mesoderm, along with the enteron ribbons, lines the most lateral edges of the embryo before blastokinesis (Fig. 48). After blastokinesis, when the embryo covers the yolk mass on the ventral half, the splanchnic mesoderm and the enteron form the dorsal covering over the yolk under the body wall.

The development of *N. viridula* shows a combination of both open and closed types of coelomic sacs. The coelomic cavities of thoracic and gnathal segments, when they appear, lie inside the rudiments of the appendages of the respective segments and always remain open to the epineural sinus in the thoracic and labial segments. In the abdominal segments, the mesoderm gives rise to definite somites; the cavity of each somite later becomes contiguous with the epineural sinus.
FIG. 47  *Nezara viridula* (L.) (79-80 hr)

Cross section of the embryo through the thoracic region.

X 439.

| am  | amnion |
| ect | ectoderm |
| ent | enteron ribbon |
| np  | neuropile |
| so. mes | somatic mesoderm |
| sp. mes | splanchnic mesoderm |
FIG. 48  *Nezara viridula* (L.) (84-85 hr)

Cross section of the embryo through the abdominal region.

X 409.

am ................................. amnion
ect ................................. ectoderm
ent ................................. enteron ribbon
gng. cl. ............................ ganglionic cells
sp. mes ............................. splanchnic mesoderm
NERVOUS SYSTEM

(a) Ventral Neural Ganglia:

The nervous system in *N. viridula* differentiates at an early stage, almost at the same time the inner layer is formed. As the inner layer arises as a ridge of cells in the gastral groove, the ectodermal cells of the germ band separate into 2 lateral plates which reunite later to form a continuous ectodermal plate under the inner layer. Certain enlarged cells resembling the neuroblasts, each containing a lightly stained, enlarged, round nucleus, can be recognized in the ectodermal plate by 45 hr. These neuroblasts, which are rudiments of the median cord cells, do not form a continuous cord of cells at this stage but are located at regular space intervals along the mid-ventral line of the germ band.

The lateral neuroblasts, which give rise to the ganglionic nervous system, appear first in the thoracic region at about 53-54 hr, simultaneously with the thoracic appendages. In the next 2-3 hr, the lateral neuroblasts are differentiated throughout the embryo. The neuroblasts, at the time of their appearance, are widely separated from each other but later group together on either side of the median line to form 2 lateral cords of cells extending from the antennal segment to the abdominal segment XI. In each transverse section, the neuroblasts in each lateral cord appear as a row of 2-3 fairly large cells with faintly staining nuclei. These
neuroblasts are easily distinguishable from the ectodermal cells due to their large size and round nucleus. These cells are ectodermal in origin because, at the time of their appearance, they lie embedded in the ventral ectoderm with their outer ends still inserted between the ventral ectodermal cells. They become detached later from the ventral side.

By 56-57 hr, the central neuroblasts have proliferated neurogenic cells throughout the length of the embryo, and a median cord of nerve cells occupies the mid-ventral line of the embryo from the antennal segment to the abdominal segment X. As a result, the ectoderm of the germ band is separated into 2 lateral plates. The median cord consists of 2 groups of nerve cells in each segment, 1 lying in the anterior half of the segment and another in the posterior half. These clumps of nerve cells can be easily distinguished in the thoracic segments at this stage. The anterior median cord clump of cells is well organized and appears as a row of 5-6 columnar cells in a transverse section. The cells constituting the posterior half of the median cord in each segment are fewer in number and only 2 to 3 cells can be detected in each transverse section. In the abdominal region, the distinction between these 2 clumps of nerve cells is not very pronounced. The number of neuroblasts in each of the lateral cords is the same in the abdominal region at this stage of development (Fig. 49) but the neuroblasts have increased in
FIG. 49  *Nezara viridula* (L.) (56-57 hr)

Cross section of the embryo through the abdominal region.

X 1094.

<table>
<thead>
<tr>
<th>am</th>
<th>amnion</th>
</tr>
</thead>
<tbody>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>md. cd.</td>
<td>median cord cells</td>
</tr>
<tr>
<td>mes</td>
<td>mesoderm</td>
</tr>
<tr>
<td>nb</td>
<td>neuroblast</td>
</tr>
</tbody>
</table>
the gnathal and thoracic segments (Fig. 50). Due to higher numbers of neuroblasts in the lateral cords of the thoracic segments, the germ band ectoderm enlarges on either side to form neural ridges. These swellings are less conspicuous in the intersegmental regions. A shallow neural groove is formed on the ventral side of the germ band in the thoracic region and is covered dorsally by the median cord cells. The number of neuroblasts in the posterior half of each segment is higher at this stage than those of the anterior half of the segment (Figs. 51 and 52). The neural ridges form an inverted V-shaped neural groove in the posterior half of the segment covered dorsally by the posterior median cord cells (Fig. 51), while the neural groove in the anterior half of the segment is inverted U-shaped and is covered dorsally by the anterior median cord cells (Fig. 52).

