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By

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(Akkeshi Marine Biological Station)

(with 143 text-figures)

Introduction

Up to the present time the phylogeny of the nemerteans has been mainly discussed from the morphological point of view (Bürger, 1895; Friedrich, 1935; Hylbom, 1957). The most important characteristics of the nemerteans used for consideration of the problem lie in the relative position of the nervous system in the body wall. Bürger's conception was partly revised by Bergendal (1903), Friedrich (1935) and Hylbom (1957). The latter reported a so-called phylogenetic tree of the Palaeonemertea, in which the questionable family Cephalothricidae was placed near the bottom of the group. It has been generally accepted that the palaeonemerteans are closely related to the heteronemerteans, but the phylogeny of the Hoplonemertea and the Bdellonemertea remains unknown.

The nemerteans show in general two types of development, namely direct and indirect. In the indirect type, the pilidium and Desor larva occur as seen in the species belonging to the order Heteronemertea. The pilidium larvae have been described in detail for *Cerebratulus lacteus* (Metschnikoff, 1869; Wilson, 1900), *C. marginatus, Micrura leidyi,* and *M. caeca* (Coe, 1899 and 1943), and *Lineus torquatus* (Iwata, 1957). The metamorphosis of the pilidium was observed by Bütschli (1873) and Salensky (1912). On the other hand, the Desor larva of *Lineus ruber* (Desor, 1848) was studied by Barrois (1877), Hubrecht (1886), Arnold (1898), Nusbaum and Oxner (1913), and Schmidt (1934). Iwata (1958) studied the development of *Micrura akkeshiensis* and reported that the species produces a larva, modified from Desor larva. These larvae above mentioned are all the same in the characteristic that the adult ectoderm was secondarily formed. On the other hand, direct development has been observed in *Cephalothrix rufifrons* (Smith, 1935) in the order Palaeonemertea and in five species, namely *Prosorochmus viviparus* (Salensky, 1914), *Tetrastemma vermiculus* and *Drepanophorus spectabilis* (Lebedin-

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sky, 1897), *Prostoma graecense* (Reisinger, 1926; Reinhard, 1941), and *Malacobdella grossa* (Hammarsten, 1918) in the orders Hoplonemertea and Bdellonemertea.

The present paper deals with the interrelationships of the nemerteans from the viewpoint of their comparative development. The development of eight species of the nemerteans belonging to the three orders Palaeonemertea, Heteronemertea, and Hoplonemertea has been observed at the Akkeshi Marine Biological Station under the guidance of Professor Tohru Uchida since 1950. Out of the species used, the development of *Lineus torquatus* and *Micrura akkeshiensis* has been reported in detail in 1957 and 1958 respectively. Accordingly brief notes from those papers are incorporated in the present paper. As the result of these studies the writer proposes here a new revised system for Nemertea.

Before going further, the writer wishes to express his sincere thanks to Professor Tohru Uchida at whose suggestions the present study was performed and by whose kind guidance this paper has taken its shape. Hearty thanks are also due to Professor Katsuma Dan of Tokyo Metropolitan University for his helpful advice and criticism on embryological study.

Materials and Methods

The nemerteans used in the present paper are classified as follows;

- Nemertea
  - Class Anopla
    - Order Palaeonemertea
      - 1) *Procephalothrix simulus* Iwata
      - 2) *Procephalothrix filiformis* (Johnston)
      - 3) *Tubulanus punctatus* Takakura
    - Order Heteronemertea
      - 4) *Lineus torquatus* Coe
      - 5) *Lineus alborostratus* Takakura
      - 6) *Micrura akkeshiensis* Yamaoka
  - Class Enopla—Order Hoplonemertea
    - 7) *Emplectonema gracile* (Johnston)
    - 8) *Oerstedia dorsalis* var. *albolineata* Iwata

The nemerteans here considered have all been reported by Yamaoka (1940) and by Iwata (1954). The mature adult worms were obtained from between the tide marks from the shores of Akkeshi and Daikoku-jima, a small island 2 miles off the Akkeshi coast. They were brought to the laboratory; the males and females of each species were kept respectively in shallow finger bowls containing sea water and the *Tubulanus* and *Lineus* in water tanks, about 30 cm in diameter. The filtrated sea water in each bowl was renewed once per day, no food was given. After the spawning was accomplished the males and the females were generally separated and then eggs deposited by the females were inseminated with sperm.

The materials were fixed in Bouin's fluid. Before embedding, the materials were put into the lower portion of the petals of a dahlia stored in 70% alcohol; they were sectioned in 8 micra and stained with Delafield's haematoxylin and eosin or Heidenhain's azan. The latter staining resulted very favorably for observations.

Results

1) Development of *Procephalothrix simulus*

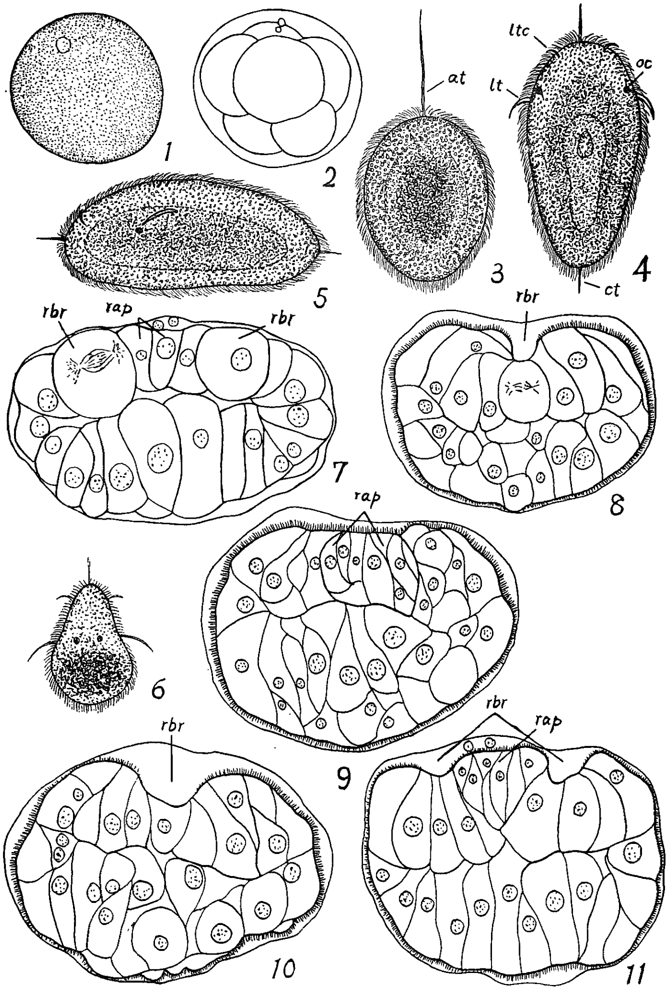
Smith (1935) worked in detail on *Cephalothrix rufifrons*. *Cephalothrix galatheae,* of which Dieck (1874) reported observations on the development of external charatcers, seems to be identical with *Carcinonemertes carcinophila* (Coe, 1902; Wijnhoff, 1913).

The writer observed the spawning habit of *P. simulus* on July 26th, 1953. A year later, artificial inseminations were made for the eggs spawned on the 28th July, 1954. The larvae lived for 41 days. On July 27th, 1956 the eggs were spawned and artificially inseminated. The materials were fixed at 1.4, 24, 91, 114, 123, 138, 147, 162, 169, 189, 209, 225, and 245 hours after fertilization. On July 11th, 1959, eggs were obtained artificially by cutting the gonads transversely and were inseminated. The materials were fixed at 5, 13, 20, 25, 31, 43, 50, 55, 61, 67, 74, 79, 85, 89, 100, 107, 114 and 120 hours after fertilization and stained with Delafield's haematoxylin and eosin. For observations the latter materials were used principally.

Cleavage and gastrulation. The eggs are discharged simultaneously through the gonoducts; they are embedded in a loose jelly substance secreted from the epidermis of the females. The jelly dissolves sooner or later after spawning. The egg is yellow-orange in color, opaque with rich yolk and measures about 0.19 mm in diameter. The germinal vesicle is represented as a small clear zone. The egg membrane is thin. The spermatozoon is a nematosperm composed of a head, a middle piece and a tail. The head, about 0.003 mm in length, is elliptical in shape and provided with a pointed acrosome. The middle piece appears as a small spot, while the tail measures about 0.04 mm in length.

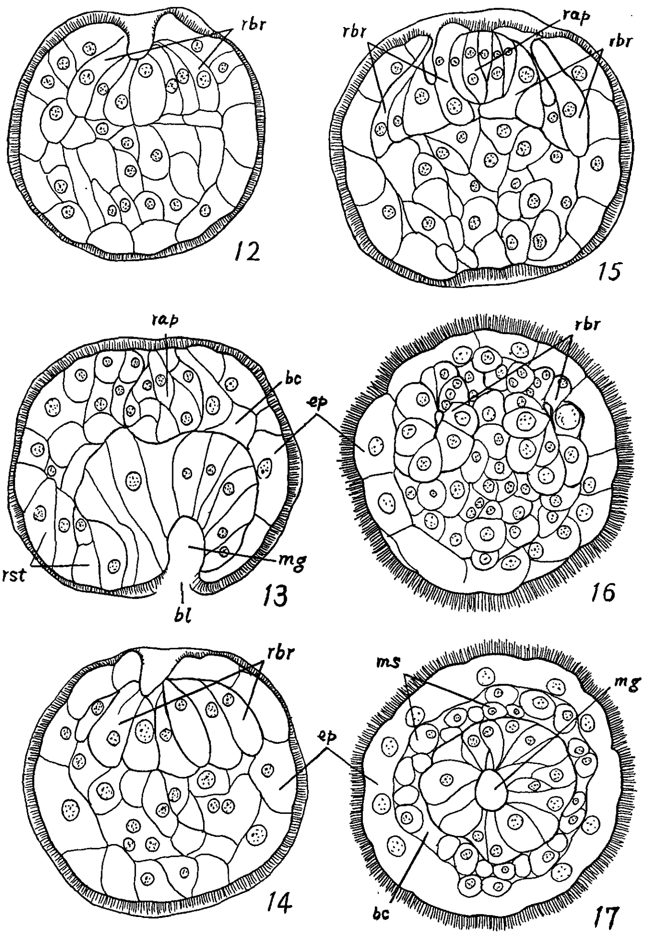
The first polar body is formed about 40 minutes after fertilization. Cleavage of the spiral type leads to the formation of a flattened blastula. The first cleavage plane passes through the animal pole. The second cleavage plane is vertical and at right angles to that of the first cleavage. The third cleavage is dexiotropic and the blastomeres of the first quartet are far larger than the macromeres (Fig. 2).

Thirteen hours after fertilization the blastula flattens on the animal and the vegetal polar sides (Fig. 7). The blastomeres on the vegetal polar



Procephalothrix simulus Iwata. Fig. 1. Egg immediately after liberation. ×105. Fig. 2. 8-cell stage. ×105. Fig. 3. 46-hour larva. ×105. Fig. 4. 165-hour larva. ×85. Fig. 5 165-hour larva from the lateral view. ×85. Fig. 6. 41-day larva. ×105. Fig. 7. Section of the 13-hour blastula through the polar body. ×340. Figs. 8–10. Sagittal sections of the 20-hour blastula, showing the rudiments of the brain and the lateral nerves (*rbr*) and of the apical plate (*rap*). ×340. Fig. 11. Horizontal section of the 20-hour blastula, showing the rudiments of the brain and the lateral nerves (*rbr*) and of the apical plate (*rap*). ×340. *at*: apical tuft. *ct*: caudal tuft. *lt*: lateral tuft. *ltc*: lateral tuft derived from the cerebral plate. *oc*: ocellus. *rap*: rudiments of the apical plate. *rbr*: rudiment of the brain and lateral nerves.

side are higher than those of the animal polar side. Under the polar bodies there come to appear a few small blastomeres which will differentiate afterward into the apical plate (*rap*). The large and round blastomeres arranged in a pair on the animal polar side differentiate into the brain and lateral nerves of the future adult worm (Fig. 7, *rbr*). The situation of the rudiments of the nervous system determines the right-left side of the future adult worm. The animal polar side, in which the rudiments of the



Procephalothrix simulus Iwata. Figs. 12–14. Sagittal sections of the 25-hour gastrula, showing the rudiments of the brain and the lateral nerves (*rbr*) and of the apical plate (*rap*). ×340. Fig. 15. Horizontal section of the 25-hour gastrula, showing the rudiments of the brain and the lateral nerves (*rbr*) and of the apical plate (*rap*). ×340. Figs. 16 and 17. Transverse sections of the 31-hour-larva, showing the rudiment of the brain and lateral nerves extending inside the epidermis. ×340. *ap*: apical plate. *bc*: blastocoel. *bl*: blastopore. *ep*: epidermis. *mg*: midgut. *ms*: mesoderm cells. *rbr*: rudiment of the brain and lateral nerves. *rst*: rudiment of the stomodaeum.

