Embryonic Development of the Nervous System and Other Ectodermal Derivatives in the Primitive Moth, *Endoclita sinensis* (Lepidoptera, Hepialidae)

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Synopsis

The formation of the nervous system, oenocytes, spiracles, and pleuropodia in the embryo of the heptalid moth, *Endoclita sinensis*, is described. The ventral nerve cord originates in large part from neuroblasts arising in 3 gnathal, 3 thoracic, and 10 abdominal segments. The median cord seems to participate in the formation of both transverse commissure and neurilemma of the ventral nerve cord. The development of the brain is typical of insects. The stomatogastric nervous system develops from 3 evaginations in the dorsal wall of the stomodeum. The anteriormost evagination gives rise to the frontal ganglion, and the middle and posterior ones the recurrent nerve and stomodeal glands respectively. The corpus cardiacum seems to be derived from the posterior evagination. Oenocytes arise in the first 8 abdominal segments. Spiracular evaginations appear in pro- and metathorax, and the first 9 abdominal segments, but the spiracles of the metathorax and the 9th abdominal segment degenerate during the later embryonic stages. Invaginated pleuropodia are formed in the first abdominal segment.

Introduction

Although much embryological work has been done on the higher (ditrysian) Lepidoptera, nothing was known about the embryology of the primitive (non-ditrysian) Lepidoptera until several years ago. Since 1976, we have worked on the embryogenesis of the primitive Lepidoptera, especially on the heptalid moths, *Endoclita sinensis* (This species has been mistakenly treated as *E. signifer* Walker in Japan. Accordingly, all the name of *E. signifer* recorded in our previous papers cited below must be changed to *E. sinensis*) and
E. exorescens, and the microerygid moth, Neomicropteryx nipponensis, and some of the results have been published in several papers (Ando and Tanaka, 1976, 1980; Ando and Kobayashi, 1978; Kobayashi et al., 1981; Kobayashi and Ando, 1981, 1982, 1983). In our previous paper (Kobayashi et al., 1981) on the embryology of E. sinensis, we described the changes in the external form of the embryo and the formation of the alimentary canal in this species, but the embryogenesis of other organs remained undescribed.

This paper deals with the ectodermal organogenesis, i.e., the formation of the nervous system, oenocytes, spiracles, and pleuropodia, in the embryo of E. sinensis.

Materials and Methods

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

Stage numbers used in this paper correspond with those in our previous paper (Kobayashi et al., 1981).

Observations

1. Nervous system

In stage 6, neuroblasts appear first in the gnathal and then head and thoracic regions, and later in the abdominal one (Fig. 1). In stage 8, the brain, premandibular, three gnathal, three thoracic, and ten abdominal ganglia become discernible (Fig. 2). The stomatogastric nervous system also shows a differentiation at this time. Then the premandibular ganglia move forwards to lie at lateral sides of the stomodeum and later become the tritocerebrum. In stage 10, three gnathal ganglia, i.e., the mandibular, maxillary, labial, fuse together to form the suboesophageal ganglion (Fig. 3). The frontal ganglion is also formed at this time. In stage 11, the ninth and tenth abdominal ganglia fuse together (Fig. 5), and then the eighth abdominal ganglion unites with the former (Fig. 6), thus forming the definitive eighth abdominal ganglion. When revolution of the embryo is completed, a fundamental composition of the nervous system is established (Fig. 4).

Ventral nerve cord

Early in stage 6, neuroblasts first appear in the gnathal ectoderm on both sides of the midline of the germ band. They are discernible in large size from other ectodermal cells. A little later, neuroblasts appear in the thoracic and the first two abdominal segments. Their appearance in posterior part of the abdomen occurs much later. The number of lateral neuroblasts counts three to five in cross section, and five to seven in longitudinal section of each segment of the gnatho-thoracic region (Fig. 7). In the abdominal region, their number is more reduced (Fig. 8). Neuroblasts also exist in the intersegmental region until 50 hr after oviposition, but thereafter they move into the intrasegmental one.

