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Embryonic Development of Alimentary Canal in the Primitive Moth, *Endoclita signifer* WALKER (Lepidoptera, Hepialidae)*

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Synopsis The formation of alimentary canal in the embryo of the monotrysian moth, *Endoclita signifer*, is described in reference to other Lepidoptera and Trichoptera. The stomodaeum is formed as an invagination at the medio-posterior portion of the cephalic regions of the embryo. After revolution, paired longitudinal folds like a conduit arise from the inner wall of the stomodaeum along its ventro-midline. Such a strange structure has never been known in other insect embryos. The proctodaeum arises asan extension of the amnio-proctodaeal cavity, i.e., the dorsal proctodaeal wall originates from the amnion and the ventral one from the posterior region of the telson. The mid-gut epithelium originates exclusively from the anlage arising from the blind end of the stomodaeum, thus belonging to the unipolar formation which is uncommon in insects. Malpighian tubules arise as three pairs of evaginations from the blind end of the proctodaeum.

Introduction

Much work has been done on the ditrysian lepidopteran embryogenesis, but nothing is known about the organogenesis of the monotrysian Lepidoptera. In our previous papers (ANDO & TANAKA, 1976, 1980; TANAKA, 1981) on the early embryology of monotrysian moths, *Endoclita signifer* and *E. excrescens*, we found a germ rudiment formation in these species quite dissimilar to those in the ditrysian Lepidoptera known so far, but similar to those in *Neomicropteryx nipponensis* (ANDO

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& KOBAYASHI, 1978; KOBAYASHI & ANDO, 1980, 1981) belonging to the most primitive suborder Zeugloptera, and in *Stenopsyche griseipennis* (MIYAKAWA, 1973, 1974) belonging to the Trichoptera.

This paper deals with the organogenesis of *E. signifer* centering on the formation of its alimentary canal.

Materials and Methods

Eggs of various ages were fixed, sectioned, and examined according to procedures previously described by ANDO and TANAKA (1980).

Observations

1. *Stages of embryonic development*

The duration of development from oviposition to hatching for *E. signifer* (incubated at 25°–30°C) was about 280 hours. For the convenience of description the period is divided into 14 stages as follows.

Stage 1 (0–40 min): Maturation division and fertilization.

Stage 2 (1–6 hr): Cleavage, formation of blastoderm.

Stage 3 (7–15 hr): Formation of germ rudiment and germ band.

Stage 4 (16–18 hr): Differentiation of protocephalon and formation of inner layer (Figs. 1, 2, 3). Detail histological description on the Stages 1 to 4 has been given in the previous paper (ANDO & TANAKA, 1980).

Stage 5 (29–35 hr): Segmentation of gnatho-thoracic region, appearance of labral and antennal anlagen and formation of neutral groove (Figs. 4, 5).

Stage 6 (35–48 hr): Appearance of gnathal (premandibular, mandibular, maxillary and labial) and thoracic appendages, and formation of stomodaeum (Figs. 6, 7).

Stage 7 (49–55 hr): The longest germ band, and appearance of pleuropodia in the 1st abdominal segment (Fig. 8).

Stage 8 (56–67 hr): Formation of proctodaeum (Fig. 9).

Stage 9 (68–80 hr): Appearance of ectodermal invagination in gnathal and thoracic region, and formation of leg anlagen on each abdonimal segment (Figs. 10, 11).

Stage 10 (81–120 hr): Consolidation of cephalognathal region and completion of abdominal legs in segments 3–6 and 10 (Figs. 12, 13, 14).

Stage 11 (121–140 hr): Revolution of embryo (Fig. 15).