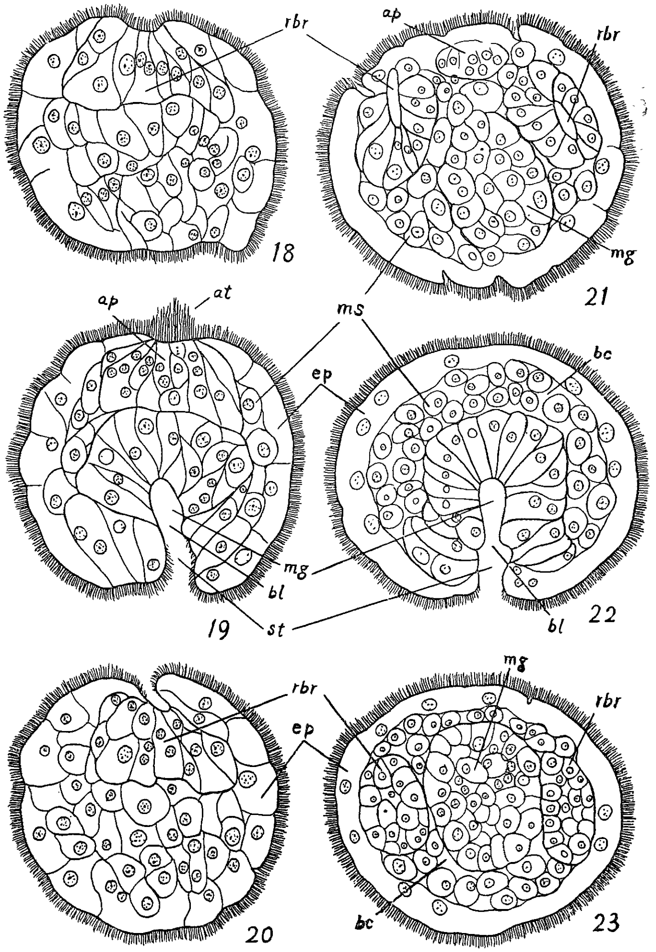
apical plate and of the nervous system are present, becomes to the head of the adult worm.

In the 20-hour blastula the rudiments of the nervous system invaginate somewhat toward the vegetal polar side as a result of rapid cell-division (Figs. 8, 10 and 11). The rudiment of the apical plate is triangular in shape in transverse section (Fig. 11). Numerous fine cilia come to appear on the body surface.

Between 20 and 25 hours after fertilization the gastrula becomes rounded in shape owing to the polar ingression (Fig. 13). The midgut is composed of large columnar cells arranged in a row; it inclines toward the lateral side of the embryo which becomes to the dorsal side of the future adult worm (Fig. 13, *mg*). The vegetal polar side and the remainder lateral side of the embryo becomes to the ventral side of the adult worm. The rudiment of the stomodaeum is represented as a small number of the columnar large cells located posterior to the midgut (Fig. 13, *rst*). The invaginations of the rudiments of the nervous system are shown in Figs. 12, 14 and 15 (*rbr*). The apical plate becomes saccular in shape (Figs. 13 and 15, *rap*). About 25 hours after fertilization the gastrula creeps out from the egg membrane, though the gastrulation of the embryo proceeds as yet. In the 31-hour larva the invaginated canal of the embryo is divided into two portions; namely the midgut and the ectodermal stomodaeum (Fig. 19, *mg, st*). The blastopore is situated at the boundary between them (*bl*). The archenteron inclines toward the portion opposite to the dorsal side of the body. The stomodaeum is distinguishable from the midgut by different arrangement of the constituent cells. The midgut is more deeply stained with eosin than the stomodaeum which shows the same affinity as the epidermis. The gastrulation of the embryo is completed in the 43-hour larva (Fig. 24). The blastopore narrows in horizontal section and is located posterior to the broad stomodaeum (Fig. 22, *bl*). The midgut again directs its anterior tip toward the dorsal side of the body (Fig. 24).

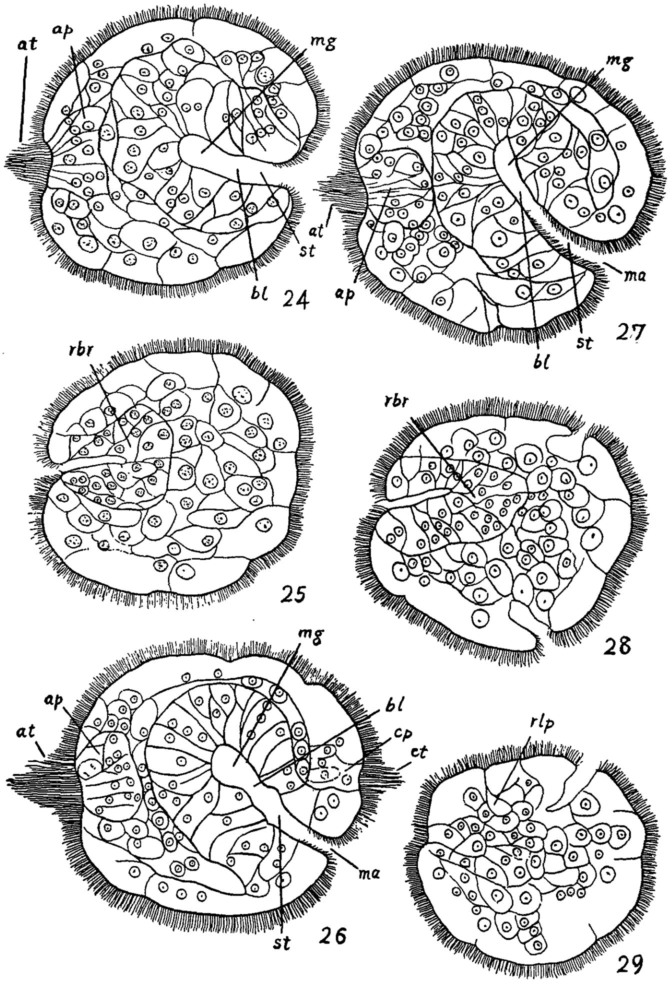
In the 31-hour larva a small number of the mesoderm cells are differentiated in the blastocoel (Fig. 19, *ms*). In the 43-hour larva the blastocoel widens and the mesoderm cells increase in number (Figs. 21-23). The apical plate produces a bundle of long flagella forming an apical tuft in the 31-hour larva (Fig. 19, *ap, at*).

Larva. The young larva just escaped from the egg membrane is spherical in shape and is provided with a long apical tuft. The larva swims in a straight line along the long axis of the body. At the 46th hour the larva becomes elongated and measures about 0.22 mm in length and 0.16 mm in width. The larva is dull yellow in color. The apical tuft measures about 0.15 mm in length. A dark contour line running inside the epidermis is clearly distinguishable from above (Fig. 3). At the 50th



Procephalothrix simulus Iwata. Figs. 18–20. Sagittal sections of the 31-hour larva, showing the rudiment of the brain and lateral nerves (*rbr*), the apical plate (*ap*), and the stomodaeum (*st*). ×340. Figs. 21 and 22. Horizontal sections of the 43-hour larva, showing the rudiment of the brain and lateral nerves (*rbr*) and the stomodaeum (*st*). ×340. Fig. 23. Transverse section of the 43-hour larva, showing the rudiment of the brain and lateral nerves extending inside the epidermis. ×340. *ap*: apical plate. *at*: apical tuft. *bc*: blastocoel. *bl*: blastopore. *ep*: epidermis. *mg*: midgut. *ms*: mesoderm cells. *rbr*: rudiment of the brain and lateral nerves. *st*: stomodaeum.

hour a short caudal tuft is found at the posterior end of the body (Fig. 26, *ct*). At the 85th hour there come to appear three pairs of the ocelli, of the lateral tufts and of the lateral tufts of the cerebral plates in the cephalic region (Figs. 4 and 5, *lt, ltc, oc*). The ocelli are blackish brown in color.



Procephalothrix simulus Iwata. Figs. 24 and 25. Sagittal sections of the 43-hour larva, showing the midgut (*mg*), stomodaeum (*st*), and the rudiment of the brain and lateral nerves (*rbr*). ×340. Fig. 26. Sagittal section of the 50-hour larva through the apical plate. ×340. Figs. 27–29. Sagittal sections of the 55-hour larva through the apical plate (27) and the rudiments of the brain and lateral nerves (28) and of the lateral plate (29). ×340. *ap*: apical plate. *at*: apical tuft. *bl*: blastopore. *cp*: caudal plate. *ct*: caudal tuft. *ma*: mouth of the future adult worm. *mg*: midgut. *rbr*: rudiment of the brain and lateral nerves. *rlp*: rudiment of the lateral plate. *st*: stomodaeum.

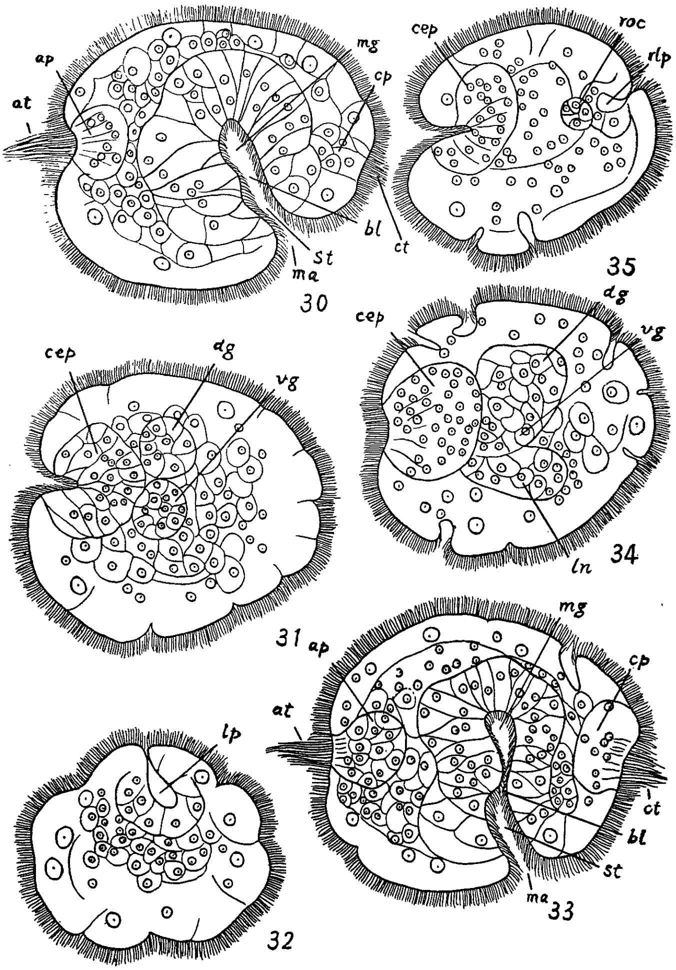
The lateral tufts are situated at the lateral sides of the ocelli. The lateral tufts of the cerebral plates are shorter in length than the lateral tufts and are found at the dorso-lateral aspect of the body between the apical and the lateral tufts. At the 95th hour the larva measures about 0.33 mm in length. Figs. 4 and 5 show the larvae at the age of 165 hours after fertilization. The digestive canal is divided into two portions, of which the anterior one is the stomodaeum while the posterior the midgut. The cilia are denser and longer in the stomodaeum than in the midgut. The larva becomes smaller in size because no food was given; it measures about 0.16 mm in length and 0.1 mm in width in the 41 day larva (Fig. 6).

Formation of the ectodermal organs.

Epidermis. The apical, the caudal, the lateral, and the cerebral plates and the ocelli are formed as larval organs in the epidermis. The formation of the epidermis is completed in the 43-hour larva when the gastrulation of the embryo ends. The sagittal section of the epidermis in the 43-, 50-, 55-, 61-, 67-, 74-, 79-, and 85-hour larva is shown in Figs. 24, 26, 27, 30, 33, 38, 39, and 42. The rapid cell-division of the epidermis on the dorsal side of the body causes the posterior elongation of the body and the downward movement of the stomodaeum. The stomodaeum gradually shifts nearer to the apical plate. The epidermis is composed of a row of flattened cells containing large nuclei in the gastrular stage of development, while in later stages it becomes gradually obscure in its cellular boundary and contains nuclei irregularly arranged. The boundary between the epidermis and the mesodermal layer also becomes obscure. In the 85-hour larva the epidermis becomes higher and contains a number of nuclei. A small number of large gland cells come to appear in the epidermis (Figs. 42 and 44).

The sagittal section of the apical plate in the 43-, 50-, 55-, 61-, 67-, 74-, 79-, and 85-hour is shown in Figs. 24, 26, 27, 30, 33, 38, 39, and 42. The apical plate is clearly demarcated from the epidermis throughout the larval stage of development and protrudes posteriorly into the blastocoel. The caudal plate is at first distinguishable in shape at the 50th hour but becomes indistinct in the 85-hour larva (Figs. 26, *cp* and 42). The caudal tuft composed of a bundle of long flagella is derived from the caudal plate. The sagittal section of the caudal plate in the 67 and 74-hour larva is shown in Figs. 33 and 38 (*cp*). The transverse section of the lateral plate in the 85-hour larva is shown in Fig. 40 and the horizontal section is shown in Fig. 44 (*lp*). The lateral plate comes to appear at first as a pair of invaginations of the epidermis (Figs. 29 and 35, *rlp*) and produces a bundle of long cilia forming the lateral tuft (Figs. 40 and 44).