When the neuroblasts appear, the primitive groove is retained on the ventral midline of the germ band. However, as they begin to produce their daughter cells, the neural groove appears and deepens, thereby they are divided into two lateral groups. The neural groove

Fig. 1. Longitudinal section of embryo at stage 6, showing distribution of neuroblasts.
Fig. 2. Longitudinal section of embryo at stage 8, showing segmentation of neuroblasts.
Fig. 3. Longitudinal section of embryo at stage 10, showing formation of ganglia.
Fig. 4. Longitudinal section of embryo at stage 14, showing definitive central nervous system.
Fig. 5. Longitudinal section through abdominal end at early stage 11.
Fig. 6. Longitudinal section through abdominal end at late stage 11.
agl, 8-10 1st. and 8th to 10th abdominal ganglion, br brain, fr. g frontal ganglion, mes, mesoderm, nb neuroblast, pce protocephalon, pmd premandibular segment, pmd.g premandibular ganglion, proc protocebrum, proc proctodeum, sog.g suboesophageal ganglion, tg1 1st thoracic ganglion.
is deep and narrow in the gnatho-thoracic region, but shallow and wide in the abdomen. The bottom of the neural groove then becomes the median cord. This change begins first in the gnatho-thoracic region, and then in the posterior region of the germ band. The intersegmental median cord is wider than the intrasegmental one, and contains one or two neuroblasts which later undergo mitosis. The development of the median cord is delayed in the posterior region of the germ band.

The first mitoses of the neuroblasts are observed at about 50 hr after oviposition in the gnatho-thoracic region. Their spindle lies in the dorso-ventral direction, but in further divisions it turns right and left alternately. Accordingly the neuroblasts produce two columns of daughter or ganglion cells on themselves (Figs. 8, 9). In stage 9, the number of daughter cells in each column counts six to seven in the gnatho-thoracic region, and two to three in a posterior part of the abdomen.

Early in stage 10, the neuropile develops in each of the gnatho-thoracic ganglia, and then two ganglia on both sides of the median cord in each segment become connected by the transverse commissures arching the median cord (Fig. 9). It seems probable that some cells in the dorsal side of the median cord participate in the formation of the transverse commissure. The longitudinal connectives develop later than the commissure. The median cord at this stage is in contact with the epidermis at the bottom of the neural groove, and its structure retains the condition of early stages. The columnar ganglion cells above each neuroblast are now arranged in other rule.

In the middle period of stage 10, walls of both sides of the neural groove meet each other, and then the groove disappears. A little later, the median cord detaches from the ventral epidermis. The mesodermal cells covering the dorsal side of the ganglion disappear; consequently the transverse commissures become independent from neighbouring tissues. At about 109 hr after oviposition, three gnathal ganglia fuse with each other and thus the suboesophageal ganglion is formed. The neuroblasts decrease in size and become as small as the daughter cells except for one or two neuroblasts situated in the most lateral sides, but they continue further divisions. Soon later, however, all the neuroblasts stop their division. The neuropile, transverse commissures, and longitudinal connectives undergo further development, and the ganglia separate from the ventral epidermis completely.

Near the end of stage 10, a layer of cells appears on the dorsal surface of the neuropile. This is the primordium of the neurilemma. It seems to be originated from two components, the one from the ganglion cells located at the most lateral side of the ganglion, and the other from the median cord cells.

At the end of stage 11, certain flat cells are observed on the outer surface of the neuropile. They are the cells of the inner glial sheath. Similar cells are found in the area where both lateral halves of the ganglion meet. At this time some cells of the median cord survive. As development proceeds, the inner glial sheath becomes more conspicuous especially in the suboesophageal ganglion.

Brain In stage 6, neuroblasts appear in the inner (dorsal) side of ectoderm of the protocerebral lobes. These form the anlage of the protocerebrum. In longitudinal sections, they distribute uniformly throughout the protocerebral lobes (Fig. 10), but in cross sections they are separable into three groups on each of the lobes, corresponding to three protocerebral lobes, of which the third (median) lobe is the largest (Fig. 11). The deutocerebral anlage, which is composed of three neuroblasts at each hemisphere in a frontal section, arises
in the location between the stomodeum and antennal rudiments (Fig. 12). The tritocerebral anlage appears in the premandibular or intercalary segment, whose condition is quite similar to that in the mandibular segment (Fig. 12).