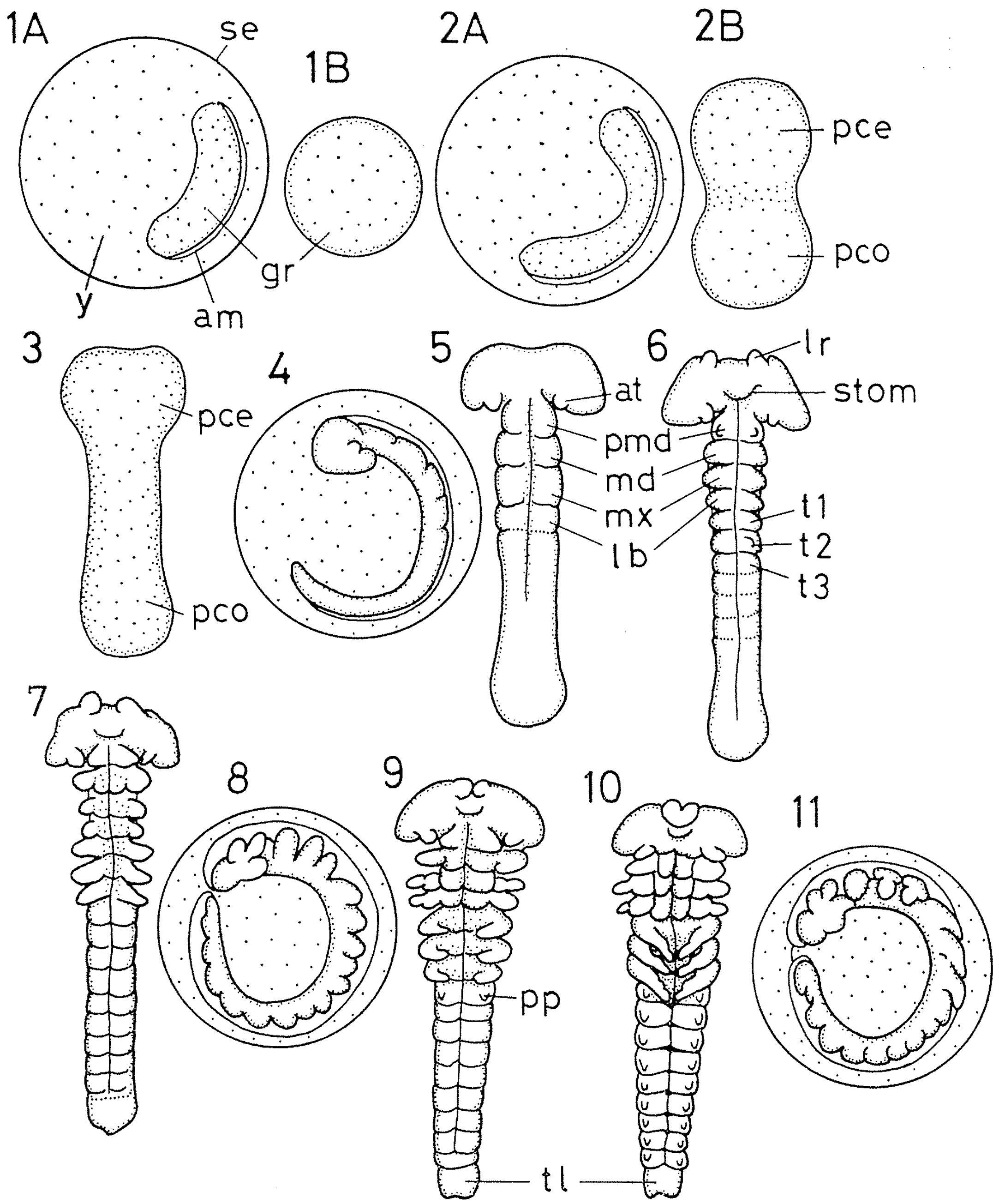
Stage 12 (141–180 hr): Completion of dorsal closure.

Stage 13 (181–240 hr): Embryonic moulting and formation of basic form of 1st instar larva (Fig. 16).

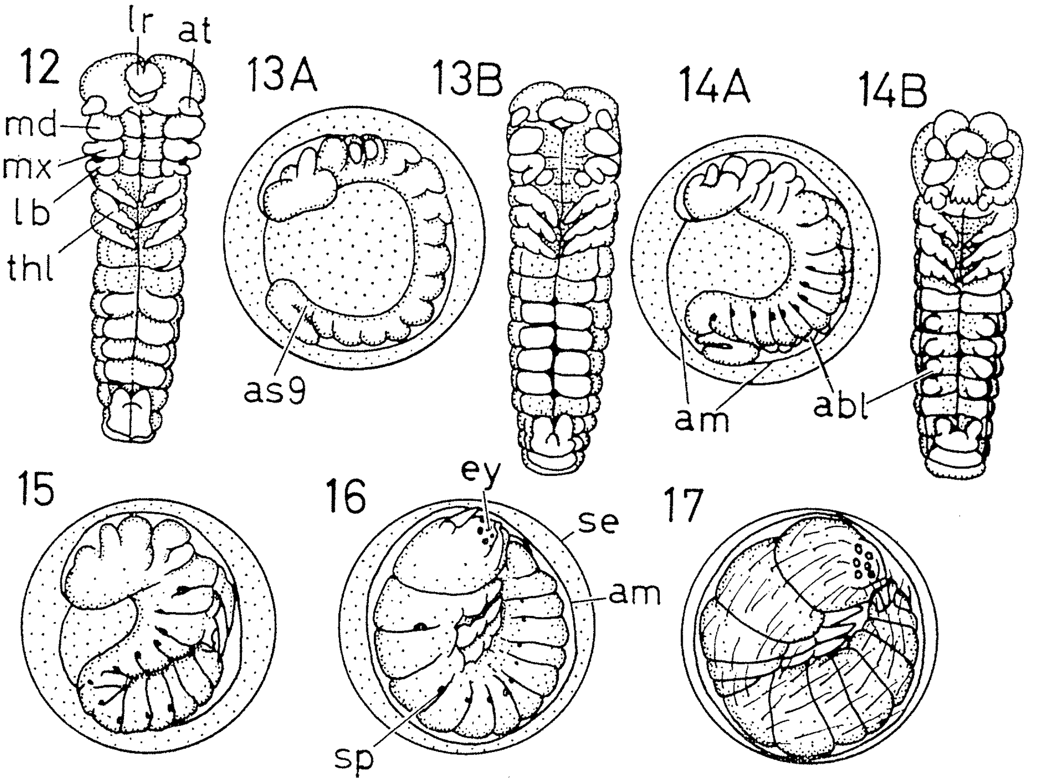
Stage 14 (241–280 hr): Completion of 1st instar larva (Fig. 17).