So, in *N. viridula*, the median cord neuroblasts appear earlier than those of the lateral cord. Typical neurogenic cells, the nucleus of each containing a single nucleolus, and the chromatin distributed around the periphery, are also proliferated earlier in the median cord and occupy the mid-ventral line of the germ band from the abdominal segment X to the intersegmental area between antennal and mandibular segments. According to Springer (1967), the median cord in *Oncopeltus* also consists of an alternation of 2 groups of cells which differ from each other in the staining reaction as well as the size of the nuclei of their cells. There is
FIG. 50  *Nezara viridula* (L.) (56-57 hr)

Cross section of the embryo through the thoracic region. X 1354.

- **ect**: ectoderm
- **md. cd.**: median cord cells
- **mes**: mesoderm
- **nb**: neuroblast
- **neu. grv.**: neural groove
- **neu. rdg.**: neural ridge
FIG. 51 Nezara viridula (L.) (56-57 hr)

Cross section of the embryo through the posterior half of thoracic segment, II. X 1215.

nb ............... neuroblast
neu. grv. ............ neural groove
neu. rdg. ............ neural ridge
pt. md. cd. ............ posterior median cord cells
FIG. 52  *Nezara viridula* (L.) (56-57 hr)

Cross section of the embryo through the anterior half of thoracic segment, II.  X 1309.

- **an. md. cd.** ............... anterior median cord cells
- **nb** ..................... neuroblast
- **neu. grv.** ................. neural groove
no difference in size or staining of the nuclei of the two groups of cells in the median cord in *N. viridula*. They differ only in their cellular organization.

As development proceeds, the concentration of neuroblasts in the anterior half of the segment is initiated and continues until the completion of typical ganglia formation. By 61-62 hr, the neural ridges in the anterior half of thoracic segments become thicker. The neural ridges appear to be composed mostly of the neuroblasts, and a few ectodermal cells line their ventral side (Fig. 53). The neural groove is shallower, at this stage, in the mandibular and maxillary segments than that of thoracic and labial segments (Fig. 54). Posterior to the abdominal segment I, the germ band does not indicate the formation of a neural groove as yet (Fig. 55). The proliferation of daughter cells by the neuroblasts of lateral cords occurs between 62-67 hr. The daughter cells, which are darker and resemble the mesodermal cells at the time of their production, are proliferated towards the dorsal side while the neuroblasts occupy the ventral position near the ectoderm (Fig. 56). The daughter cells have been observed to divide again. During this time, when the daughter cells are being produced by the neuroblasts, the neural groove increases to its maximum depth. The neural groove, which was lined earlier mostly by the neuroblasts, becomes lined with the ectodermal cells forming a pocket around each lateral ganglion mass. As a result of concentration, the numbers of
FIG. 53 Nezara viridula (L.) (61-62 hr)

Cross section of the embryo through the anterior half of a thoracic segment. X 1094.

an. md. cd. ................. anterior median cord cells
ect ....................... ectoderm
ep. sin ...................... epineural sinus
mes ......................... mesoderm
nb ......................... neuroblast
neu. grv. ................... neural groove
FIG. 54 *Nezara viridula* (L.) (61-62 hr)

Cross section of the embryo through the maxillary segment. X 688.

am .................... amnion
ect .................... ectoderm
md. cd. .................... median cord cells
mes .................... mesoderm
nb .................... neuroblast
neu. grv. .................... neural groove
FIG. 55  *Nezara viridula* (L.) (61-62 hr)

Cross section of the embryo through the abdominal region.  X 1031.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ect</td>
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</tr>
<tr>
<td>md. cd.</td>
<td>median cord cells</td>
</tr>
<tr>
<td>mes</td>
<td>mesoderm</td>
</tr>
<tr>
<td>nb</td>
<td>neuroblast</td>
</tr>
</tbody>
</table>
FIG. 56 *Nezara viridula* (L.) (66-67 hr)

Cross section of the embryo through the anterior half of a thoracic segment. X 1094.

- an. md. cd. ............... anterior median cord cells
- ect ..................... ectoderm
- gng. cl. ................. ganglionic cells
- nb ...................... neuroblast
- neu. grv. ............... neural groove
neuroblasts and daughter nerve cells increase in the anterior end of the segment, thus widening the neural mass in that region. At the same time, the cells of the anterior median cord also become concentrated in the anterior of each segment and lie arched over the ganglia of each segmental pair. The anterior median cord cells still maintain their cellular organization and have started losing their connection with the underlying open neural groove due to development of vacuoles in their ventral cytoplasm. The cells of the posterior median cord also shift anteriorly and lie wedged between the posterior halves of the lateral ganglion mass (Fig. 57). These cells are connected to the ectoderm of the closed neural groove and possess reticulate cytoplasm.