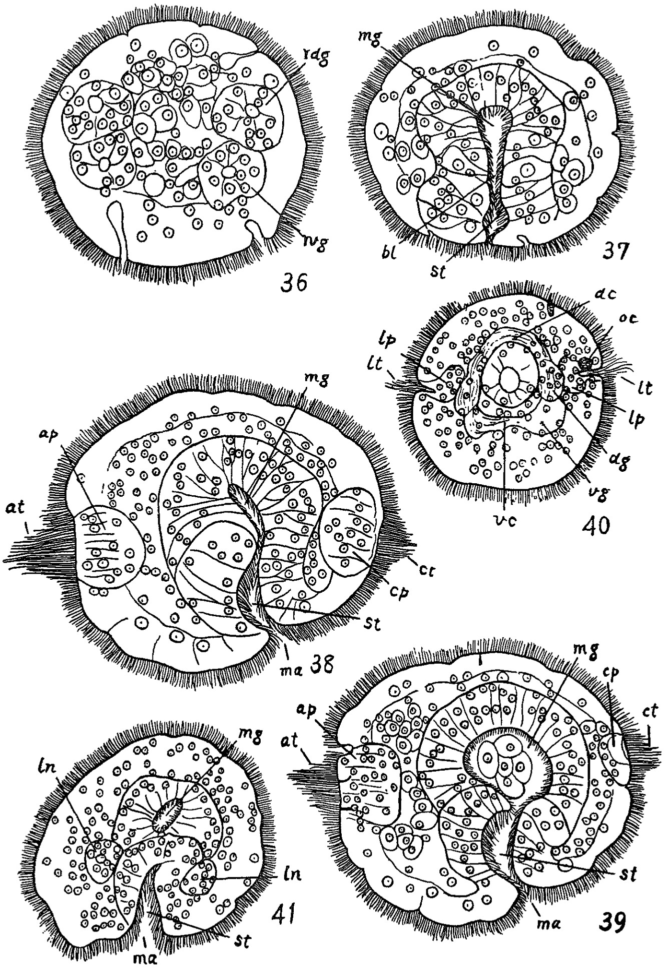
At the 67th hour the ocelli are differentiated on the lateral sides of the lateral plates (Fig. 35, *roc*). They are composed of several small cells. At the 85th hour many fine granules stained blackish brown in color are found in the ocelli (Figs. 40 and 44, *oc*). The cerebral plate is derived from the rudiment of the nervous system. The sagittal section of the



Procephalothrix simulus Iwata. Figs. 30–32. Sagittal sections of the 61-hour larva through the apical plate (30), the nervous system (31), and the lateral plate (32). ×340. Figs. 33–35. Sagittal sections of the 67-hour larva through the apical plate (33), the nervous system (34), and the lateral plate (35). ×340. *ap*: apical plate. *at*: apical tuft. *bl*: blastopore. *cep*: cerebral plate. *cp*: caudal plate. *dg*: dorsal ganglion. *ln*: lateral nerve. *ma*: mouth of the future adult worm. *mg*: midgut. *rlp*: rudiment of the lateral plate. *roc*: rudiment of the ocellus. *st*: stomodaeum. *vg*: ventral ganglion.

cerebral plate in the 61- and 67-hour larvae is shown in Figs. 31, 34, and 35 (*cep*). Its horizontal section in the 85-hour larva is shown in Fig. 43. The lateral tuft of the cerebral plate composed of a bundle of long cilia is shorter than the lateral tuft (Fig. 4, *lt, ltc*).

Oesophagus. The oesophagus of the adult worm originates from the ectodermal stomodaeum. The formation of the stomodaeum is completed in the 50-hour larva when the underlying epidermis forms the mouth of



Procephalothrix simulus Iwata. Figs. 36 and 37. Transverse sections of the 67-hour larva through the brain (36) and the midgut (37). ×340. Fig. 38. Sagittal section of the 74-hour larva through the apical plate. ×340. Fig. 39. Sagittal section of the 79-hour larva through the apical plate. ×340. Figs. 40 and 41. Transverse sections of the 85-hour larva through the brain (40) and the stomodaeum (41). ×340. *ap*: apical plate. *at*: apical tuft. *bl*: blastopore. *cp*: caudal plate. *ct*: caudal tuft. *dc*: dorsal commissure of the brain. *dg*: dorsal ganglion. *ln*: lateral nerve. *lp*: lateral plate. *lt*: lateral tuft. *mg*: midgut. *oc*: ocellus. *rdg*: rudiment of the dorsal ganglion. *rvg*: rudiment of the ventral ganglion. *st*: stomodaeum. *vc*: ventral commissure of the brain. *vg*: ventral ganglion.

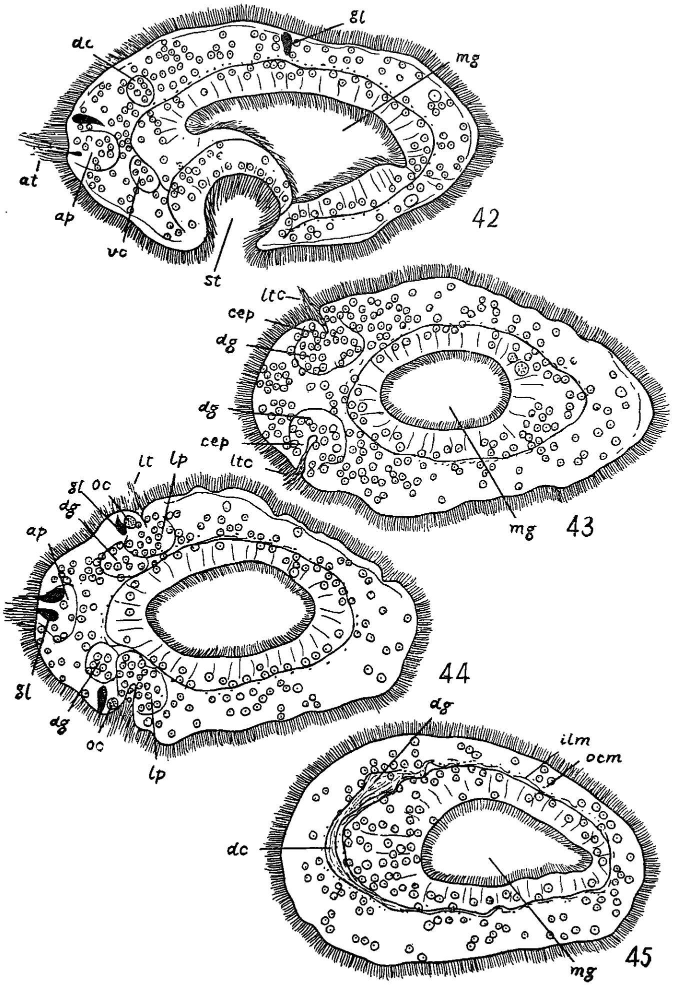
the future adult worm (Figs. 13, 19, 24, and 26, *rst, st*). The saggital section of the stomodaeum in the 55-, 61-, 67-, 74-, and 79-hour larva is shown in Figs. 27, 30, 33, 38, and 39. In the 31-hour larva the stomodaeum runs along the egg axis (Fig. 19), while in the 74-hour larva it runs at right angles to the long axis of the body (Fig. 38). The transition of the stomodaeum toward the anterior portion of the body is caused by posterior elongation of the epidermis on the dorsal side of the body. At the 79th hour the stomodaeum becomes convex toward the anterior portion of the body (Fig. 39). Along with the posterior extension of the body in the 85-hour larva the stomodaeum moves near the anterior part of the body and widens along the long axis of the body (Fig. 42). The midgut extends on the dorsal side of the stomodaeum. The midgut becomes broader while the opening of the stomodaeum into the midgut shrinks in sagittal section (Fig. 42).

Brain and lateral nerves. The brain and the lateral nerves of the adult worm are originated from a pair of the large and rounded blastomeres on the animal polar side of the 13-hour blastula (Fig. 7, *rbr*). The sagittal section of the rudiments of the nervous system at the 43th and 55th hours is illustrated in Figs. 25 and 28 (*rbr*). The horizontal section of the rudiments at the 43th hour is shown in Fig. 21. The rudiments invaginate posteriorly into the blastocoel (Figs. 21 and 23). At the 61st hour the rudiments differentiate into the anterior cerebral plate and the posterior dorsal and ventral ganglia (Fig. 31, *cep, dg, vg*). The lateral nerve derived from the ventral ganglion extends posteriorly along the lateral side of the body in the 67-hour larva (Fig. 34 *ln*). The transverse section of the dorsal and the ventral ganglia at the 67th hour is reproduced in Fig. 36. The dorsal and ventral commissures of the brain are differentiated in the 85-hour larva (Fig. 40, *dc, vc*). The lateral nerves are situated inside the epidermis extending posteriorly along the lateral sides of the stomodaeum and the midgut (Fig. 41, *ln*).

Proboscis and nephridia. The proboscis and the nephridia could not be observed at all during the present study.

Formation of the mesodermal organs.

Circular and longitudinal muscle layers of the body wall. The mesoderm cells differenciate into the circular and the longitudinal muscle layers of the body wall. The mesoderm cells never fill up the blastocoel during development and their cellular boundary becomes indistinct upon staining. The mesoderm cells in the 50-, 55, 61-, 67, 74, and 79-hour larva are shown in Figs. 26, 27, 30, 33, 38, and 39. At the 85th hour the mesoderm cells differentiate into a thin layer of the outer circular and inner longitudinal muscles (Figs. 45, *ilm, ocm*).



Procephalothrix simulus Iwata. Fig. 42. Sagittal section of the 85-hour larva through the apical plate. ×340. Figs. 43–45. Horizontal sections of the 85-hour larva through the cerebral plate (43), the lateral plate (44), and the dorsal commissure of the brain (45). ×340. *ap*: apical plate. *at*: apical tuft. *cep*: cerebral plate. *dc*: dorsal commissure of the brain. *dg*: dorsal ganglion. *gl*: gland cell. *ilm*: inner longitudinal muscles. *lt*: lateral tuft. *ltc*: lateral tuft derived from the cerebral plate. *mg*: midgut. *oc*: ocellus. *ocm*: outer circular muscles. *st*: stomodaeum. *vc*: ventral commissure of the brain.

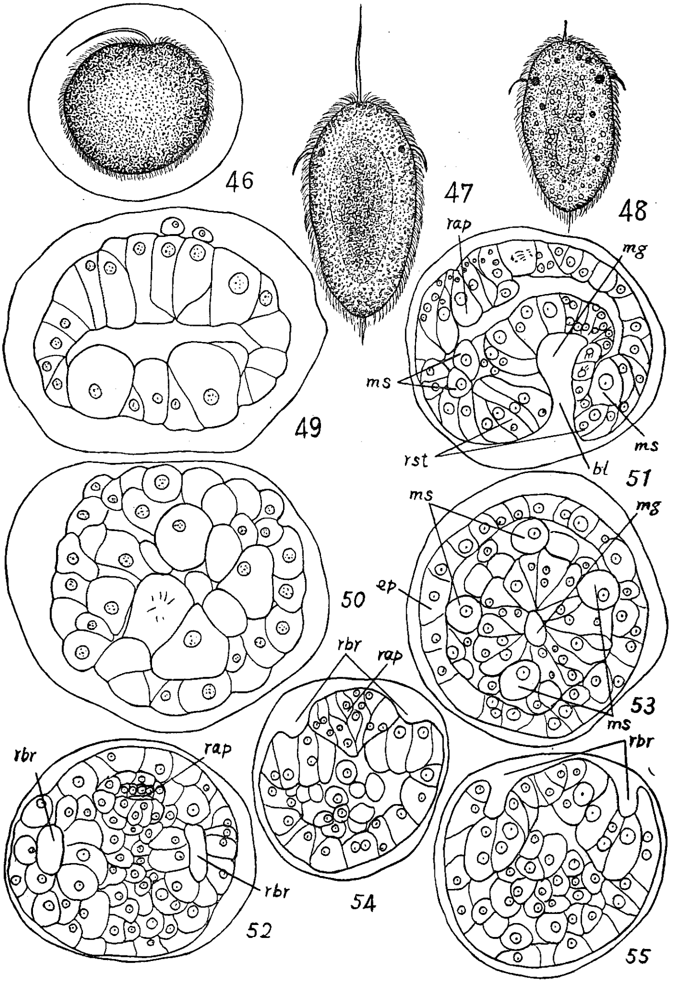
Formation of the endodermal organs.

Intestine. Between 43 and 61 hours after fertilization the midgut extends toward the dorsal side of the larva (Figs. 24, 26, 27, and 30). At the 67th hour the midgut runs at right angles to the long axis of the body (Fig. 33). In later stages the midgut narrows downward and inclines toward the anterior portion of the body (Figs. 33 and 38). In the 79-hour larva the midgut expands axially along the long axis of the body and contains a small number of oval cells (Fig. 39). The cilia come to appear at first in the 61-hour larva (Fig. 30). At the 85th hour the midgut extends posteriorly along the long axis of the body (Figs. 42-45). The formation of the anus could not be observed in the present study.

2) Development of *Procephalothrix filiformis*

Observations on the spawning habit were first made on August 3rd, 1954; the larvae lived for 23 days. On July 28th, 1957, spawning was again observed; the materials were fixed at intervals of half a day during 9 days and stained with Delafield's haematoxylin and eosin. On July 19th, 1958, the egg mass was obtained and the materials were fixed at 4, 17, 25, 45, 53, 58, 71, 77, 92, 102, 117, 141, 149, 165, 172, 188, 196, 213, 221, 237, 245, 262, 286, 310, 334, 358, 381, and 405 hours after fertilization and stained with Heidenhain's azan. For observations the latter materials were mainly used.