At the end of stage 7, the protoccephalic neuroblasts commence mitosis to produce their daughter or ganglion cells on the dorsum as observed in the ventral nerve cord formation. At this time the three protocerebral lobes, viz., the median, lateral, and optic ones, become clearly separable from each other. In the meantime the epithelium of the protocerebral lobes becomes thinner. The deutocerebral neuroblasts located in the bases of the antennal rudiments likewise produce their daughter cells. Meanwhile the premandibular segment is pushed forwards into the posterior part of the cephalic region. Consequently the mandibular segment becomes to situate between the antennal rudiments.

Early in stage 10, the cephalic region is filled by the ganglion cells. Its dorsal part is occupied by the protocerebrum, intero-lateral sides of the stomodeum by the deutocerebrum, and lateral sides of the stomodeum by the tritocerebrum. On the inner side of each cerebral part, the neuropile appears, and soon later its commissures and connectives develop.

In the middle period of stage 10, the supraoesophageal commissure is formed by the ganglion cells of the median protocerebral lobes, which runs laterally towards the dorsal side of the stomato gastric nervous system. Meanwhile the protocerebral neuropile becomes connected to the deutocerebral one of its own side. The deutocerebrum is connected posteriorly with the tritocerebrum, but lacks the transverse commissure (Fig. 13). The tritocerebrum is divided into two halves at lateral sides of the stomodeum. Its neuropiles are continuous to the suboesophageal commissure, and face to the oesophagus (Fig. 13). It sends, on the other hand, longitudinal nerve cords as the circumoesophageal connectives to the suboesophageal ganglion. The neuroblasts in each part of the brain, at this time, become smaller, but they are discernible and some of them still undergo mitosis.

At the end of stage 10, the neurilemma becomes apparent on the surface of the brain neuropiles. Its origin seems to be most lateral ganglion cell. At this time most of the neuroblasts become indistinguishable from other ganglion cells, but one or two large ganglion cells are found in the most lateral sides of the protocerebrum and of the deutocerebrum. These are of neuroblast origin and seem to develop into the neurosecretory cells. They exist thereafter throughout the embryonic period.

The relative position of cerebral components alters accompanying revolution of the embryo. Before revolution, the protocerebrum is located at the most anterior position, and then the deutocerebrum, tritocerebrum and suboesophageal ganglion follow in this order. When the revolution is finished, however, the protocerebrum shifts dorswards, so that the deutocerebrum occupies the most anterior position among them. As development advances, this dorso-posteriorward shift of the brain further continues resulting in that the protocerebrum and deutocerebrum come dorsal to the suboesophageal ganglion, and the tritocerebrum occupies the most anterior position in the head capsule.

**Stomatogastric nervous system** The stomatogastric nervous system arises in the dorsal wall of the developing stomodeum. It comprises the frontal ganglion, recurrent nerve, and stomodeal glands. The last named glands later change into the corpus cardiacum.

In stage 7, the stomodeal wall is thicker in the dorsal side than in the ventral, and the dorsal wall is covered by the mesoderm connected with the labral coelom (Fig. 14). At this time two cell-masses, the anlagen of the stomodeal glands, appear along the midline of the
dorsal stomodaecal wall, the one at middle point, the other near the posterior end of the stomodeum. At the anterior end of the dorsal stomodaecal wall another cell-mass composed of two or three large cells is found. This is the rudiment of the frontal ganglion.

As development proceeds, the stomodaecal invagination rapidly deepens, and in stage 9, the anlage of the frontal ganglion evaginates as a conspicuous cell-mass from the stomodeum. The two anlagen of the stomodaecal glands likewise evaginate (Figs. 16, 17), and the recurrent nerves develop from both glands along the dorsal stomodaecal wall, bridging the frontal ganglion and stomodaecal glands.