By 71-72 hr, additional ganglionic cells have been produced but the neuroblasts still exist on the ventral side. The ganglionic cells have given rise to the neuropile on the dorsal side (Fig. 58). The anterior median cord cells lie arched over the anterior regions of the paired ganglia and have lost their connection with the neural groove. The processes of the anterior median cord cells become continuous with the neuropile of the respective ganglionic mass of either side. The posterior median cord cells have organized to form a gangliomere in the middle of the neural mass which lies at the dorsal level of the lateral ganglionic mass (Fig. 59). Posteriorly, this gangliomere is continuous and lies sandwiched between the neural masses (Fig. 60). The
FIG. 57 *Nezara viridula* (L.) (66-67 hr)

Cross section of the embryo through the middle of a thoracic segment. X 1165.

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>gng. cl.</td>
<td>ganglionic cells</td>
</tr>
<tr>
<td>mes</td>
<td>mesoderm</td>
</tr>
<tr>
<td>nb</td>
<td>neuroblast</td>
</tr>
<tr>
<td>pt. md. cd.</td>
<td>posterior median cord cells</td>
</tr>
</tbody>
</table>
FIG. 58 *Nezara viridula* (L.) (71-72 hr)

Cross section of the embryo through the anterior portion of labial segment. X 692.

<table>
<thead>
<tr>
<th>Term</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>an. md. cd.</td>
<td>anterior median cord cells</td>
</tr>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>nb</td>
<td>neuroblast</td>
</tr>
<tr>
<td>neu. grv.</td>
<td>neural groove</td>
</tr>
<tr>
<td>np</td>
<td>neuropile</td>
</tr>
</tbody>
</table>
FIG. 59  *Nezara viridula* (L.) (71-72 hr)

Cross section of the embryo through the middle of labial segment.  X 692.

- **ect**: ectoderm
- **lat. gam.**: lateral ganglionic mass
- **neu. grv.**: neural groove
- **pt. md. cd.**: posterior median cord cells
FIG. 60 *Nezara viridula* (L.) (71-72 hr)

Cross section of the embryo through the labial segment posterior to Fig. 59. X 692.

- ect: ectoderm
- ent: enteron ribbon
- gng. cl.: ganglionic cells
- neu. grv.: neural groove
- pt. md. cd.: posterior median cord cells
- sp. mes: splanchnic mesoderm
numbers of neuroblasts and daughter cells are fewer in the posterior half of the thoracic segments. The neural groove in the abdominal region has been formed by this time and the neuroblasts have given rise to daughter cells (Fig. 61). The sub-neural sinus has appeared on the ventral side of the lateral ganglionic mass of the thoracic region. The sub-neural sinus first arises between the neuroblasts and the ectoderm of the posterior half of the lateral ganglionic mass.

In later development, the ectodermal walls of the neural groove in the antero-ventral side of the ganglionic mass fuse and disintegrate, but the groove still exists in the posterior half of the segment by 79-80 hr. The 2 lateral ganglionic masses become continuous anteriorly and give rise to the rudiments of typical ganglia. The anterior median cord cells, after giving rise to cross commissures, become incorporated into the antero-dorsal portions of the ganglia. The neuropile becomes elongated longitudinally but is still open dorsally by 79-80 hr. The existing median cord, at this stage, is the posterior median cord. The cells of the posterior median cord migrate anteriorly along with the concentration of the ganglionic tissue with the result that the gangliomere of the posterior median cord comes to occupy the mid-dorsal position of the partially united ganglionic masses and lies posterior to the cross commissure (Fig. 62). Posteriorly, this gangliomere is continuous with the median
FIG. 61 *Nezara viridula* (L.) (71-72 hr)

Cross section of the embryo through the abdominal region. 

X 497.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>am</td>
<td>amnion</td>
</tr>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>neu. grv.</td>
<td>neural groove</td>
</tr>
<tr>
<td>so. mes</td>
<td>somatic mesoderm</td>
</tr>
<tr>
<td>sp. mes</td>
<td>splanchnic mesoderm</td>
</tr>
</tbody>
</table>
FIG. 62 *Nezara viridula* (L.) (79-80 hr)