Cleavage and gastrulation. At the beginning of spawning the males and the females come together and interwind with each other. After that by peristaltic movement of the body the eggs and the spermatozoa are discharged simultaneously through the gonoducts. The jelly substance secreted from the epidermis of the females forms a large egg mass. The spermatozoa penetrate freely into the jelly. The egg is pale yellow brown in color, opaque with rich yolk and measures about 0.15 mm in diameter. The egg membrane is thick, measuring about 0.06 mm in thickness. The 17-hour blastula flattens on the animal and the vegetal polar sides (Fig. 49). The animal polar side is composed of large columnar cells and is higher than the vegetal polar side. Four large blastomeres found on the vegetal polar side are arranged in rectangular position (Fig. 50). In the 25-hour gastrula the rudiments of the nervous system come to appear on the animal polar side as a pair of invaginations (Figs. 52, 54, and 55, *rbr*). Between them the rudiment of the apical plate is lying as in the case of *Procephalothrix simulus* (Figs. 51 and 54, *rap*). The polar bodies disappear at this time, but it is conceivable that the rudiment of the apical plate is situated at the animal pole. The polar ingression takes place toward one lateral side of the embryo, which becomes the dorsal side of the future adult worm (Fig. 51). The animal polar side becomes to the head of the adult worm, in which the apical plate is present, while the vegetal polar side and the remainder of the lateral side of the embryo becomes to the ventral side of the adult. The right-left side of the adult is determined by the situation of the rudiments of the nervous system.



Procephalothrix filiformis (Johnston). Fig. 46. Larva about 60 hours after fertilization. ×105. Fig. 47. Larva about 130 hours after fertilization. ×105. Fig. 48. Larva about 220 hours after fertilization. ×105. Fig. 49. Sagittal section of the 17-hour blastula through the polar body. ×340. Fig. 50. Horizontal section of the vegetal polar side of the 17-hour blastula. ×340. Fig. 51. Sagittal section of the 25-hour gastrula through the apical plate, showing the formation of the midgut and the stomodaeum. ×340. Figs. 52 and 53. Horizontal sections of the 25-hour gastrula through the rudiment of the brain and lateral nerves (52) and the midgut (53). ×340. Figs. 54 and 55. Transverse sections of the 25-hour gastrula through the apical plate (54) and the rudiment of the brain and lateral nerves (55). ×340. *ap*: apical plate. *bl*: blastopore. *ep*: epidermis. *mg*: midgut. *ms*: mesoderm cells. *rap*: rudiment of the apical plate. *rbr*: rudiment of the brain and lateral nerves. *rst*: rudiment of the stomodaeum.

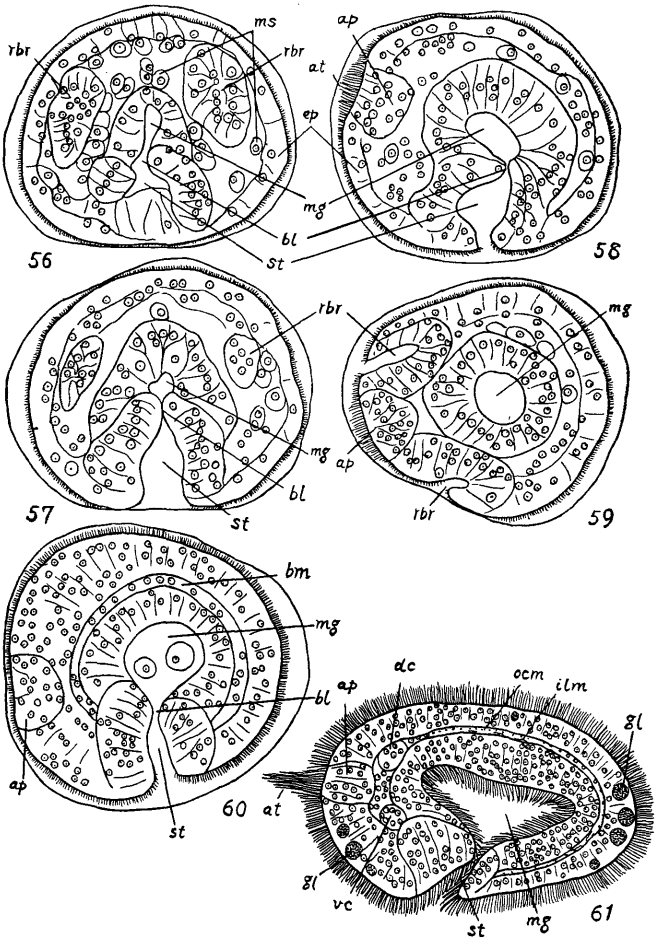
The rudiment of the ectodermal stomodaeum consists of a small number of large columnar cells posterior to the midgut (Fig. 51, *rst*). The blastopore is situated at the portion between the midgut and the stomodaeum (*bl*). The oval mesoderm cells found near the vegetal polar side of the embryo are arranged in rectangular position (Fig. 53, *ms*). Two of them are arranged along the sagittal plane of the embryo. As is shown in Fig. 51, it seems that the blastomeres at the portion between the endoderm and the ectoderm push the mesoderm cells out into the blastocoel.

Larva. The young larva just liberated from the egg membrane is round in shape and provided with a long apical tuft. The larva swims in a straight line parallel to the long axis of the body. The larva is wider anteriorly and tapers posteriorly, measuring about 0.28 mm in length and 0.14 mm in width (Fig. 47). The apical tuft measures about 0.15 mm in length. The ocelli situated at the lateral sides of the cephalic region appear as a pair of spots, blackish brown in color. The lateral tufts come to appear at the lateral sides of the ocelli. The caudal tuft is found at the posterior end of the body; it is shorter than the apical tuft. The larva becomes gradually smaller in size because no food was given. The decreased larva in Fig. 48 measures about 0.23 mm and 0.12 mm in width. The apical and the caudal tuft become shorter, while the ocelli become larger. The digestive canal is divided into two portions, the anterior of which become the stomodaeum, while the posterior one forms the midgut. In the epidermis there come to appear a small number of gland cells, blackish brown in color.

Formation of the octodermal organs.

Epidermis. The apical, the caudal, and the lateral plates and the ocelli are formed as larval organs in the epidermis. At the 25th hour the epidermis is composed of a row of rectangular cells extending on the body surface except the rudiments of the nervous system and of the stomodaeum. It is clearly demarcated from the underlying blastocoel. In the 45-hour embryo the epidermis becomes indistinct in its cellular boundary and the nuclei are arranged in a row. At the 58th hour the epidermis widens dorsoventrally and contains a number of nuclei irregularly arranged (Fig. 60). The blastocoel becomes narrowed in width. In the 77-hour larva a small number of large rounded gland cells are differentiated on the anterior and the posterior portions of the body. The apical plate at the 45th hour is saccular in shape and protrudes posteriorly into the blastocoel (Fig. 58, *ap*). The sagittal section of the apical plate at the 102 hour is shown in Fig. 61. The formation of the lateral and the caudal plates and the ocelli could not be observed in the present study.

Oesophagus. The oesophagus of the adult worm originates from the



Procephalothrix filiformis (Johnston). Figs. 56 and 57. Serial transverse sections of the 45-hour embryo through the rudiment of the brain and lateral nerves. ×340. Fig. 58. Sagittal section of the 45-hour embryo through the apical plate. ×340. Fig. 59. Horizontal section of the 45-hour embryo through the apical plate. ×340. Fig. 60. Sagittal section of the 58-hour embryo through the apical plate. ×340. Fig. 61. Sagittal section of the 102-hour larva through the apical plate. ×340. *ap*: apical plate. *at*: apical tuft. *bl*: blastopore. *bm*: blastocoel filled with the mesoderm cells. *ep*: epidermis. *dc*: dorsal commissure of the brain. *gl*: gland cells. *ilm*: inner longitudinal muscles. *mg*: midgut. *ms*: mesoderm cells. *ocm*: outer circular muscles. *rbr*: rudiment of the brain. *st*: stomodaeum. *vc*: ventral commissure of the brain.

ectodermal stomodaeum. At the 45th hour the stomodaeum is demarcated histologically from the midgut and the epidermis (Figs. 56-58, *st*). It narrows at both ends and broadens in the middle portion. The epidermis lying under the stomodaeum forms the mouth of the future adult worm. At the 58th hour the stomodaeum becomes narrow in its inner lumen (Fig. 60). The sagittal section of the stomodaeum in the 102-hour larva is shown in Fig. 61. The anterior dorsal side of the stomodaeum is much higher than the posterior ventral side. Along with the extension of the epidermis on the dorsal side of the body, the stomodaeum moves near the anterior portion of the body where the apical plate is situated. At the 45th hour the stomodaeum runs at right angles to the body axis from the apical plate to the posterior end of the body (Fig. 58), while it inclines anteriorly in the sagittal section of the 102-hour larva (Fig. 61).

Brain and lateral nerves. As noted in the preceeding pages the brain and the lateral nerves of the future adult worm originate from a pair of invaginations of the blastomeres on the animal polar side (Figs. 52, 54, and 55, *rbr*). At the 45th hour the rudiments of the nervous system form a pair of narrow canals invaginated into the blastocoel (Fig. 59), and posteriorly reach the anterior portion of the midgut. They are longer than wide dorsoventrally in transverse section (Figs. 56 and 57). At the 92nd hour the dorsal and the ventral ganglia are differentiated from the rudiments and are respectively connected with the dorsal and the ventral commissures. The lateral nerve derived from the ventral ganglion extends posteriorly along the lateral side of the stomodaeum and the midgut. The dorsal and the ventral commissures of the brain in the 102-hour larva are shown in Fig. 61 (*dc, vc*).

Proboscis and nephridia. The formation of the proboscis and the nephridia could not be studied in the present study.

Formation of the mesodermal organs.

Circular and longitudinal muscle layers of the body wall. At the 45th hour there come into appearance a small number of mesoderm cells scattered in the broad blastocoel (Figs. 56-59, *ms*). In the 58-hour embryo the blastocoel is much reduced in width and contains a row of the nuclei of the mesoderm cells. At the 102nd hour the mesoderm cells differentiate into a thin layer of the outer circular and inner longitudinal muscles.

Formation of the endodermal organs.

Intestine. The midgut extends posteriorly toward the dorsal side of the body in the gastrular stage of development. Accompanying the elongation of the body in the direction of the long axis of the body the midgut expands axially (Figs. 58, 60, and 61, *mg*). The cilia are dense and long in the 102-hour larva (Fig. 61). The formation of the anus has not been observed in the present study.

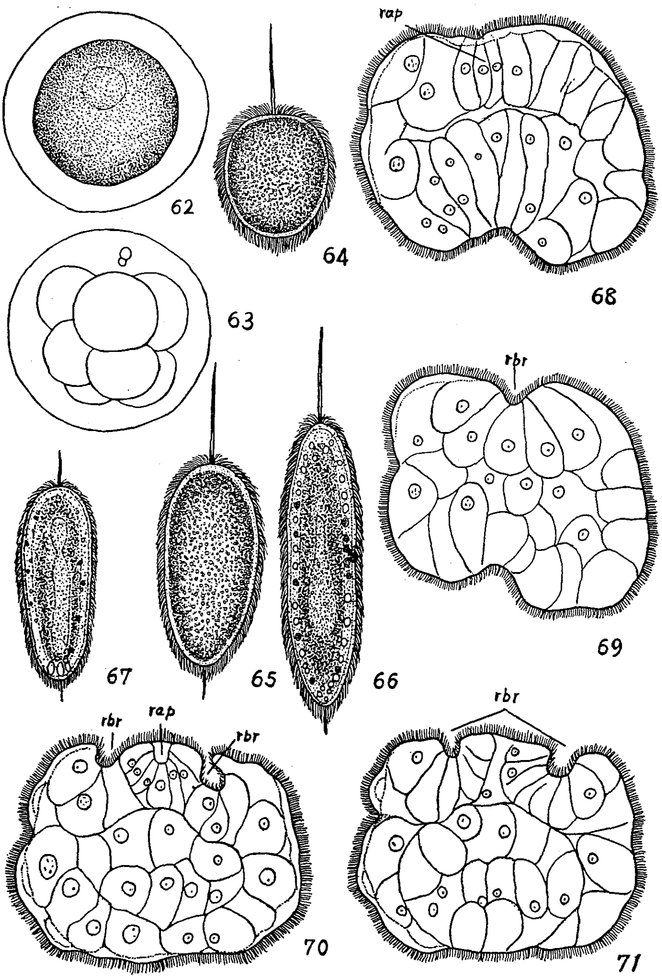
3) Development of *Tubulanus punctatus*

Observations on the spawning habit were made on July 30th, 1953 and the larvae lived during 50 hours after fertilization. On August 4th, 1954, eggs were spawned and the larvae lived for 15 days. On July 25th, 1955, eggs were artificially inseminated. The materials were fixed at 2, 15, 21, 37, 50, 71, 87, 94, 110, 118, 140, 163, 191, 215, and 286 hours after fertilization and stained with Delafield's haematoxylin and eosin.

Cleavage and gastrulation. The eggs are all discharged simultaneously through the gonoducts. One can find a large number of the eggs embedded in mucus secreted from the epidermis of the females. The mucus dissolves sooner or later after spawning. The egg is reddish brown in color, opaque with rich yolk, and measures about 0.1 mm in diameter. The germinal vesicle appears as a small clear zone. The egg membrane measures about 0.03 mm in thickness. The spermatozoon is a nematosperm, composed of a head, a middle piece and a tail. The head is spherical in shape and measures about 0.003 mm in length. The middle piece measures about 0.0015 mm in length, while the tail measures about 0.05 mm in length. The acrosome is not detectable.

After the first and second polar bodies are shed, cleavage of the spiral type leads to the formation of a flattened blastula. The first cleavage plane passes through the animal pole. The second cleavage plane is vertical and at right angles to that of the first cleavage. The third cleavage is dexiotropic and the blastomeres of the first quartet are far larger than the macromeres (Fig. 63).