2. *Formation of stomodeaum*

Early in the Stage 6, the stomodaeum begins to form as an invagination at the



Figs. 1–11. External forms of the embryo of *Endoclita signifer*.——1. About 18-hr-old, lateral (A) and ventral (B) view.——2. About 20-hr-old, lateral (A) and ventral (B) view.——3. About 25-hr-old, vetnral view.——4. About 32-hr-old, lateral view.——5. About 32-hr-old, ventral view.——6. About 38-hr-old, ventral view.——7. About 43-hr-old, ventral view.——8. About 51-hr-old, lateral view.——9. About 58-hr-old, ventral view.——10. About 70-hr-old, ventral view.——11. About 70-hr-old, lateral view.

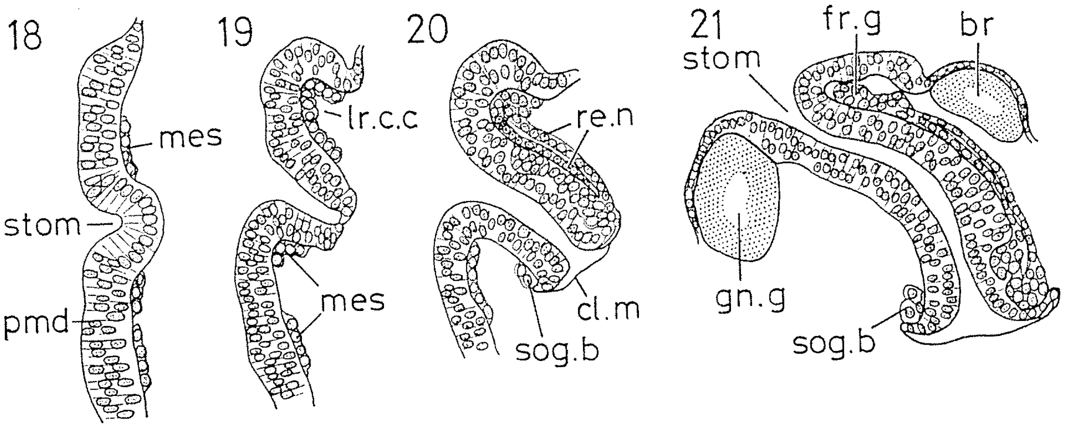


Figs. 12–17. External forms of the embryo of *E. signifer*. —— 12. About 95-hr-old, ventral view. —— 13. About 110-hr-old, lateral (A) and ventral (B) view. —— 14. About 120-hr-old, lateral (A) and ventral (B). —— 15. About 130-hr-old, lateral view. —— 16. About 200-hr-old, lateral view. —— 17. About 243-hr-old, lateral view.

medio-posterior portion of the cephalic region. The stomodeal wall is composed of columnar cells with the nucleus at an inner side (Fig. 18). At this time it is not lined with the inner layer or mesoderm. As development proceeds, the stomodaeum grows dorso-posterior wards (Fig. 19).

In the Stage 7, the blind end of developing stomodaeum consists of monocellular membrane and this part then becomes very thin (Fig. 20). The stomodaeal wall increases in thickness along the dorso-midline and is covered by the circumstomodaeal mesodermal cells. In the Stage 8, the anlagen of the stomatogastric nerve appear as three cell-masses protruded from the dorsal wall of the stomodaeum, and then form a longitudinal strand along its dorso-midline. On the other hand, the anlagen of the suboesophageal bodies appear as 2 or 3 cells attached on the ventral side of the stomodaeum.