Cross section of the embryo through the anterior portion of a thoracic segment showing part of cross commissure and posterior median cord gangliomere. X 1082.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>cr. com.</td>
<td>cross commissure</td>
</tr>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>gng. cl.</td>
<td>ganglionic cells</td>
</tr>
<tr>
<td>nb</td>
<td>neuroblast</td>
</tr>
<tr>
<td>np</td>
<td>neuropile</td>
</tr>
<tr>
<td>pt. md. cd.</td>
<td>posterior median cord cells</td>
</tr>
</tbody>
</table>
cord cells which still separate the posterior half of the lateral ganglionic mass and are connected to the neural groove (Fig. 63). Certain darkly staining cells are proliferated from the posterior median cord which cover dorsally the neuropiles of lateral ganglia. These are the rudiments of the inner sheath. As development continues, the lateral ganglionic cells cover the neuropile dorsally and typical ganglia are formed after blastokinesis. The later development of ventral ganglia prior to and after blastokinesis has not been described because it follows essentially the same pattern as reported for Oncopeltus. An excellent account of the developmental changes in ventral neural ganglia of O. fasciatus during and after blastokinesis has been presented by Springer (1967).

Thus, in N. viridula, the cells of the median cord are neurogenic in origin and contribute to the formation of ganglia depending upon their location and stage of development of the ganglia. The anterior median cord cells contribute to the anterior portions of ganglia and become indistinguishable earlier than the posterior median cord cells which contribute to the posterior regions of the ganglia in later development. The anterior median cord cells give rise to cross commissures while the posterior median cord cells give rise to a single gangliomere between lateral ganglion masses.
FIG. 63 Nezara viridula (L.) (79-80 hr)

Cross section of the embryo through the middle of a thoracic segment. X 1082.

<table>
<thead>
<tr>
<th>Abb</th>
<th>Meaning</th>
</tr>
</thead>
<tbody>
<tr>
<td>ect.</td>
<td>ectoderm</td>
</tr>
<tr>
<td>gng. cl.</td>
<td>ganglionic cells</td>
</tr>
<tr>
<td>nb</td>
<td>neuroblast</td>
</tr>
<tr>
<td>neu. grv.</td>
<td>neural groove</td>
</tr>
<tr>
<td>np</td>
<td>neuropile</td>
</tr>
<tr>
<td>pt. md. cd.</td>
<td>posterior median cord cells</td>
</tr>
</tbody>
</table>
(b) **Brain:**

The mode of participation of neural tissue of different segments to give rise to the brain of insects is a very controversial issue. There is no doubt that the supra-oesophageal brain, in its definite shape, is composed of 3 ganglia, protocerebrum, deutocerebrum and tritocerebrum. The brain is connected to the sub-oesophageal ganglia by means of circum-oesophageal connectives. The sub-oesophageal part of the brain is composed of the 3 ganglia of the gnathal segments. Most workers in embryology have reported that the tritocerebrum arises from an intercalary segment which lies between the antennal and mandibular segments. A few workers have even reported the presence of rudimentary appendages and definite mesodermal somites corresponding to that segment. According to them, all the characteristics of the segment are lost soon after their appearance except for the nervous tissue. In Hemiptera, such a segment has also been reported by Mellanby (1936) in *R. prolixus*. Butt (1949) has also reported the presence of a tritocerebrum in *Oncopeltus* but has not explained the origin of this pair of ganglia. In *N. viridula*, an intercalary segment and the various associated embryological tissue were not found. The brain in the typical form is composed of 3 pairs of ganglia, but I believe that the tritocerebral ganglia develop on the lateral sides of the labral appendages.

By the time the amniotic cavity is closed anteriorly,
the head lobes lie on the dorsal side of the egg and are flexed over the dorsal side of the germ band, where their lateral edges, composed of small ectodermal cells, are continuous with the amniotic cells. The bases of the head lobes are in the same plane with the ventral surface of the germ band. A median plate of ectodermal cells extends, sandwiched between the bases of the head lobes, from the antennal segment to the anterior end of the germ band by 50-51 hr. The head lobes are thus composed of two kinds of cells at this stage. The darkly staining ectodermal cells occupy the middle of their bases and the postero-dorsal ends while the rest of the head lobes consist of neuroblast-like cells.

The labral appendages arise as lateral evaginations from the anterior end of the germ band by 55 hr of development and the invagination of ectoderm between them gives rise to the stomodaeum. By 56-57 hr, the labral appendages, as well as the stomodaeum, become prominent. Each head lobe at this stage is divided into 2 lobes; 1 inner lobe lying next to the labral appendages, and 1 external or lateral lobe (Fig. 64). The inner lobe is thus the rudiment of the labral ganglion and the lateral lobe that of the protocerebrum (protocerebral ganglion). By 61-62 hr, the ectodermal cells of the posterior margin of the lateral lobes grow inward to separate off a postero-dorsal lobe, the optic lobe. So by this stage, each head lobe consists of 3 parts.
FIG. 64 *Nezara viridula* (L.) (56-57 hr)

Cross section of the embryo through the head lobes.