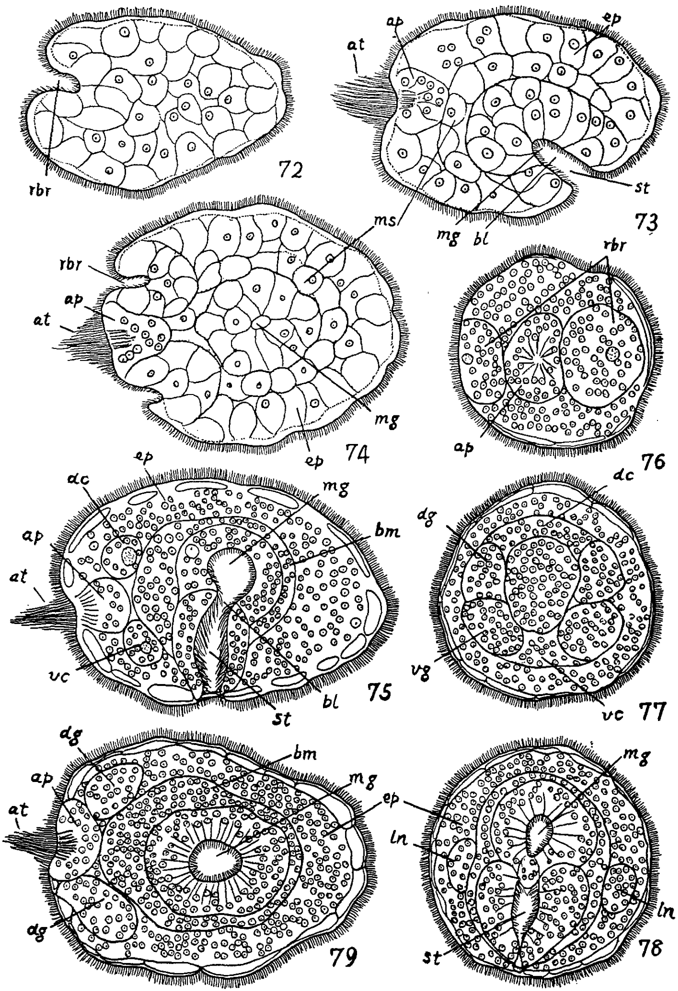
About 15 hours after fertilization, the embryo becomes flattened on the animal and the vegetal polar side and begins to revolve slowly in the horizontal plane. The egg membrane is broken down by the ciliary movement of the embryo. The vegetal polar side of the embryo composed of a row of large columnar cells is much deeper than the animal polar side (Fig. 68). The gastrulation of the embryo begins at this time. The rudiment of the apical plate is differentiated at the animal pole. It is composed of a small number of columnar cells clearly distinguishable from the surrounding cells, and somewhat invaginates downward (Figs. 68 and 70, *rap*). The rudiments of the nervous system come to appear at this time too. They are situated at the lateral sides of the apical plate (Figs. 67-71, *rbr*) and invaginate downward as does also the apical plate. The right-left side of the future adult worm is determined with the appearance of the rudiments of the nervous system. The animal polar side, in which the rudiments of the apical plate and of the nervous system are located, becomes the head of the adult worm. As is shown in Fig. 68, one lateral side of the embryo in sagittal section elongates more than the opposite lateral side owing to rapid cell-division and becomes the dorsal side of the



Tubulanus punctatus Takakura. Fig. 62. Egg immediately after liberation. ×180. Fig. 63. 8-cell stage. ×180. Fig. 64. 22-hour larva. ×95. Fig. 65. 69-hour larva. ×95. Fig. 66. 138-hour larva. ×95. Fig. 67. 283-hour larva. ×95. Figs. 68 and 69. Sagittal sections of the 15-hour gastrula through the rudiments of the apical plate (68) and of the brain and lateral nerves (69). ×430. Figs. 70 and 71. Serial transverse sections of the 15-hour gastrula through the rudiments of the apical plate (70) and of the brain and lateral nerves (71). ×430. *rap*: rudiment of the apical plate. *rbr*: rudiment of the brain and lateral nerves.

adult. The vegetal polar side and the opposite lateral side of the embryo becomes the ventral side of the adult. The epidermal cells inflate in the outer part and are paler in staining with eosin.

The polar ingression takes place toward the animal polar side. At



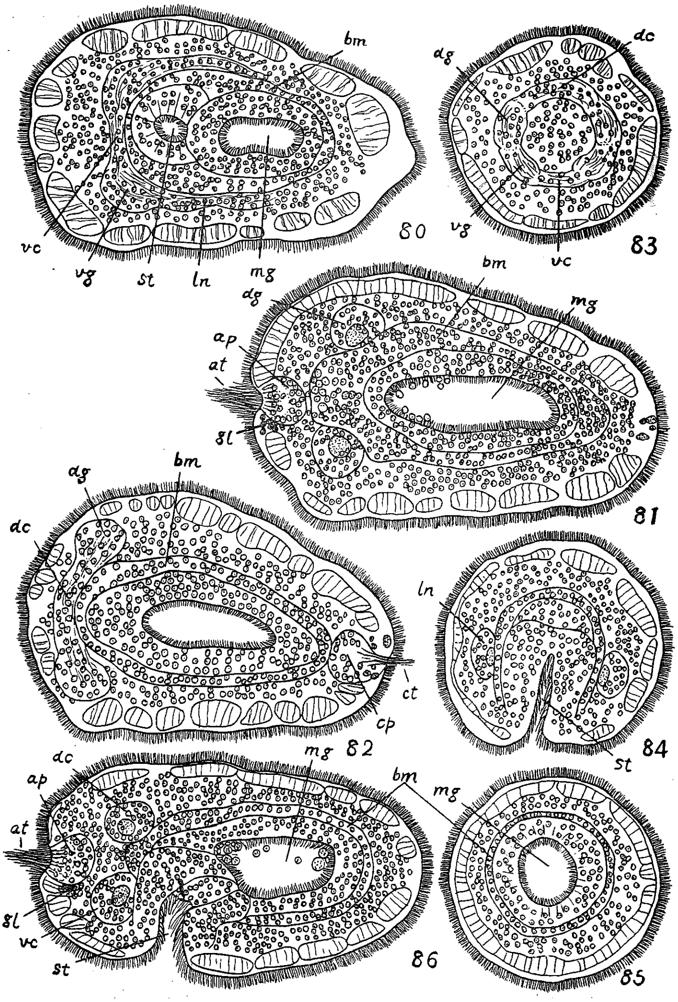
Tubulanus punctatus Takakura. Figs. 72 and 73. Sagittal sections of the 21-hour larva through the rudiment of the brain and lateral nerves (72) and the apical plate (73). ×430. Fig. 74. Horizontal section of the 21-hour larva through the apical plate. ×430. Fig. 75. Sagittal section of the 37-hour larva through the apical plate. ×430. Figs. 76–78. Transverse sections of the 37-hour larva through the apical plate (76), the brain (77), and the stomodaeum (78). ×430. Fig. 79. Horizontal section of the 37-hour larva through the apical plate. ×430. *ap*: apical plate. *at*: apical tuft. *bl*: blastopore. *bm*: blastocoel filled with mesoderm cells. *dc*: dorsal commissure of the brain. *dg*: dorsal ganglion. *ep*: epidermis. *ln*: lateral nerve. *mg*: midgut. *ms*: mesoderm cells. *rbr*: rudiment of the brain and lateral nerves. *st*: stomodaeum. *vc*: ventral commissure of the brain. *vg*: ventral ganglion.

the 21st hour the midgut and the ectodermal stomodaeum have been formed and the blastopore is situated at the portion between them (Fig. 73, *bl*, *mg*, *st*). The midgut is better stained with eosin than the stomodaeum which shows the same affinity as the epidermis. Numerous fine cilia are found in the inner lumen of the invaginated canal. The dorsal side of the larva is longer than the ventral side and the archenteron inclines toward the ventral side of the body (Fig. 73). The apical plate is saccular in shape and the central portion contains roots of the long flagella which form the apical tuft. The rudiments of the nervous system invaginate deeply in the epidermis and form a pair of narrow canals (Fig. 74, *rbr*). The mesoderm cells are arranged in a row in the blastocoel between the epidermis and the midgut (Figs. 73 and 74, *ms*). The epidermis is composed of a row of large oval cells containing large nuclei. The inflated outer part of the cells in the epidermis forms a thin layer showing weak affinity to eosin.

Larva. Between 15 and 22 hours after fertilization, the embryo becomes elongated along the egg axis running through the animal and the vegetal poles. The larva swims in a straight line along the long axis of the body. The 22-hour larva measures about 0.16 mm in length and 0.13 mm in width (Fig. 64). The apical tuft measures about 0.12 mm in length. The larva is pale yellowish brown in color. The cortical part of the larva is paler in color. At the 37th hour the larva is elliptical in shape, measuring about 0.28 mm in length and 0.13 mm in width (Fig. 65). The 138-hour larva is more than two times the length of the 22-hour larva; it measures about 0.38 mm in length and 0.08 mm in width (Fig. 66). The colorless vacuoles and the gland cells, blackish brown in color, are scattered in the epidermis. The digestive canal is divided into two portions, of which the anterior narrowed one is the stomodaeum, while the posterior broader one is the midget. At later stages the larva becomes smaller in size because no food was given. The 283-hour larva measures about 0.26 mm in length and 0.09 mm in width (Fig. 67). The apical and the caudal tufts decrease in length. The stomodaeum situated at the anterior portion of the body appears as an oval canal from above. The blastopore situated between the stomodaeum and the midgut becomes narrowed. The midgut is long and slender in shape. A small number of colorless vacuoles are found at the posterior end of the body.

Formation of the ectodermal organs.

Epidermis. The formation of the epidermis is complete in the 37-hour larva (Figs. 75-79). Numerous nuclei are irregularly scattered in the epidermis. The epidermis is clearly demarcated from the underlying mesodermal layer of the body; it contains the nervous system and the apical and caudal plates. At the 37th hour the epidermis forms discontinuous flattened spaces in the outer part which does not show affinity to eosin. At the 50th hour these spaces are provided with numerous fine



Tubulanus punctatus Takakura. Figs. 80–82. Horizontal sections of the 50-hour larva through the stomodaeum (80), the apical plate (81), and the dorsal commissure of the brain (82). ×430. Figs. 83–85. Transverse sections of the 50-hour larva through the brain (83), the stomodaeum (84), and the midgut (85). ×430. Fig. 86. Sagittal section of the 50-hour larva through the apical plate. ×430. *ap*: apical plate. *at*: apical tuft. *bm*: blastocoel filled with mesoderm cells. *cp*: caudal plate. *ct*: caudal tuft. *dc*: dorsal commissure of the brain. *dg*: dorsal ganglion. *gl*: gland cells. *ln*: lateral nerve. *mg*: midgut. *st*: stomodaeum. *vc*: ventral commissure of the brain. *vg*: ventral ganglion.

fibrous strings running dorsoventrally (Fig. 80). The caudal plate comes to appear in the 50-hour larva and is saccular in shape (Fig. 82, *cp*). The sagittal section of the apical plate at the 15th, 21st, 37th, 50th, 71st, and 94th hours is shown in Figs. 68, 73, 75, 86, 88, and 91 (*ap*), while the horizontal section is illustrated in Figs. 70, 74, 79, 81, 87, and 89. In the 37-hour larva the apical plate turns downward in its anterior end, while in the 71-hour larva it is situated at the dorsal side of the body (Figs. 75 and 88). A small number of gland cells stained with haematoxylin come to appear at the ventral side of the apical plate (Figs. 86, 88, and 91, *gl*).

Oesophagus. The oesophagus of the adult worm is ectodermal in origin. At the 37th hour the stomodaeum is situated in the middle portion of the body running at right angles to the long axis of the body (Fig. 75, *st*). At this time the mouth is covered with the epidermis already extended. The blastopore (*bl*) narrows somewhat in shape. The stomodaeum is longer than the midgut and slender; the inner margin is well stained with eosin and provided with numerous long cilia. The cilia of the stomodaeum are longer and denser than those of the midgut. At the 50th hour the mouth opens, while the blastoporal region is packed with nuclei (Fig. 86). Concomitant with the extension of the body, the stomodaeum comes near the anterior portion of the body and inclines anteriorly. At the 70th hour the blastoporal region of the stomodaeum opens again into the midgut (Fig. 88). The gland cells are differentiated in the anterior portion of the stomodaeum (Fig. 91).

Brain and lateral nerves. At the 37th hour the rudiments of the nervous system differenciate into the dorsal and the ventral ganglia and the dorsal and the ventral commissures (Figs. 75-79, *dg, vg, dc, vc*). The lateral nerve derived from the ventral ganglion extends posteriorly along the lateral side of the body. It must be noted that the nervous system is formed in the epidermis.