As development advances, the developing stomodaeum increases in length, and the anlagen of the stomatogastric nerve differentiate into the frontal ganglion, recurrent nerve and corpus cardiacum respectively at the Stage 10 (Fig. 21). In the middle period of the Stage 12, paired longitudinal folds arise at the inner wall of the stomodaeum along its ventro-midline, and they occupy posterior two thirds of



Figs. 18–21. Sagittal sections through developing stomodaeum. —— 18. Stage 6. —— 19. Stage 7. —— 20. Stage 9. —— 21. Stage 10.

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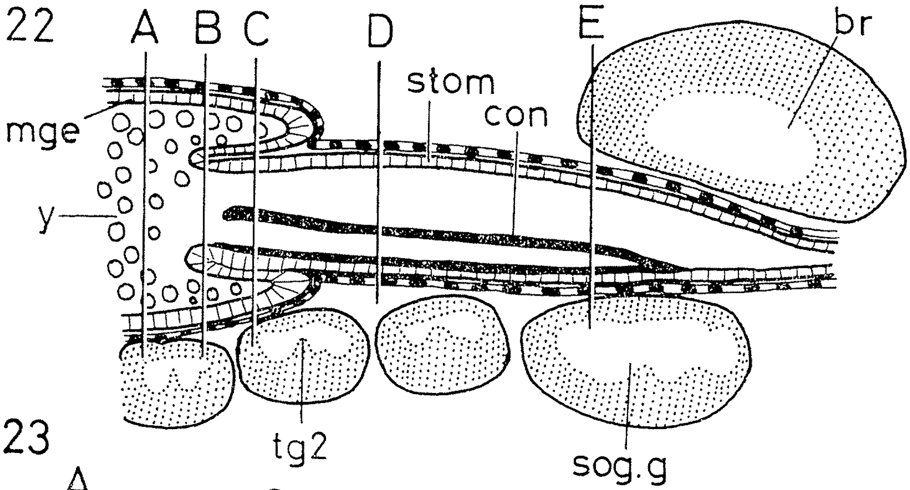

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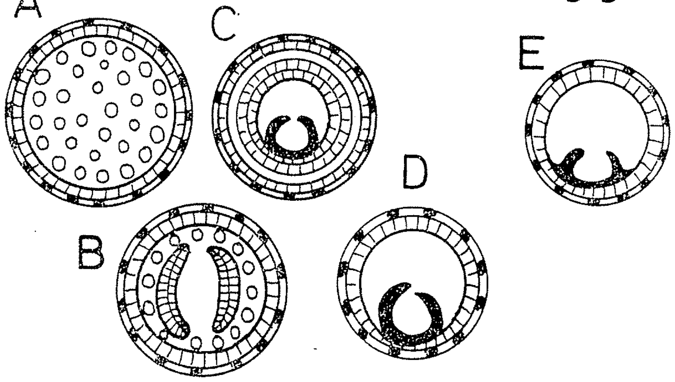


Fig. 22. Sagittal section through stomodeaum at stage 13, showing conduit-like structure, schematic.

Fig. 23. Cross sections of mid-gut and stomodaeum at leavels A-E in Fig. 22, schematic.

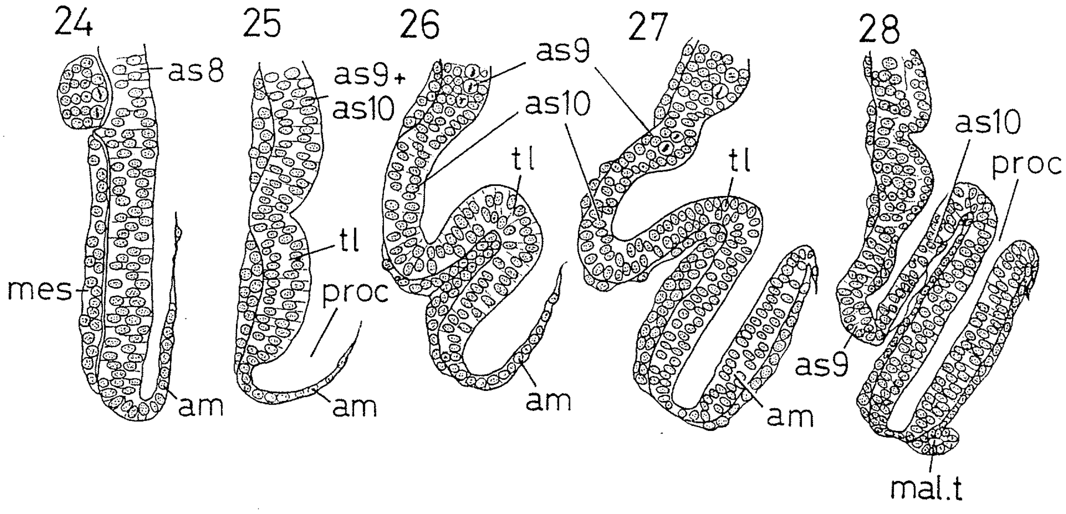
the stomodaeum. Then they develop interiorly to form a longitudinal lumen like a "conduit" (Figs. 22, 23). In the stage 13, the stomodaeum elongates and its posterior end reaches the level between the meso- and metathorax. The bottom of the stomodaeum differentiates into the stomodaeal valve and stretches into the mid-gut lumen, but it disintegrates at 220 hr after oviposition as will be described below.