X 540.

inn. lb. ................................................. inner lobe
lat. lb. ................................................. lateral lobe
lr ...................................................... labrum
mes .................................................... mesoderm
The inner lobes, which lie on either side of the labral appendages, are ventral in position (Fig. 65). The lateral lobes are biggest in size and are the rudiments of protocerebral ganglia. The latero-dorsal lobes, which are rudimentary optic lobes, lie at the same level as the anterior portion of the antennal segment (Fig. 66). The ectoderm of the antennal segment in the anterior half becomes divided as a result of the stomodeal invagination, and the neuroblasts which will give rise to antennal ganglia lie on either side of the blind end of the stomodaeum (Fig. 67). Therefore, at this stage all three lobes of each head lobe contain neuroblasts. The posterior half of the antennal segment is mostly occupied by median cord cells which extend posteriorly and are continuous with the anterior median cord cells of the mandibular segment (Figs. 68 and 69).

As development continues, the ectodermal cells of the bases of the labrum, as well as those of the postero-dorsal side of the head lobes, unite to form a dorsal covering over the protocerebral ganglia by 66-67 hr (Fig. 70). These ectodermal cells will later form a covering also on the ventral side of the protocerebrum. The number of neuroblasts in the protocerebrum has increased and they have become arranged in longitudinal rows (Fig. 71). The lateral ectoderm of the germ band in the antennal segment becomes continuous with the ectodermal cells of the dorsal side of the head lobes. As the cephalic lobes increase in size, this growth is accommodated
FIG. 65 Nezara viridula (L.) (61-62 hr)

Cross section of the embryo through the anterior portion of the head lobes. X 542.

inn. lb. .......................... inner lobe
lat. lb. .......................... lateral lobe
lr ............................... labral appendage
mes .............................. mesoderm
FIG. 66 *Nezara viridula* (L.) (61-62 hr)

Cross section of the embryo through the anterior half of the antennal segment. X 430.

<table>
<thead>
<tr>
<th>mes</th>
<th>mesoderm</th>
</tr>
</thead>
<tbody>
<tr>
<td>op. lb.</td>
<td>optic lobe</td>
</tr>
<tr>
<td>st</td>
<td>stomodaeum</td>
</tr>
</tbody>
</table>
FIG. 67  Nezara viridula (L.) (61-62 hr)

Cross section of the embryo through the anterior half of the antennal segment. X 690.

ect .......................... ectoderm
mes .......................... mesoderm
nb .......................... neuroblast
st .......................... stomodaeum
FIG. 68  Nezara viridula (L.) (61-62 hr)

Cross section of the embryo through the posterior half of the antennal segment.  X 544.

| an. msn. | .......... | anterior mesenteron rudiment |
| ant     | .......... | antenna                     |
| md. cd. | .......... | median cord cells           |
| mes     | .......... | mesoderm                    |
| nb      | .......... | neuroblast                  |
FIG. 69 *Nezara viridula* (L.) (61-62 hr)

Cross section of the embryo through the intersegmental region between antennal and mandibular segments. X 540.

<table>
<thead>
<tr>
<th>ant</th>
<th>antenna</th>
</tr>
</thead>
<tbody>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>md. cd.</td>
<td>median cord cells</td>
</tr>
</tbody>
</table>
FIG. 70 *Nezara viridula* (L.) (Figure 70)

Cross section of the head lobes anterior to the stomodaeum

- ect: ectoderm
- inn. lb.: inner lobe
- lat. lb.: lateral lobe
- lr: labrum
- mes: mesoderm
FIG. 70  *Nezara viridula* (L.) (66-67 hr)

Cross section of the embryo through the head lobes anterior to the stomodaeum. X 422.

- ect: ectoderm
- inn. lb.: inner lobe
- lat. lb.: lateral lobe
- lr: labrum
- mes: mesoderm
FIG. 71 Nezara viridula (L.) (66-67 hr)

Cross section of the embryo through the head lobes anterior to the stomodaeum. X 677.