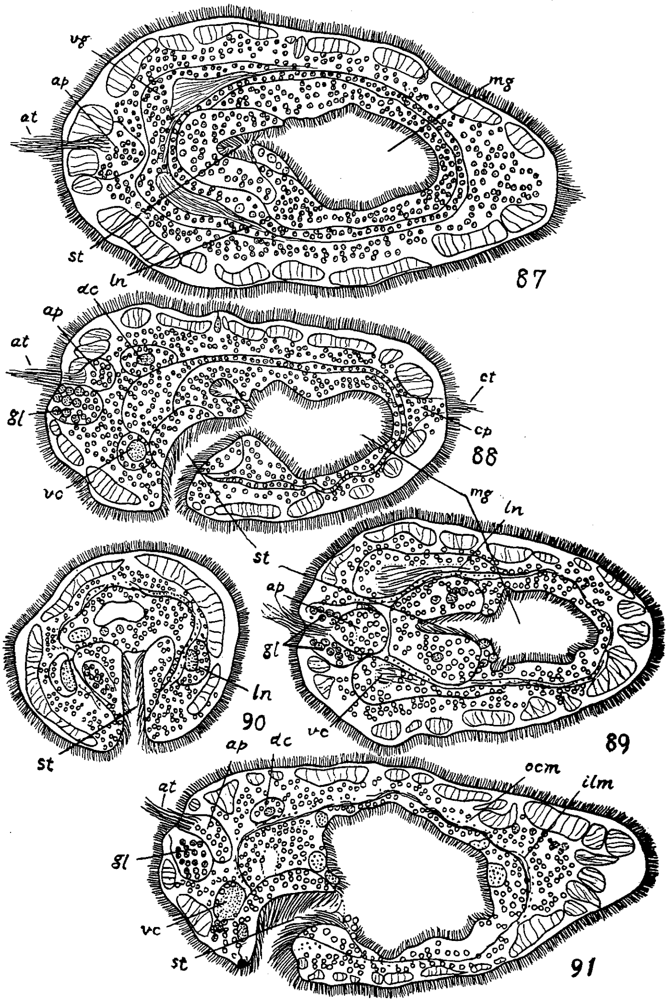
Proboscis and nephridia. The formation of the proboscis and the nephridia has not been observed in the present study.

Formation of the mesodermal organs.

Circular and inner longitudinal muscle layers of the body wall. At the 37th hour the mesoderm cells increase in number, showing many nuclei alone in the mesoderm layer. In the 94-hour larva a thin layer of outer circular and inner longitudinal muscles is differentiated from the mesoderm cells (Fig. 91).

Formation of the endodermal organs.

Intestine. At the 37th hour the midgut is round in shape in sagittal section and is situated in the middle portion of the body. Along with the elongation of the body the midgut extends axially and widens dorsoventral-



Tubulanus punctatus Takakura. Fig. 87. Horizontal section of the 71-hour larva through the apical plate. ×430. Fig. 88. Sagittal section of the 71-hour larva through the apical plate. ×430. Fig. 89. Horizontal section of the 94-hour larva through the apical plate. ×430. Fig. 90. Transverse section of the 94-hour larva through the stomodaeum. ×430. Fig. 91. Sagittal section of the 94-hour larva through the apical plate. ×430. *ap*: apical plate. *at*: apical tuft. *bm*: blastocoel filled with mesoderm cells. *cp*: caudal plate. *ct*: caudal tuft. *dc*: dorsal commissure of the brain. *gl*: gland cells. *ilm*: inner longitudinal muscles. *ln*: lateral nerve. *mg*: midgut. *ocm*: outer circular muscles. *st*: stomodaeum. *vc*: ventral commissure of the brain. *vg*: ventral ganglion.

ly (Figs. 75, 86, 88, and 91). The formation of the anus has not been observed in the present study.

4) Development of *Lineus torquatus*

The eggs after fertilization show the cleavge of typical spiral type and the larva at the age of five days indicates clearly the shape characteristic of the pilidium (Iwata, 1957).

5) Development of *Lineus alborostratus*

Observations on the early development of this species were made on the 16th of August, 1956.

Cleavage and gastrulation. A large number of eggs were obtained by cutting the females in the intestinal region of the body after which the eggs were artificially inseminated. The eggs immediately after liberation from the ovary are irregularly oval in shape, transparent, and not surrounded by the egg membrane. About 30 minutes later the ovum has become round in shape and measures about 0.12 mm in diameter (Fig. 92). The germinal vesicle is large and round with a diameter of about 0.06 mm. Cleavage of the spiral type leads to the formation of a round blastula. Fourteen hours after fertilization the embryo is opaque in the central portion owing to the poor yolk (Fig. 93). Numerous fine cilia come to appear at this time. The 22-hour larva becomes elongated along the egg axis (Fig. 94). The vegetal half of the body becomes opaque, while the animal half is transparent; a row of large columnar cells is arranged dorsoventrally. At this time the larva becomes to have an apical plate and an apical tuft. The apical plate is small and round in shape. The apical tuft measures about 0.09 mm in length. The vegetal polar side of the larva is wider than the animal one. Forty-eight hours after fertilization the larva has grown markedly in size and measures about 0.19 mm in length and 0.16 mm in width (Fig. 95). The vegetal polar side flattens and the archenteron bends laterally in the inner portion. A small number of the cells provided with a few short fibres are scattered in the blastocoel. The cilia on the vegetal polar side are longer and denser than those on the dorsal surface of the body. The apical tuft measures about 0.14 mm in length. In the 88-hour larva the lateral lappets, in a pair, expand vertically downward on both sides of the stomodaeum and then the larva exhibits completely the shape characteristic of pilidium (Fig. 96). The archenteron is divided into two portions: the endodermal midgut and the stomodaeum which is ectodermal. The blastopore is situated at the boundary between them. The midgut is large and round in shape and provided with long cilia. The stomodaeum is thinner than the midgut. The body measures about 0.34 mm in width and 0.3 mm in height. The apical tuft measures about 0.2 mm in length. The muscle cells which are provided with long fibres are scattered on the inner wall of the body. Several muscle fibres are arranged on the lateral lappets. On the basal portion of the lateral lappet there is found a horizontal narrow band of the stomodaeum. The cilia arranged on the ventral margin of the body are closely set and longer than those on the dorsal side.

6) Development of *Micrura akkeshiensis*

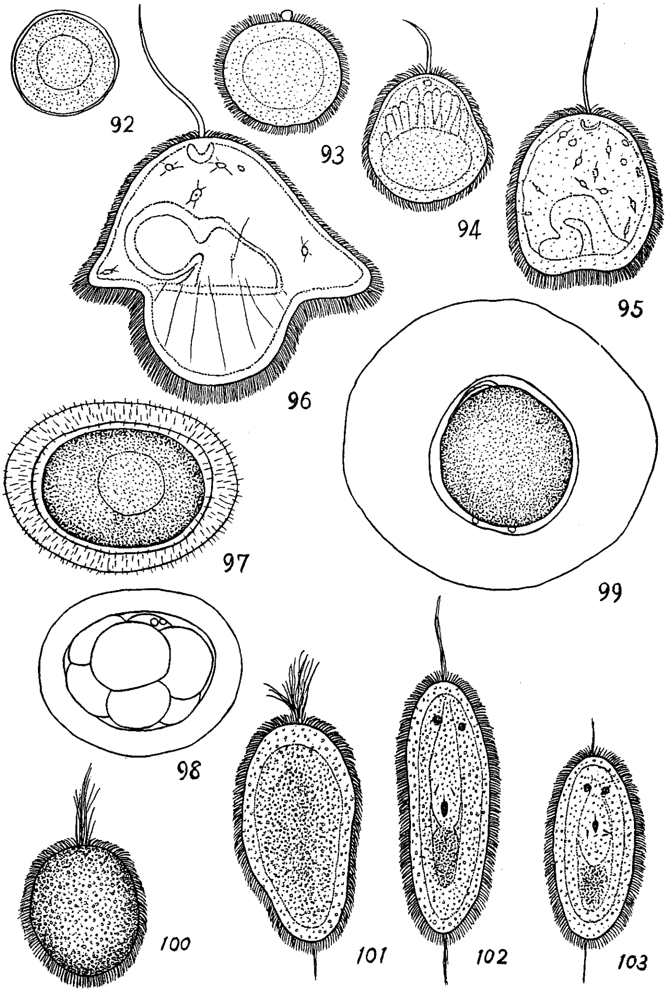
The egg shows the spiral type of cleavage. The blastopore is situated between the midgut and the stomodaeum. The young worm metamorphoses within the larval ectoderm. The adult ectoderm is derived from the five amniotic invaginations of the larval ectoderm. These five invaginations are a pair of head invaginations, a pair of trunk invaginations and an unpaired dorsal invagination. By the fusion of the blastodiscs which close in from the larval surface, the adult epidermis is formed under the larval skin, the latter of which is shed off when metamorphosis is completed. The cephalo-caudal axis of the larva and the axis of the metamorphosed adult growing inside the larva run in opposite directions from each other. The oesophagus is secondarily formed from the dorsal diverticulum of the stomodaeum. The cerebral sense organs are originated from the stomodeal invaginations. The epidermis of the proboscis and the nervous system are differentiated from the head blastodiscs. The lateral nerves run posteriorly in the blastodiscs. The nephridia are derived from the stomodaeum. The circular and the inner longitudinal muscle layers of the body wall, the muscle layers of the proboscis and of the proboscis sheath, and the blood lacunae are of mesodermal origin. The intestine is of endodermal origin. It is concluded that the present larva is an intermediate type between the pilidium and Desor larva (Iwata, 1958).

7) Development of *Emplectonema gracile*

Delsman (1918) published a report on the early development of this species in detail, though restricted only to external characters.

In the present study the eggs were obtained on August 8th, 1956. They were fixed at 1, 1.5, 4, 23, 27, 37, 43, 62, 73, 88, 136, 151, and 174 hours after fertilization and stained with Delafield's haematoxylin and eosin. The eggs fertilized on July 28th, 1957 were fixed at 2, 9, 15, 22, 35, 47, 62, 70, 86, 95, 107, 117, 134, 141, 160, 166, 182, 189, 208, 230, 254, and 278 hours and stained with Heidenhain's azan. For observations the latter materials were mainly used.

Cleavage and gastrulation. The eggs are laid through the gonoducts. The eggs immediately after liberation from the ovary are elliptical in shape, pale yellow in color, and opaque with rich yolk. The egg membrane is thin. The glutinous membrane with brilliant spine-like objects is thick and measures about 0.05 mm in thickness (Fig. 97). About 20 minutes later the egg has become round in shape, measuring about 0.17 mm in



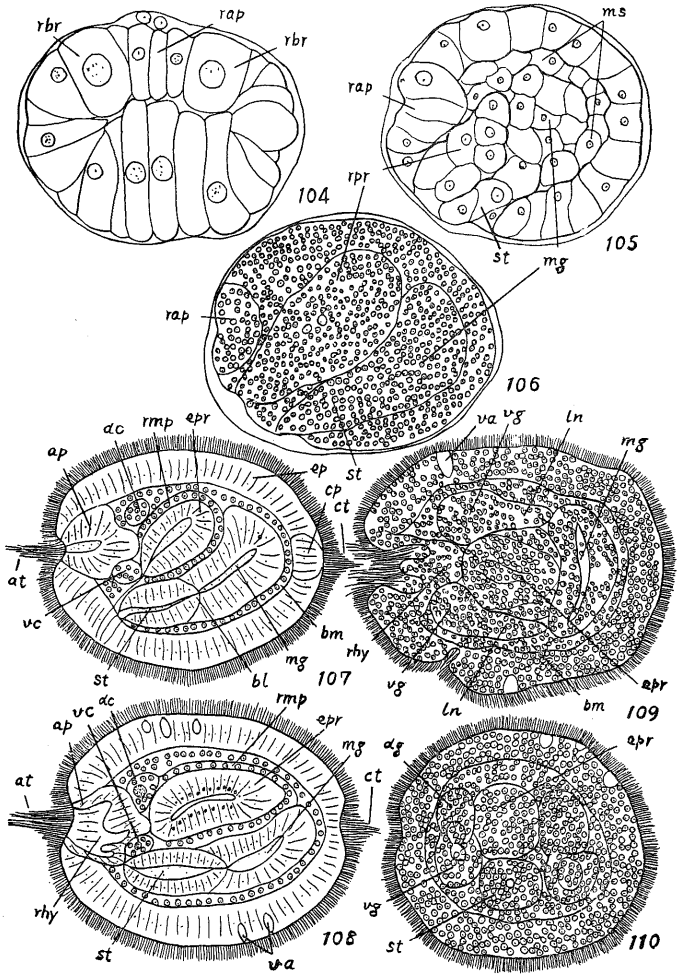
Lineus alborostratus Takakura. Fig. 92. Egg immediately after liberation. ×105. Fig. 93. 14-hour gastrula. ×105. Fig. 94. 22-hour larva. ×105. Fig. 95. 48-hour larva. ×105. Fig. 96. 88-hour pilidium larva. ×105. *Emplectonema gracile* (Johnston). Fig. 97. Egg immediately after liberation. ×105. Fig. 98. 8-cell stage. ×105. Fig. 99. 17-hour gastrula. ×105. Fig. 100. 29-hour larva. ×105. Fig. 101. 51-hour larva. ×105. Fig. 102. 99-hour larva. ×105. Fig. 103. 17-day larva. ×105.

diameter. The germinal vesicle is large, measuring about 0.08 mm in diameter.

After the first and the second polar bodies are formed, cleavage of the spiral type leads to the formation of a round blastula. The first cleavage plane passes through the animal pole. The second cleavage plane is vertical and at right angles to that of the first cleavage. The third cleavage is dexiotropic and the blastomeres of the first quartet are far larger than the macromeres (Fig. 98). Seventeen hours after fertilization the embryo provided with a long apical tuft and numerous fine cilia begins to rotate slowly within the egg membrane (Fig. 99).