Early in stage 10, the frontal ganglion detaches from the stomodaecal wall and the neuropile appears at its ventral side. The stomodaecal glands develop as somewhat flat ellipsoid bodies on the dorsal midline of the stomodeum, and their diameter in cross section is a third of that of the stomodaeum. These bodies show a glandular character with radially scattered nuclei near the body surface (Fig. 18). The posterior stomodaecal gland is more developed than the anterior.

In the middle period of stage 10, the frontal ganglion is nearly completed and lies between two halves of the deutocerebrum, just anterior to the supraoesophageal commissure. The frontal ganglion sends thick nerves from its anterior corner to the tritocerebrum, and receives the recurrent nerve from the anterior stomodeal gland at its postero-ventral end which likewise receives at its dorsal side the recurrent nerve from the posterior stomodeal gland (Fig. 15). As development proceeds the stomodaecal glands lose their glandular character, and at the end of stage 10, the anterior stomodeal gland degenerates, and then the posterior one shrinks and detaches from the stomodaecal wall. As their degeneration proceeds, the cell multiplication occurs at the posterior end of the recurrent nerve and at the dorsal side of the degenerating stomodaecal glands, and these cells thus reconstruct a new cell mass. Early in stage 12, it lies as a flat ellipsoid body on the dorsum of posterior end of the stomodeum. This is the anlage of the corpus cardium.

2. Oenocytes

Early in stage 9, in each of the first to the eighth abdominal segment a pair of cell-mass having large nuclei is observed in the ectoderm just posterior to the tracheal invagination (Figs. 19, 20). This is the anlage of the oenocytes. It then changes into a tissue composed of large cells with a large ellipsoid nucleus. As development proceeds, the nucleus decreases in size, and the cytoplasm becomes eosinophilic with an inconspicuous cell membrane. Accompanying the dorsal upgrowth of the body walls, the oenocytes move latero-dorsally to lie at the lateral side of the embryo. This position is retained until about 220 hr after oviposition. The oenocytes never show mitosis during the period of their differentiation and development.

3. Spiracles

When the division of the head and thorax becomes evident and the abdominal appendages are completed (late stage 10), a pair of spiracular invaginations appears in each lateral wall of the pro- and metathorax, and the first nine abdominal segments (Fig. 19). The prothoracic

Ectodermal derivatives of *Endoclitida*

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**Fig. 14.** Sagittal section through stomodeum at stage 7, showing rudiments of stomatogastric nervous system.

**Fig. 15.** Sagittal section through stomodeum at late stage 10, showing stomatogastric nervous system.

**Fig. 16.** Cross section through stomodeum at stage 10, showing posterior stomodeal gland.

**Fig. 17.** Cross section through stomodeum just anterior position of Fig. 16 at stage 10.

**Fig. 18.** Cross section through posterior stomodeal gland at late stage 10.

**Fig. 19.** Longitudinal section through the 3rd abdominal segment at stage 10, showing oenocytes and spiracular invagination.

**Fig. 20.** Cross section through the 4th abdominal segment at stage 10, showing oenocytes, as3,4 3rd and 4th abdominal segment, a.st.g anterior stomodeal gland, cl.m closing membrane, fr.g frontal ganglion, gl.c glandular cell, l.n.b lateral neuroblast, l.r.c.c lral coelomic cavity, mes mesoderm, ng neural groove, oec oenocyte, p.st.g posterior stomodeal gland, re.n recurrent nerve, sog.b suboesophageal body, spr spiracular invagination, stom stomodeum.
spiracles invaginate at the latero-posterior part of the segment, while metathoracic ones appear in the medio-ventral part (near the base of the thoracic leg) of the segment. All the abdominal spiracles are situated on the dorsal part of oenocytes.

After revolution, the metathoracic spiracles are gradually reduced and disappear, and then the spiracles of the ninth abdominal segment also degenerate. Consequently, in a 165-hr embryo (stage 12), spiracles remain in the prothoracic and the first eight abdominal segments.