ect .......................... ectoderm
inn. lb. .......................... inner lobe
lat. lb. .......................... lateral lobe
lr ................................ labrum
mes ............................. mesoderm
nb ............................. neuroblast
by a slight rotation of the head on its antero-posterior axis, and contraction of the germ band takes place. The bases of the antennae shift near to the postero-dorsal side of the protocerebral lobes. The labrum and the oral opening move posteriorly on the ventral side of the germ band (Fig. 16). Therefore, by 71-72 hr, the antennae have become attached to the postero-dorsal side of the protocerebral lobes near the median line, and the labrum, with partially united distal ends, forms a flap over the oral opening, which has also shifted posteriorly. The neuroblasts have given rise to ganglion cells in the respective lobes of the brain. A transverse section through the anterior end of the germ band indicates that the protocerebral ganglia are very large and are covered on the dorsal as well as the ventral sides by ectodermal cells (Fig. 72). A transverse section through the middle of the head lobes, anterior to the stomodaeum, shows that the labral ganglia lie close to the oral opening. The optic rudiment is dorso-lateral while the antennal ganglia lie on the dorsal side and are thus postero-dorsal to the protocerebral ganglia (Fig. 73). The region between the mandibular and antennal segments is filled with median cord cells which become divided into two groups due to posterior penetration of the stomodaeum close to the ventral ectoderm. While the ventral neural ganglia, including the gnathal ganglia, have given rise to neuropiles, ganglion cells of the brain do not show neuropiles at this stage.
FIG. 72 *Nezara viridula* (L.) (71-72 hr)

Cross section of the embryo through the anterior end of the head lobes. X 594.

- **ect**: ectoderm
- **gng. cl.**: ganglionic cells
- **lr**: labrum
- **nb**: neuroblast
FIG. 73 *Nezara viridula* (L.) (71-72 hr)

Cross section of the embryo through the middle of the head lobes anterior to the stomodaeum. X 553.

ant. ggl. ................................ antennal ganglion
lr ........................................... labrum
lr. ggl. ....................................... labral ganglion
op ............................................. optic rudiment
During subsequent development, nerve cells in the brain start to give rise to neural processes. The definitive shape of the brain is initiated. A frontal section close to the ventral ectoderm (Fig. 74), shows only part of the protocerebral and the labral ganglia. A frontal section a little posterior to Fig. 74 shows that the protocerebral ganglia have become continuous with each other on the dorsal side of the stomodaeum (Fig. 75). Note that only a part of the deutocerebral ganglia (the antennal ganglia) appears in this section. A frontal section of the head, passing through the postero-dorsal side of the protocerebral lobes, shows the exact location and the point of insertion of the antennae and the antennal ganglia (deutocerebrum) (Fig. 76). Thus, the antennae and the deutocerebrum, which arise post-orally early in development, become pre-oral due to their shifting to the postero-dorsal side of the protocerebral lobes.

As folding of the embryo continues in the following hours prior to blastokinesis, more ganglionic cells are proliferated and the neuropiles arise in the brain ganglia. The median cord cells anterior to the mandibular segment contribute to the labral ganglia (which may now be called the tritocerebrum) and also form the circum-oesophageal connectives. The sub-oesophageal commissure between the tritocerebral ganglia is formed by the median cord cells. A transverse section of the head of the embryo after blastokinesis shows all 3 ganglia of the brain (Fig. 77).
FIG. 74  *Nezara viridula* (L.) (75-76 hr)

A frontal section through the anterior end of the embryo.
X 511.

ent  ......................  enteron ribbon
lr. ggl.  ....................  labral ganglion
md. cd.  ......................  median cord cells
mes  .........................  mesoderm
pro. ggl.  .....................  protocerebral ganglion
FIG. 75  *Nezara viridula* (L.) (75-76 hr)

Frontal section of the embryo posterior to Fig. 74.

X 529.

ant. ggl.  ......................  antennal ganglion
ent  .........................  enteron ribbon
lr. ggl.  ......................  labral ganglion
mes  .........................  mesoderm
pro. ggl.  ......................  protocerebral ganglion
st  .........................  stomodaeum
FIG. 76 *Nezara viridula* (L.) (75-76 hr)

A frontal section of the embryo posterior to Fig. 75.

X 443.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ant</td>
<td>antenna</td>
</tr>
<tr>
<td>ant. ggl.</td>
<td>antennal ganglion</td>
</tr>
<tr>
<td>ent</td>
<td>enteron ribbon</td>
</tr>
<tr>
<td>lr</td>
<td>labrum</td>
</tr>
<tr>
<td>mb. ggl.</td>
<td>mandibular ganglion</td>
</tr>
<tr>
<td>sp. mes</td>
<td>splanchnic mesoderm</td>
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</tbody>
</table>
FIG. 77 Nezara viridula (L.) (99 hr)

Cross section of the egg passing through the head of the embryo at the anterior pole. X 551.

| deut | .......... | deutocerebrum (antennal ganglion) |
| prot | .......... | protocerebrum                     |
| trit | .......... | tritocerebrum (labral ganglion)    |
DIGESTIVE SYSTEM

The alimentary canal consists of 3 parts, the foregut, midgut and the hindgut. These sections of the alimentary canal originate separately and then unite to form the typical adult digestive system. The mode of development of midgut in insects has been the most controversial topic among the early workers. It appears that the diverse interpretations of the mesenteron rudiment in insects by early workers have been due to their efforts to homologize it with the endoderm of a typical invertebrate gastrula. The latest review of the early theories regarding the origin of endoderm in insects has been presented by Johannsen and Butt (1941).