The 9-hour blastula becomes flattened on the animal and the vegetal polar side, which is about twofold the thickness of the former side. The blastocoel is found as a narrow and flattened space. The rudiment of the apical plate comes to appear on the animal polar side as a small number of columnar cells situated under the polar bodies (Fig. 104, *rap*). As in the case of *Procephalothrix simulus* there are found a pair of large blastomeres on the animal polar side (Fig. 104, *rbr*). It seems that they differentiate into the nervous system of the adult worm. The right-left side of the adult worm is determined by the appearance of the rudiments of the nervous system. The animal polar side becomes to the head of the adult, while the vegetal polar side and one lateral side of the embryo in sagittal section become to the ventral side of the adult. The other lateral side of the embryo becomes to the dorsal side of the adult. Between 9 and 15 hours after fertilization, the polar ingression takes place toward the dorsal side of the adult worm. The body becomes elliptical in shape owing to rapid cell-division of the blastomeres on the dorsal side of the future adult worm (Fig. 105). The rudiment of the epidermis of the proboscis comes to appear as a small number of cells found between the apical plate and the ectodermal stomodaeum (*rpr*). The apical plate approaches the vegetal polar side of the embryo. The ectodermal stomodaeum posterior to the midgut appears as a row of a small number of cells (*st*). The mesoderm cells are arranged in a row in the blastocoel. Twenty-two hours after fertilization, numerous small nuclei are found irregularly scattered in the body; the boundary features of the apical plate, the proboscis, and the digestive system are not clearly recognizable.

Larva. About 25 hours after fertilization the embryo escapes from the egg membrane. The just-escaped larva swims in a straight line parallel to the long axis of the body. The 25-hour larva measures about 0.16 mm in length and 0.13 mm in width (Fig. 100). The apical tuft composed of a bundle of long flagella measures about 0.12 mm in length. At the 51st hour the larva is provided with a short caudal tuft (Fig. 101). The epidermis is clearly recognizable form above as a thick layer surrounding the body. The 99-hour old larva, about 0.31 mm long and 0.1 mm wide, is provided with a pair of large ocelli which are blackish brown in color (Fig. 102). The proboscis is divided into two portions: the anterior portion is long and contains two pouches of accessory stylets and a base with a central stylet; the posterior portion is short and rounded, brownish



Emplectonema gracile (Johnston). Fig. 104. Transverse section of the 9-hour blastula through the polar body, showing the rudiments of the brain and lateral nerves (*rbr*) and of the apical plate (*rap*). ×430. Fig. 105. Sagittal section of the 15-hour blastula through the rudiment of the apical plate. ×430. Fig. 106. Sagittal section of the 22-hour gastrula through the rudiment of the apical plate. ×430. Fig. 107. Sagittal section of the 35-hour larva through the apical plate. ×430. Fig. 108. Sagittal section of the 47-hour larva through the apical plate. ×430. Fig. 109. Horizontal section fo the 47-hour larva through the apical plate. ×430. Fig. 110. Transverse section of the 47-hour larva through the brain. ×430. *ap*: apical plate. *at*: apical tuft. *bc*: blastocoel. *bl*: blastopore. *bm*: blastocoel filled with mesoderm cells. *cp*: caudal plate. *ct*: caudal tutf. *dc*: dorsal commissure of the brain. *dg*: dorsal ganglion. *ep*: epidermis. *epr*: epidermis of the proboscis. *ln*: lateral nerve. *mg*: midgut. *ms*: mesoderm cells. *rap*: rudiment of the apical plate. *rbr*: rudiment of the brain and lateral nerves. *rhy*: rhynchodaeum. *rmp*: rudiment of the muscle layers of the proboscis and of the proboscis sheath. *rpr*: rudiment of the proboscis. *st*: stomodaeum. *va*: colorless vacuoles. *vc*: ventral commissure of the brain. *vg*: ventral ganglion.

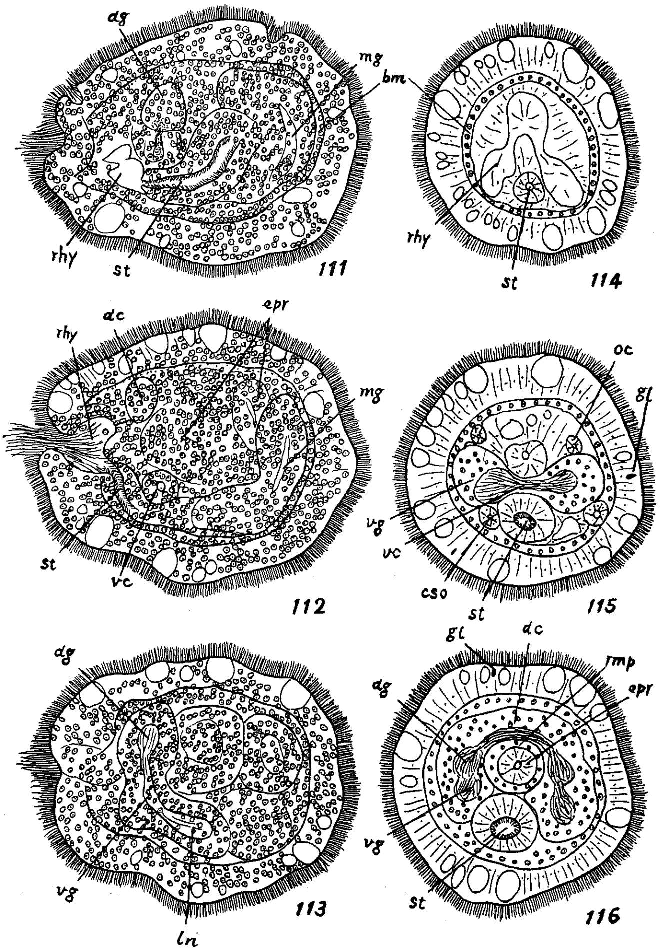
in color. At the age of 17 days the larva shortens to about 0.23 mm in length (Fig. 103). The larva does not shed off the primordial epidermis differing from the observation reported by Delsman (1915).

Formation of the ectodermal organs.

Epidermis. In the 35-hour larva the epidermis is clearly demarcated from the mesodermal layer (Fig. 107). The caudal plate is formed in the epidermis at the posterior end of the body (*cp*). In the 47-hour larva a small number of colorless vacuoles come to appear in the epidermis (Figs. 108-110, *va*). The caudal plate somewhat invaginates inward and disappears in its cellular boundary (Fig. 108). In the 70-hour larva the epidermis differentiates a small number of gland cells which are stained reddish with Heidenhain's azan (Figs. 115-118, *gl*).

Oesophagus. At the 22nd hour the midgut and the ectodermal stomodaeum are not recognizable histologically in their boundary; they contains numerous small nuclei (Fig. 106, *mg, st*). In the 35-hour larva the formation of the stomodaeum and the midgut is completed in shape (Fig. 107, *mg, st*). The blastopore is situated at the portion between them (*bl*). The narrow canal of the stomodaeum is filled with cytoplasm. The stomodaeum is enclosed inside the epidermis. In the 47-hour larva the stomodaeum anteriorly attaches to the posterior wall of the apical plate which becomes the rhynchodaeum of the future adult worm (Fig. 108, *rhy, st*). The anterior portion of the stomodaeum lies under the ventral commissure of the brain (*vc*). In the 70-hour larva the stomodaeum opens anteriorly into the rhynchodaeum (Figs. 111 and 112, *rhy, st*) and posteriorly into the midgut (Fig. 117, *os*).

Proboscis. In the 35-hour larva the proboscis is enclosed inside the body as an elongated sac. It is composed of the epidermis of the proboscis and the muscle layers of the proboscis and of the proboscis sheath, the latter of which appears as a thin layer containing a row of mesoderm cells (Fig. 107, *epr, rmp*). In the 47-hour larva the proboscis is attached anteriorly to the back of the rhynchodaeum (Fig. 108). Glandular globules are arranged in the epidermis of the proboscis. The proboscis becomes larger in size and longer, lying on the digestive canal. Two lateral pouches of the proboscis containing a few accessory stylets and the base of the proboscis come into appearance in the 70-hour larva (Fig. 117, *as, bcs, las*). The base is surrounded by glandular globules. In the 86-hour larva the proboscis is divided into three portions (Fig. 119). The anterior portion is coelomic and opens into the rhynchodaeum (*anp*). The middle one contains the proboscidial apparatus (*mp*), while the posterior portion is round and short, being filled with cytoplasm dark in color with Heidenhain's azan staining (*ppr*). The base of the proboscis becomes larger in



Emplectonema gracile (Johnston). Figs. 111–113. Sagittal sections of the 70-hour larva through the stomodaeum (111 and 112) and the lateral nerves (113). ×430. Figs. 114–116. Transverse sections of the 70-hour larva through the stomodaeum (114), the cerebral sense organs (115), and the brain (116). ×430. *ap*: apical plate. *bm*: blastocoel filled with mesoderm cells. *cso*: cerebral sense organ. *dc*: dorsal commissure of the brain. *dg*: dorsal ganglion. *ep*: epidermis. *epr*: epidermis of the proboscis. *gl*: gland cell. *ln*: lateral nerve. *mg*: midgut. *oc*: ocellus. *rhy*: rhynchodaeum. *rmp*: rudiment of the muscle layers of the proboscis and of the proboscis sheath. *st*: stomodaeum. *vc*: ventral commissure of the brain. *vg*: ventral ganglion.

size and is pyriform. The central stylet of the base is found in the 208-hour larva (Fig. 122).

Rhynchodaeum. At the 35th hour the apical plate has invaginated inward and forms a narrow canal filled with cytoplasm (Fig. 107, *ap*). The posterior portion of the apical plate is situated between the dorsal and the ventral commissures of the brain. In the 47-hour larva the rhynchodaeum thus formed widens dorsoventrally and posteriorly attaches to the proboscis and the stomodaeum (Fig. 108). The sagittal section of the rhynchodaeum which opens into the proboscis and the stomodaeum at the 86th hour is shown in Fig. 119.

Brain and lateral nerves. As in the cases of *Procephalothrix simulus* and *P. filiformis,* the nervous system of the present adult worm is formed in the blastocoel which is filled with mesoderm cells. The dorsal and the ventral commissures of the brain are first found in the 35-hour larva (Fig. 107, *dc, vc*). The dorsal and the ventral ganglia are distinguishable in shape in the 47-hour larva in transverse section (Fig. 110, *dg, vg*). The lateral nerve derived from the ventral ganglion extends along the lateral side of the body (Fig. 109, *ln*). In the 70-hour larva the brain and the lateral nerves consist of a central portion containing numerous nerve fibres and an external portion containing the nerve cells (Figs. 116 and 117).

Cerebral sense organs. The cerebral sense organs make their appearance at first in the 70-hour larva in front of the brain (Fig. 115, *cso*).

Ocelli. At the 70th hour a pair of ocelli situated on the dorsal side of the head are recognized in the mesodermal layer (Fig. 115, *oc*).

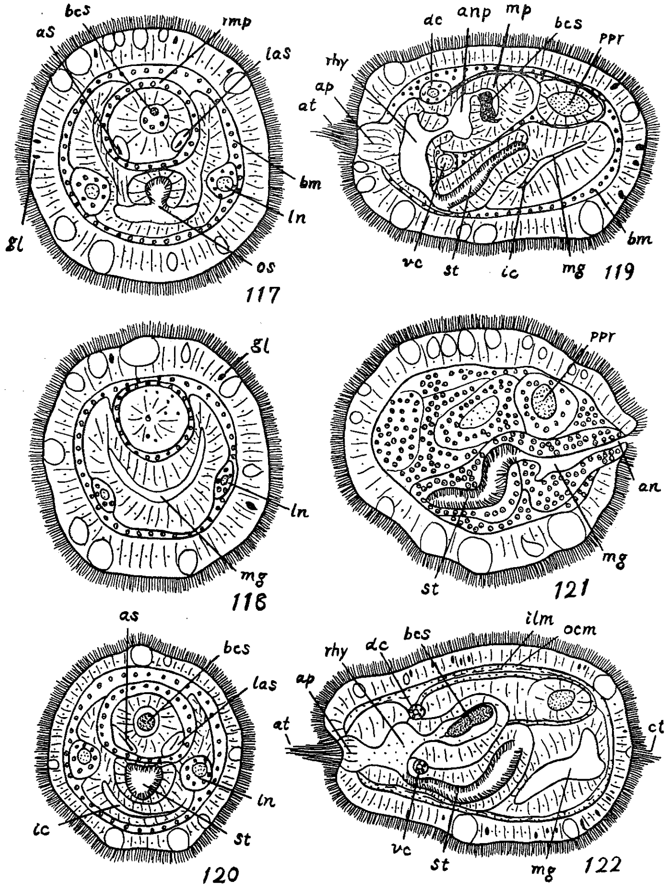
Formation of the mesodermal organs.

Circular and longitudinal muscle layers of the body wall. The muscle layers of the body wall are originated from the mesoderm cells found in the 15-hour gastrula (Fig. 105, *ms*). At the 35th hour a thin mesodermal layer of the embryo containing a row of the nuclei of the mesoderm cells is clearly found among the epidermis, proboscis and digestive system (Fig. 107, *bm*). The mesoderm cells differentiate into the outer circular and inner longitudinal muscles in the 208-hour larva (Fig. 122, *ilm, ocm*).

Muscle layers of the proboscis and proboscis sheath. In the 35-hour larva the epidermis of the proboscis is surrounded by a thin layer containing a row of the nuclei of the mesoderm cells (Fig. 107, *rmp*). The mesodermal layer of the proboscis does not differentiate into the muscle layers of the proboscis and of the proboscis sheath. The formation of the rhynchocoel has not been observed in the present study.