3. *Formation of proctodaeum*

In the stage 6, the mesodermal segmentation in the first 8 abdominal segments have occurred, but the rest of inner layer in the posterior abdominal region does not yet show any metamerism (Fig. 24). The amnion at the posterior end of the embryo is thicker than that in elsewhere, and consists of cubic cells with a round nucleus.

Late in the Stage 7, the abdominal region is 10 segmented in the ectoderm including the telson. In this region the last two segments are larger than the other segments, and have the mesoderm or inner layer on their dorsal side (Fig. 25). In the stage 8, the 10th abdominal segment and the telson increase in length and bend as shown in Fig. 26, consequently the posterior half of the telson and the thicker amnion continue to form a pouch. The pouch is the anlage of the protodaeum.

As development proceeds, the proctodaeum elongates, and its lateral and dorsal walls thicken, and the thickeness of proctodaeal walls except for its blind end now becomes almost uniform throughout. The dorsal and ventral surface of the proctodaeum is surrounded by the mesodermal layer (Fig. 27). On both sides of the blind end of the proctodaeum three pairs of evaginations arise to form the anlagen of the malpighian tubules (Fig. 28). After revolution, the blind end of the proctodaeum reaches the level of the 6th abdominal segment. It then gradually extends anteriorly and finally reaches the level of the middle part of the 4th



Figs. 24–28. Sagittal sections through abdominal end. —— 24. Stage 6. —— 25. Stage 7. —— 26. Stage 8. —— 27. Stage 9. —— 28. Stage 10.

abdominal segment at 214 hr after oviposition, thus the developing proctodaeum is complete.

4. *Formation of mid-gut*

The mid-gut epithelium originates exclusively from the blind end of the stomodaeum. Early in the Stage 6, when the stomodaeal invagination appears, the mid-gut epithelium anlage is undiscernible. At the end of this stage, however, a pair of cell-masses different from neighbouring cells is observed on ventro-lateral sides of the blind end of the developing stomodaeum (Fig.31). This is the anlage of the mid-gut epithelium.

Early in the Stage 7, the mid-gut anlagen grow posteriorwards as paired ribbons on both sides just inside the splanchnic mesoderm (Fig. 29). They are monocellular

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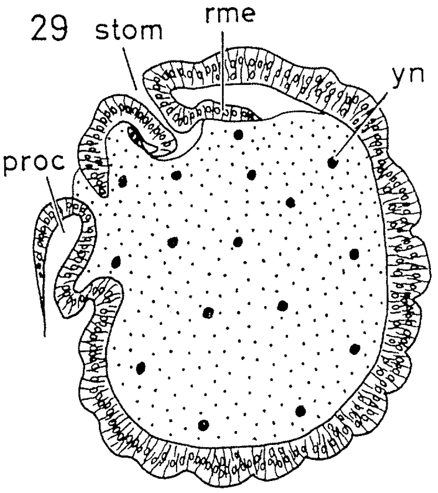

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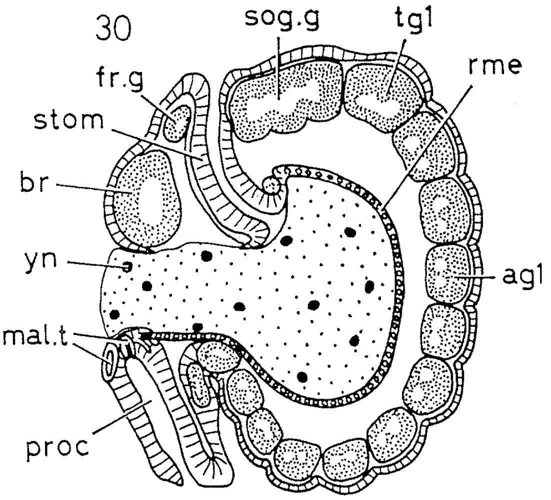