The mesenteron rudiments in N. viridula are differentiated before the appearance of stomodaeum and proctodaeum. The anterior mesenteron rudiment occupies the posterior antennal segment and extends into intersegmental region between antennal and mandibular segments by 50-51 hr of development (Fig. 29). The posterior mesenteron rudiment occupies the posterior extremity of the germ band (Fig. 30). Thus the rudiments of midgut arise earlier than the appearance of foregut and hindgut rudiments and lie dorsal to the ectoderm.

The stomodaeum appears as a shallow invagination in the ventral ectoderm immediately posterior to the rudiments of labral appendages at 53-54 hr of development. In the following hours, the stomodaeum and labral appendages become
prominent. As the stomodaeum invaginates, the cells of the anterior mesodermal cluster surround its basal region while the distal end extends up to the anterior mesenteron rudiment. As a result of cell multiplication, the anterior mesenteron rudiment becomes larger than the posterior mesenteron rudiment by 61-62 hr and lines the blind end of the stomodaeum as a compact mass of cells (Figs. 67 and 68). As development continues, this compact mass of cells forms a multicellular layer occupying the mid-dorsal area of the germ band. Cell proliferation occurs in the form of two posteriorly growing ribbons on the dorsal side of the germ band (Fig. 78). By 71-72 hr, the enteron ribbons from the anterior mesenteron rudiment extend posteriorly to the labial segment. In the next 3-4 hr, these ribbons reach the third thoracic segment. Both enteron ribbons lie dorsally in the lateral sides of the epineural sinus and are closely associated with the splanchnic mesoderm (Fig. 45). These enteron ribbons do not extend beyond the thoracic region but are joined later at 79-80 hr by the anteriorly growing enteron ribbons of the posterior mesenteron rudiment.

The proctodeal invagination becomes visible in the germ band a little later than the stomodaeum. At 50-51 hr, the posterior end of the germ band is continuous on the ventral side with the amnion cells which are similar to the ectodermal cells. The ectoderm and amnion then fold back by 53-54 hr and form a small pouch at the posterior end. In the
FIG. 78 Nezara viridula (L.) (71-72 hr)

Cross section of the embryo passing anterior to the mandibular segment. X 694.

ect ........................................... ectoderm
ent ............................................ enteron ribbon
next 2-3 hr, the rudiment of the proctodaeum arises as a shallow invagination of the ectoderm of the pouch. At this stage the dorsal wall of the rudiment is continuous with the amnion cells. The proctodaeum then develops anteriorly as a tube on the underlying mesoderm with its blind end in direct contact with the posterior mesenteron rudiment. The posterior mesenteron rudiment, which is located now at the abdominal segment X, does not proliferate cells until later. By 66-67 hr, the proctodaeum extends up to the abdominal segment IX (Fig. 14). The primordia of the malpighian tubules appear as four lateral ectodermal evaginations on the ventral side of the proctodaeum by 71-72 hr. A transverse section near the anterior end of the proctodaeum (Fig. 79) shows the relationship of only 2 of them to the proctodaeum. The malpighian tubules are surrounded by mesodermal cells. Ectodermal cells in the dorsal wall of proctodaeum multiply and form a thick layer by 79-80 hr (Fig. 80).

Cell proliferation from the posterior mesenteron rudiment begins at 71-72 hr of development. The enteron ribbons from the posterior mesenteron rudiment line the dorsal side of the splanchnic mesoderm of the abdominal segments VII and VIII. By 75-76 hr, the posterior enteron ribbons extend forward to the abdominal segment IV. By 79-80 hr, both the anterior and posterior enteron ribbons become continuous with each other and so the rudiment of the midgut is laid down on the dorsal side of the germ band as 2 continuous lateral ribbons which
FIG. 79 *Nezara viridula* (L.) (71-72 hr)

Cross section of the embryo through the caudal end.

X 659.

mes .......................... mesoderm
mp. tub. ........................ malpighian tubule
pr ............................... proctodaeum
FIG. 80 *Nezara viridula* (L.) (79-80 hr)

Cross section of the embryo through the abdominal segment X. X 521.

- **ect**: ectoderm
- **gng. cl.**: ganglionic cells
- **mes**: mesoderm
- **mp.**: malpighian tubule
- **pr**: proctodaeum
are closely associated with the splanchnic mesoderm.