4. Pleuropodia

In stage 8, rudimentary appendages become evident on all abdominal segments of the embryo. Among them, the appendages in the first abdominal segment are well developed and sharp in their extremities, and are located more medially than other ones (Fig. 21). In this stage, distinct coelomic cavities are observed on both sides of the first abdominal segment.

In stage 9, somewhat large nuclei appear in the body wall near the base of the rudimentary appendages of the first abdominal segment (Fig. 22). Then the cells having these nuclei aggregate and separate from the body wall to form the rudiment of the pleuropodium, located between the ventral nerve cord and oenocytes in the first abdominal segment. In stage 10, the projections of rudimentary appendages are reduced, but the pleuropodium, on the contrary, well develops (especially its cytoplasm elongates externally), and forms a conical, glandular structure of about 20 μm in diameter and 35 μm in height. As development advances, the cytoplasm of the pleuropodial cells further grow, and their distal ends protrude from the body wall of the embryo (Fig. 23). Shortly after that the distal region becomes rounded and swells.

During revolution of the embryo (stage 11), the pleuropodia are situated beneath oenocytes, and their distal ends are faced laterally. At 162 hr after oviposition, ringed depressions of body walls are observed around the pleuropodial projections. After revolution, the pleuropodial cells continue their development, and the pleuropodia attain the maximum state of 55 μm in length and 12 μm in diameter of the swollen, distal end by 200 hr after oviposition (stage 13). This state (Fig. 24) persists until stage 14 when the basic form of the first instar larva is established. The pleuropodia then degenerate and disappear, but we could not observe its exact process.

Discussion

1. Nervous system

As exactly summarized by Springer and Rutschky (1969), there are many opinions about the fate of the median cord. In the Lepidoptera such as Pieris rapae (Eustham, 1930) and Chilo suppressalis (Okada, 1960), the median cord is shown to form the transverse commissure, although in the latter species the median cord in part participates in the formation of the dorsal half of the neuropil. In Heliothis zea, however, the median cord is reported to form the glial elements at the midline of the neuropile (Springer and Rutschky, 1969). In
the primitive lepidopteran Neomicropteryx nipponensis (Kobayashi and Ando, 1983) and the trichopteran Stenopsyche griseipennis (Miyakawa, 1974), the median cord seems in part to form the dorsal cortical layer of the ganglion and in part to form the gland elements. The median cord of E. sinensis, as in C. suppressalis, seems to be destined in part to form the transverse commissure, and in part the neurilemma. In this species, however, the rest of the neurilemma seems to arise from the most lateral neuroblasts of the lateral cord. On the other hand, in P. rapae (Eastham, 1930) and N. nipponensis (Kobayashi and Ando, 1983), the neurilemma is solely formed by the peripheral lateral cord cells. At any rate, further ultrastructural studies are needed to decide the fate of the median cord and the origin of the neurilemma.

In S. griseipennis (Miyakawa, 1974), the deutocephral commissure exists in the preoral region, and the tritocerebral commissure in part fuses to the frontal ganglion, although these facts are unusual in insect embryogenesis. However, in E. sinensis as in other lepidopteran species, the deutocephral commissure is absent, and the tritocerebral one is confluent with the suboesophageal commissure, thus being clearly postoral in position. Therefore the development of the brain in E. sinensis proceeds in more general manner than in S. griseipennis.