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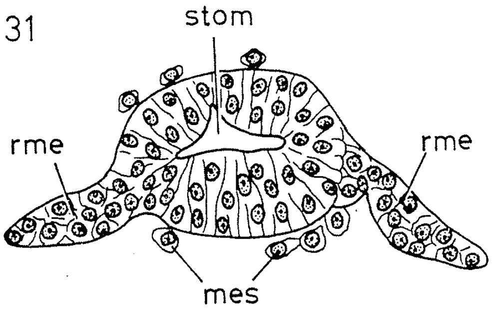

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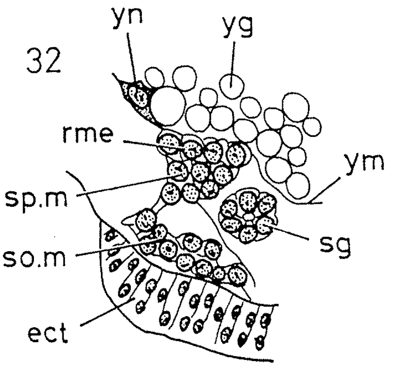

Fig. 29. Longitudinal section of embryo at Stage 7, showing the rudimentary mid-gut epithelium arising from the blind end of stomodaeum.

Fig. 30. Longitudinal section of embryo at Stage 10, showing the rudimentary mid-gut epithelium extending to the blind end of proctodaeum.

Fig. 31. Cross section near blind end of stomodaeum at Stage 7.

Fig. 32. Cross section through 1st abdominal segment at Stage 10.

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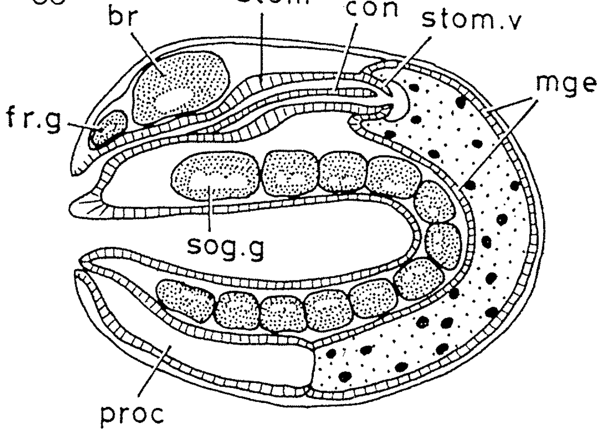

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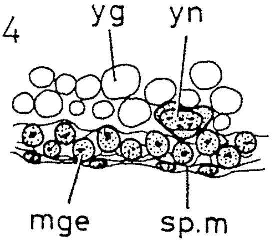

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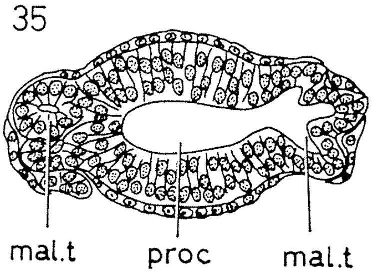

Fig. 33. Longitudinal section of embryo at Stage 12, showing completed mid-gut epithelium.
Fig. 34. Cross section through 2nd abdominal segment at Stage 12.
Fig. 35. Cross section near the blind end of protctodaeum at Stage 10, showing basal parts of malpighian tubules.

and situated between the yolk mass and the multicellular splanchnic mesoderm which connects with the somatic mesoderm. As development advances, the anlagen extend posteriorwards and reach the 9th abdonimal segment at about 85 hr after oviposition (early in the Stage 10). The posterior anlage of the mid-gut epithelium as observed in many other insect embryos could not be found.

At the Stage 10, when the embryo postures as shown in Fig. 30, the tips of the growing mid-gut epithelia reach the blind end of the proctodaeum, thus forming a pair of strands which connect the stomodaeum with the proctodaeum.

As development proceeds, the yolk mass decreases in volume owing to the comsumption of the yolk, hence the yolk membrane detatches from the germ band, causing the epinural sinus to form between them. Consequently the paired mid-gut ribbons are separated from the somatic mesoderms (Figs. 32, 36A, B). Then the paired ribbons spread over the yolk membrane and meet each other at the ventro-midline to form the ventral half of the mid-gut epithelium (Figs. 36C, D). At this stage it is composed of a single layer of flat cells. In further development the epithelium also spread dorsalwards and cover the dorsal side of the yolk mass completely (Figs. 36E, F). After revolution, the dorsal closure takes place first in the posterior region of the embryo, and then proceeds anteriorwards finishing at a thoracic region at about 170 hr after oviposition (Fig. 33).