During subsequent development, the stomodaeum and proctodaeum elongate. Due to folding of the germ band prior to blastokinesis on the ventral side, the enteron ribbons become lateral in position (Fig. 48). After blastokinesis, the enteron ribbons line the yolk mass and are covered externally by the splanchnic mesoderm as well as by the body wall (Fig. 81). The lateral margins of entodermal strands of cells then grow dorsally and meet along the dorsal line to form the tubular covering around the yolk. The splanchnic mesoderm also follows the developing midgut ribbons and will eventually form the gut musculature. A transverse section of the embryo at the posterior pole, after blastokinesis, shows that the proctodaeum is still a blind tube and the malpighian tubules in 2 lateral groups occupy the area near the rectum (Fig. 82).

A similar bipolar origin of the midgut has been described for many insects by other workers. Researchers studying the development of the midguts are divided into 2 groups. One group believes that the stomodeal and proctodeal invaginations give rise to the mesenteron rudiments while the other believes the mesenteron rudiments arise independently of the stomodeal and proctodeal invaginations. The latter group is again subdivided into 2 categories depending upon the participation of the inner layer in the origin of mesenteron rudiments. In Hemiptera, the development of the midgut from
FIG. 81  Nezara viridula (L.) (86-87 hr)

Cross section of the egg through the abdominal region of the embryo. The right side of the egg presented here. X 426.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>ect</td>
<td>ectoderm</td>
</tr>
<tr>
<td>ent</td>
<td>enteron ribbon</td>
</tr>
<tr>
<td>sp. mes</td>
<td>splanchnic mesoderm</td>
</tr>
<tr>
<td>yk</td>
<td>yolk mass</td>
</tr>
</tbody>
</table>
FIG. 82  *Nezara viridula* (L.) (99 hr)

Cross section through the caudal end of the embryo.

X 313.

as .......................... anus
mp. tub. ........................ malpighian tubule
pr ............................. proctodaeum
mesenteron rudiments has been recorded in _R. prolixus_ (Mellanby, 1936) and _P. apterus_ (Seidel, 1924). Butt (1949), failed to find such mesenteron rudiments prior to the stomodeal and proctodeal invaginations in _O. fasciatus_. He later recognized 2 masses of cells which developed independently of the inner layer and believed that these proliferated cells were from the stomodaeum and proctodaeum. This means that the midgut in _Oncopeltus_ is either ectodermal in origin or develops from certain undifferentiated cells at the tip of these ectodermal invaginations of fore-and hindgut. The midgut in _N. viridula_ develops from the 2 endodermal rudiments which arise independently of mesoderm and are carried inwards attached to the tips of the stomodaeum and proctodaeum when they develop.
SUMMARY

Embryos of *Nezara viridula* (L.) incubated at 27°C and 55-60% humidity undergo blastokinesis at 85-86 hr and hatching at 140-144 hr after egg deposition.

The inner layer (mesoderm) covers the germ band as a unicellular layer. It extends laterally from the median, follows the ectodermal evaginations of the thoracic and gnathal appendages where it forms the coelomic sacs. This occurs earlier in the thoracic region. Loose mesodermal cells from the anterior mesodermal cluster fill the lumina of the antennal and labral appendages. The lumina of the coelomic sacs of the labial and thoracic segments always remain open and contiguous with the epineural sinus. Mesodermal somites are formed in the abdominal segments. At 71-72 hr, the mesoderm splits into the somatic and splanchnic portions. The latter becomes covered dorsally by the enteron ribbons from the mesenteron rudiments. The development of the muscular and circulatory systems from the somatic mesoderm is not included in this dissertation.

A pair of labral appendages arise in the anterior end of the germ band at 53-54 hr. They fuse to form a bilobed labrum which shifts posteriorly and covers the oral opening on the ventral side of the germ band by 75-76 hr. The antennae, which arise post-orally become pre-oral and lie on the dorsal side of the head lobes by 75-76 hr.

The ventral neural ganglia follow a similar pattern of
development to that of *Oncopeltus fasciatus* (Dallas). Unlike *Oncopeltus*, the median cord cells arise earlier than the lateral cord neuroblasts and form two groups of cells in each segment. These cells are neurogenic in origin and form the cross commissures as well as contribute to the formation of segmental pair of ganglia. The tritocerebral ganglia of the brain do not arise from an intercalary segment, which is lacking in *N. viridula*, but develop lateral to the labral appendages and are pushed posteriorly to their definitive position by the protocerebral ganglia. The median cord cells between the antennal and mandibular segments contribute to the formation of the sub-oesophageal connectives.

A pair of mesenteron rudiments can be distinguished in the embryo at 50-51 hr, 1 located in the posterior half of the antennal segment and 1 near the posterior extremity of the embryo. These rudiments appear earlier than the foregut and hindgut rudiments. The midgut is formed by cell proliferation from these 2 mesenteron rudiments. The endodermal cells of the midgut completely cover the yolk mass and lumina of the foregut and hindgut are not connected with the midgut at eclosion. There are 4 malpighian tubules which are ectodermal in origin.
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