In most insects the stomatogastric nervous system originates from the three cell-masses lying on the median line of the dorsal wall of the stomodeaum (Anderson, 1972). In the Lepidoptera, slightly different observations have been reported on its formation. In P. rapae (Eastham, 1930), two cell-masses proliferate from the dorsal stomodeaum wall, and the anterior one gives rise to the frontal ganglion and the posterior one to the stomatogastric ganglion. The recurrent nerve is produced by the extension of nerve cells from the two ganglia. In C. suppressalis (Okada, 1960), the system originates from a single mass; but as the stomodeaum grows, it is divided into three masses. The anterior one gives rise to the frontal ganglion, the middle one to the recurrent nerve, and the posterior one divides into two to become the corpora cardiaca. In H. zea (Presser and Rutschky, 1957), three cell-masses are formed through the evagination of the dorsal stomodeaum wall. The anterior one becomes the frontal ganglion that gives rise to the recurrent nerve. The other two cell-masses become glandular “stomodeaum glands” that later seem to fuse and disappear. In N. nipponensis (Kobayashi and Ando, 1983), the system develops from three evaginations in the dorsal stomodeaum wall. The anteriormost evagination forms the frontal ganglion, the second the hypocerebral ganglion and corpora cardiaca, and the posterior one the ventricular ganglion. The recurrent nerve differentiate from both the second and posterior evagination. Therefore, the mode of development of the stomatogastric nervous system in E. sinensis is basically similar to that in H. zea at the point that two glandular cell-masses are formed in the dorsal stomodeaum wall, although the recurrent nerve of the former species originates from the two stomodeaum glands not from the frontal ganglion as in the latter.

2. Oenocytes

In the ditrysian Lepidoptera such as Ephestia kuehniella (Stendell, 1912), P. rapae (Eastham, 1930), H. zea (Presser and Rutschky, 1957), and Hyalophora cecropia (Harmsen and Beckel, 1960), as in other pterygote insects, the oenocytes arise segmentally in the first eight abdominal segments. The same observation was made here in E. sinensis. However, in N. nipponensis (Kobayashi and Ando, 1983) and trichopteran S. griseipennis (Miyakawa, 1974), the oenocytes occur in the first seven abdominal segments but are absent in the eighth. Kobayashi and Ando (1983) have regarded the situation in these two insects as one of the strong evidence for close affinity between N. nipponensis and the Trichoptera. It seems necessary in future to ascertain the existence of oenocytes in the eighth abdominal segment in other primitive lepidopterans.

3. Spiracles

In most of the Lepidoptera as in other Holometabola, larval spiracles are present in the metathorax and the first eight abdominal segments. However, the sites of occurrence of spiracular invaginations in their embryos slightly vary according to species. For example, in Bombyx mori (Toyama, 1902), spiracular invaginations appear first in each segment from the prothoracic to the eleventh abdominal. During embryogenesis, the spiracles on the mesothoracic, ninth, and tenth abdominal segment disappear entirely, while the remaining ten pairs of spiracles persist in the larval stage. In P. rapae (Eastham, 1930), the spiracles occur in the pro- and mesothorax, and the first eight abdominal segments; and among them only the mesothoracic ones degenerate during the embryonic stage. In Diacrisia virginica (Johannsen, 1929) and N. nipponensis (Kobayashi and Ando, 1983), spiracular invaginations are formed in the meso- and metathorax, and the first eight abdominal segments. In later embryonic stages of these species, the mesothoracic spiracles migrate onto the prothorax, and the metathoracic ones are reduced and disappear entirely. Consequently, the number and position of the spiracular invaginations in the embryonic stage of E. sinensis, in which they arise first in the pro- and metathorax, and the first nine abdominal segments, do not accord with those of any of the above-mentioned species.

4. Pleuropodia

Roonwal (1937) classified the pleuropodia into evaginate and invaginate types. In the Lepidoptera, the pleuropodia are rare and known to occur in Catocala nupta, C. fraxini (Hirschler, 1928), P. rapae (Eastham, 1930), and N. nipponensis (Kobayashi and Ando, 1983). All of these are weakly developed, and belong to the invaginate type. In E. sinensis, the pleuropodia first appear as appendage-like projections in the first abdominal segment. Their nuclei are then retracted into the body cavity, whereas their cytoplasm projects into the amniotic cavity before and during revolution. Thus the pleuropodia of E. sinensis also belong to the invaginate type.

Notice The material moth of the present study has long been regarded as Endoclita signifera Walker, but recently, the species was accurately identified with E. sinensis (Moore) (Inoue et al., 1982). Accordingly, all the name of E. signifera appeared in our previous papers (Ando and Tanaka, 1980; Kobayashi et al., 1981) should be replaced with the name of E. sinensis.
References