In the yolk mass primary yolk nuclei are present and some of them attach on the yolk membrane or just inside the mid-gut epithelium. They are distinguishable from the mig-gut epithelial cells in form, size and distribution of chromatin in the nucleus, but later they degenerate (Fig. 34). Therefore, the primary yolk cells do

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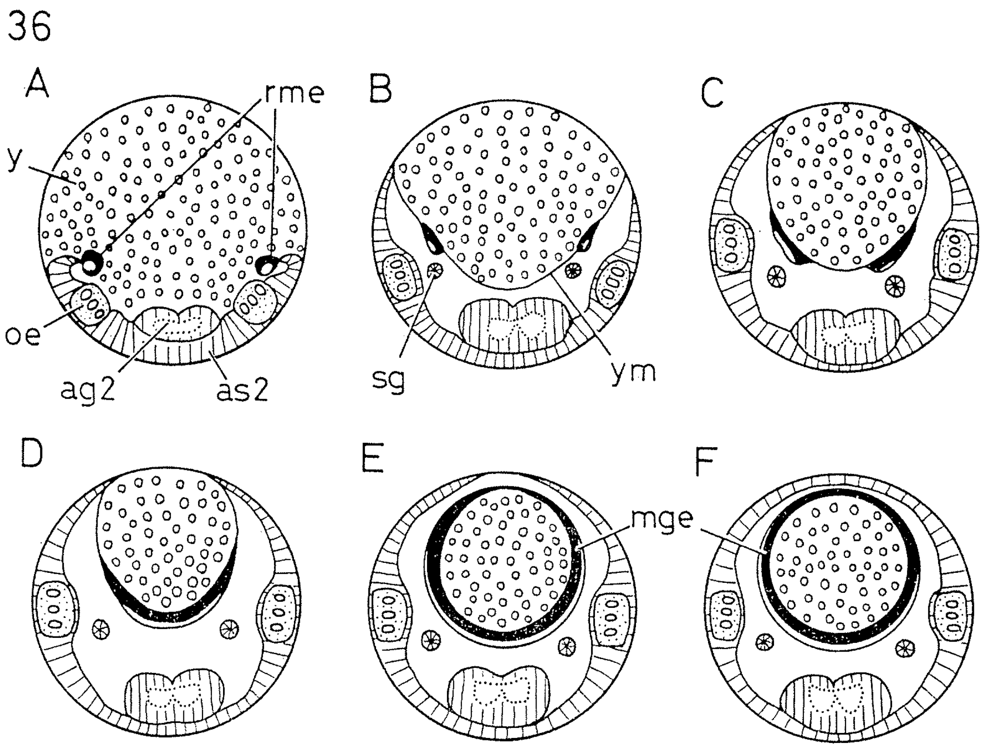

Fig. 36. The formation of mid-gut (cross sections through 2nd abdominal segment, schematic). A. middle of Stage 10; B, late in Stage 10; C, early in Stage 12; D, middle of Stage 12; E, late in Stage 12; F, early in Stage 13.

not participate in the formation of the mid-gut epithelium. In the Stage 13, the stomodaeal valve mentioned above degenerates and its constituent cells are dispersed among the yolk in the anterior part of the mid-gut and disintegrate there. The yolk is completely absorbed in the mid-gut by the end of the Stage 14.

Discussion

The stomodaeum of *E. signifer* is formed by the same process observed in other lepidopteran embryos, and is without doubt ectodermal in origin. The structure like a "conduit" on the ventral inner surface of the stomodaeum, however, is an unexpected one unknown in other insect embryos. It may be related to the food habit of this species whose larvae are at first leaf feeders then change into wood borers, although an examination as to the postembryonic state of this structure is need to prove it.

In most insects the stomatogastric nervous system originates from three cell-masses lying on the median line of the dorsal wall of the stomodaeum (ANDERSON, 1972). In the Lepidoptera, slightly different observations have been reported as to its formation. In *Pieris rapae* (EASTHAM, 1930), two cell-masses proliferate from the dorsal stomodaeal wall, and the anterior one give rise to the frontal ganglion from which the stomatogastric or recurrent nerve later arises. In *Chilo suppressalis* (OKADA, 1960), the stomatogastric system originated from a single mass, but later, as the stomodaeal invagination proceeds, it is divided into three masses. Its anterior one gives rise to the frontal ganglion and the middle one the recurrent nerve. The posterior one divides into two and becomes corpora cardiaca. In *Heliothis zea* (PRESSER & RATSCHKY, 1957), three cell-masses are formed through the invagination of the dorsal stomodaeal wall. Its anterior one becomes the frontal ganglion from which the recurrent nerve grows out. Other two cell-masses show glandular structure, referred to as stomodaeal glands. Later they seem to fuse and disappear. The stomatogastric nervous system of *E. signifer* is basically similar to that of *H. zea* in the manner of its formation, although the recurrent nerve of *E. signifer* originates from the two stomodaeal glands not from the frontal ganglion as in *H. zea*.

In the present species, the ventral wall of the proctodaeum arises from the posterior part of the telson, thus being ectodermal in origin. On the othr hand, its dorsal wall is formed from the thickened amnion which is continuous to the telson. This manner of proctodaeum formation has been observed in the Odonata (ANDO, 1962), Hemiptera (BUTT, 1949; SANDER, 1956), Coleoptera (REMPEL & CHURCH, 1969) and Trichoptera (MIYAKAWA, 1975), but has not been observed in the Lepidoptera except for the primitive moth, *Neomicropteryx nipponensis* (KOBAYASHI, unpublished).

The origin of the embryonic mid-gut epithelium is a controversial subject. In the Lepidoptrea as in other higher insects, the anlagen of the mid-gut epithelium arise bipolarly from the blind ends of the stomo- and proctodaeum in spite of different opinions as to its origin, e.g., endodermal in *Bombyx mori* (STRINDBERG, 1915), *Pieris rapae* (EASTHAM, 1930), *Ephestia kuehniella* (DRUMMOND, 1936), *Prodenia eridania* (GROSS & HOWLAND, 1940) and *Mamestra configurata* (REMPEL, 1951), ectodermal in *Bombyx mori* (TOYAMA, 1902), and "latent" endodermal in *Diacrisia virginica* (JOHANNSEN, 1929). Recent account of the origin of the bipolar mid-gut anlagen seems to be settled down in the difference in the time of occurrence of the parts, that is, the anterior and posterior mid-gut anlagen appear either before or after formation of stomodaeum and proctodaeum (MORI, in press).

The mid-gut anlagen of *E. signifer* arise exclusively from the blind end of the stomodaeum, thus belonging to "unipolar" origin. Judging merely by its superficial appearance, the anlage is ectodermal in origin, since there are no cell-masses formed independently of and prior to the stomodaeum near the place where the stomodaeal invagination is to appear. Unipolar formation of the mid-gut epithelium is unusual and so far is known in the stonefly, *Pterocarcys proteus* (MILLER, 1940), and in the coleopteran *Tribolium confusum* (STANLEY & GRUNDMANN, 1970), although their mid-gut epithelia arise solely from posterior (proctodaeal) mid-gut anlagen not from those of a stomodaeum. The unipolar condition in these species may be understood if either anlage grows quickly and arrives at the proctodaeal or stomodaeal end before the differnetiation of another anlage which is to appear in the bipolar condition. The unipolar condition also suggests the latent possibility that the whole mid-gut epithelium in the insects of bipolar condition could be formed from only either one of anterior or posterior mid-gut anlage, although a experimental work is need to prove it.

In *E. signifer* malpighian tubules arise as three pairs of evaginations from the blind end of the proctodaeum, thus being ectodermal in origin. They are formed in the same way as in other Lepidoptera, Trichoptera and Mecoptera..

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Abbreviations

abl: abdominal leg; ag1, 2: 1st and 2nd abdominal ganglion; am: amnion; as2, 8–10: 2nd and 8th to 10th abdominal segment; at: antenna; br: brain; cl.m: closing membrane; con: conduit-like structure; ect: ectoderm; ey: eye; fr.g: frontal ganglion; gn.g: gnathal ganglion; gr: germ rudiment; lb: labial rudiment; lr: labral rudiment; lr.c.c: labral coelomic cavity; mal.t: malpighian tubule; md: mandibular rudiment; mes: mesoderm; mge: mid-gut epithelium; mx: maxillary rudiment; oe: oenocyte; pce: protocephalon; pco: protocorm; pmd: premandibular segment; pp: pleuropodia; proc: proctodaeum; re.n: recurrent nerve; rme: rudimentary mid-gut epithelium; se: serosa; sg: silk gland; sog.b: suboesophageal body; sog.g: suboesophageal ganglion; so.m: somatic mesoderm; sp. spiracle; sp.m: spranchnic mesoderm; stom: stomodaeum; stom.v: stomodaeal valve; t1–3: 1st to 3rd thoracic segment; tg1, 2: 1st and 2nd thoracic ganglion; th1: thoracic leg; tl: telson; y: yolk; yg: yolk granule; ym: yolk membrane; yn: yolk nucleus.