Formation of the endodermal organs.

Intestine. In the 35-hour larva the midgut is saccular in shape; it forms a narrow canal filled with cytoplasm (Fig. 107, *mg*). In the 70-hour larva the midgut opens anteriorly into the stomodaeum (Fig. 117, *os*). The intestinal caecum of the intestine is recognizable in the anterior portion of



Emplectonema gracile (Johnston). Figs. 117–118. Transverse sections of the 70-hour larva through the lateral pouches of the accessory stylets (117) and the midgut (118). ×430. Fig. 119. Sagittal section of the 86-hour larva through the apical plate. ×430. Fig. 120. Transverse section of the 94-hour larva through the base of the central stylet. ×430. Fig. 121. Sagittal section of the 94-hour larva through the anus. ×430. Fig. 122. Sagittal section of the 208-hour larva through the apical plate. ×430. *an*: anus. *anp*: anterior part of the proboscis. *ap*: apical plate. *at*: apical tuft. *as*: accessory stylet. *bm*: blastocoel filled with mesoderm cells. *bcs*: base of the central stylet. *ct*: caudal tuft. *dc*: dorsal commissure of the brain. *gl*: gland cell. *ic*: intestinal caecum. *ilm*: inner longitudinal muscles. *las*: lateral pouch of the accessory stylets. *ln*: lateral nerves. *mg*: midgut. *mp*: middle part of the proboscis. *ocm*: outer circular muscles. *os*: opening of the stomodaeum into the midgut. *ppr*: posterior part of the proboscis. *rhy*: rhynchodaeum. *rmp*: rudiment of the muscle layers of the proboscis and of the proboscis sheath. *st*: stomodaeum. *vc*: ventral commissure of the brain.

the midgut in the 94-hour larva (Figs. 119 and 120, *ic*). The anus opens at the posterior end of the body where the caudal plate is formed (Fig. 121, *an*).

8) Development of *Oerstedia dorsalis* var. *albolineata*

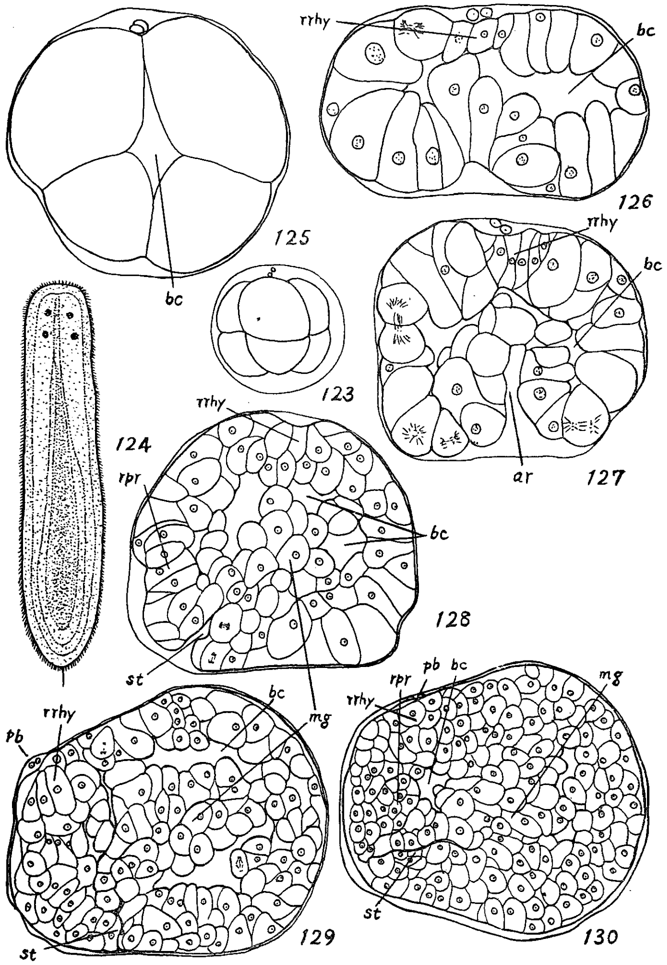
Coe (1943) has briefly reported external observations on the development of this species from eggs to young worms.

The species lives in the vicinity of Akkeshi usually attached to the hydroid *Eudendrium annulatum* Norman. The egg masses attached to the stalks of *Eudendrium* were first found on August 25th, 1953 at Daikokujima and then again on August 6th, 1955. The mature adult worms collected early in August 1956 spawned on the 12th of the same month and produced 25 egg masses, which were stained with Delafield's haematoxylin and eosin. In 1957, the adult worms collected on July 14th produced 21 egg masses, which were stained with Heidenhain's azan. For observations the latter materials were mainly used.

Cleavage and gastrulation. The jelly substance secreted from the epidermis of the females agglutinates a number of the eggs. The egg mass remains attached to the stalks of the hydroid after the females creep out. These eggs are laid through the gonoducts. The egg is milky white in color, opaque with rich yolk and measures about 0.2 mm in diameter. The egg membrane is thin. The spermatozoon is a nematosperm, of which the head is round in shape. In the eight-cell stage the blastomeres of the first quartet are budded off dexiotropically from four macromeres; the former are larger than the latter (Figs. 123 and 125).

The polar ingression takes place toward the animal polar side of the embryo (Figs. 126 and 127). The rudiment of the rhynchodaeum comes to appear in gastrular stages as a small number of small columnar cells situated under the polar bodies (Figs. 126 and 127, *rhy*). The blastocoel is broad in width and is colorless in staining. It narrows gradually in accordance with the advancement of the polar ingression. As in the case of *Emplectonema gracile* the ectoderm on one lateral side of the embryo becomes more elongated by rapid cell-division than that on the other side, in which the rudiment of the proboscis is present (Figs. 128 and 130, *rpr*). The elongated lateral side becomes to the dorsal side of the future adult worm. The animal polar side in which the rudiment of the rhynchodaeum is present becomes to the head of the adult worm, while the vegetal polar side and the other lateral side of the embryo becomes to the ventral side of the adult. The rudiment of the proboscis is represented by a small number of ectodermal cells situated on the dorsal side of the stomodaeum (Fig. 130, *rpr*).

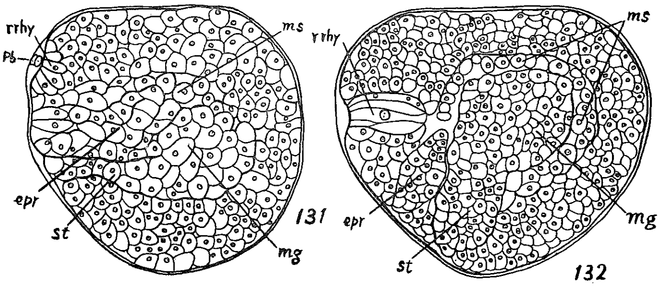
Young worm. About 2 days after fertilization the embryo begins to

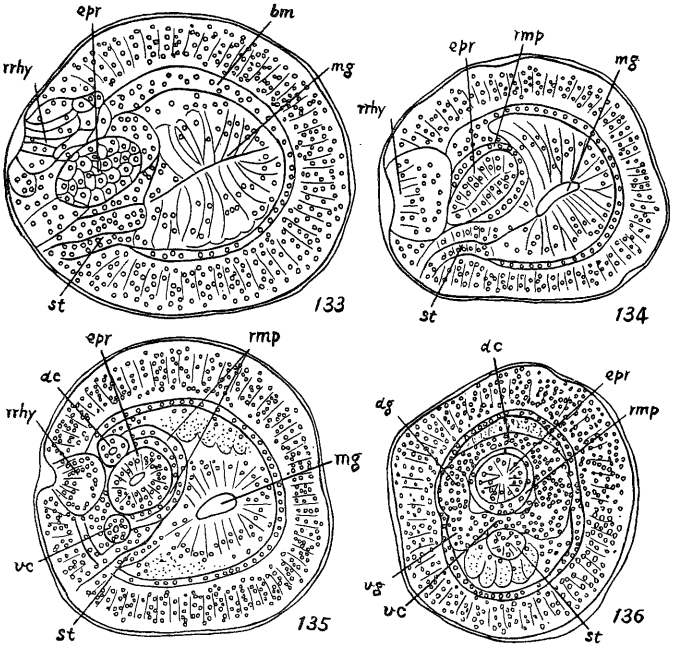


Oerstedia dorsalis var. *albolineata* Iwata. Fig. 123. 8-cell stage. ×95. Fig. 124. Young worm at the age of 4 days. ×95. Fig. 125. Section of the egg through the polar body at 8-cell stage. ×215. Figs. 126 and 127. Sections of the gastrula through the rudiment of the rhynchodaeum (*rhy*). ×215. Figs. 128–130. Sagittal sections of the embryo through the stomodaeum (*st*). ×215. *ar*: archenteron. *bc*: blastocoel. *mg*: midgut. *pb*: polar body. *rrhy*: rudiment of the rhynchodaeum. *rpr*: rudiment of the proboscis. *st*: stomodaeum.

rotate slowly within the egg membrane. Four days later the young worms creep out from the egg membrane. The young worm just escaped from the egg membrane is milky white in color; it is provided with two pairs of ocelli and a short caudal tuft composed of a bundle of long cilia and measures about 0.43 mm in length and 0.27 mm in width. The epidermis and the proboscis are observable beneath the skin. The young worm at the age of 8 days measures about 0.73 mm in length and 0.14 mm in width. The caudal tuft is not recognizable in shape.

The larva of *Oerstedia dorsalis* reported by Coe (1943) was elliptical in shape, provided with a long apical tuft and was swimming during de-





Oerstedia dorsalis var. *albolineata* Iwata. Figs. 133–135. Sagittal sections of the embryo through the rudiment of the rhynchodaeum, showing the formation of the proboscis (133), and of the stomodaeum (134) and the appearance of the dorsal and ventral commissures of the brain (135). ×215. Fig. 136. Transverse section of the embryo through the brain. ×215. *bc*: blastocoel filled with mesoderm cells. *dc*: dorsal commissure of the brain. *dg*: dorsal ganglion. *epr*: epidermis of the proboscis. *mg*: midgut. *ms*: mesoderm cells. *pb*: polar body. *rrhy*: rudiment of the rhynchodaeum. *rmp*: rudiment of the muscle layers of the proboscis and of the proboscis sheath. *st*: stomodaeum. *vs*: ventral commissure of the brain. *vg*: ventral ganglion.

velopment as was also reported in the cases of *Tetrastemma vermiculus* and *Drepanophorus spectabilis* (Lebedinsky, 1897).

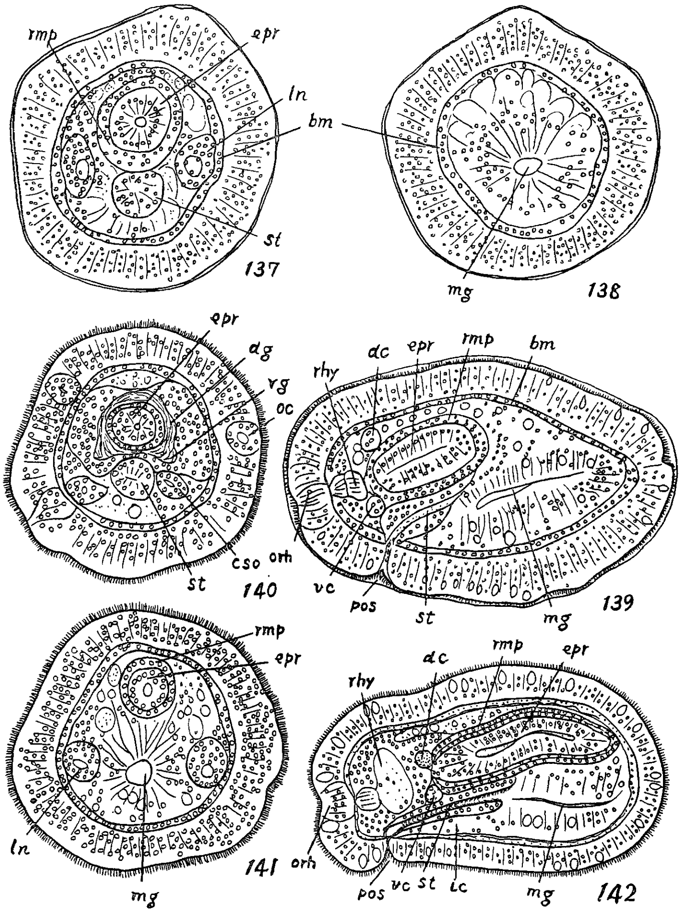
Formation of the ectodermal organs.

Epidermis. In the early stage of development the epidermis of the future adult worm is composed of ectodermal cells which are irregular in shape, arrangement and size (Figs. 126-132). The epidermis is at first not distinctly demarcated from the mesodermal layer of the body; it is composed of cells with clear boundaries between them. After the mesodermal layer is obviously formed, those boundaries become obscure (Fig. 133). The epidermis is thick and contains numerous small nuclei. The gland cells are differentiated in the young worm just escaped from the egg membrane.

Oesophagus. The oesophagus and the stomach of the adult worm are originated from the stomodeal part of the embryo formed by the polar ingression. The blastoporal region of the embryo is not detectable exactly in gastrular stages. The inner lumen of the stomodaeum is well stained with Heidenhain's azan and is reddish in color (Figs. 128-130, *st*). The sagittal section of the stomodaeum in later stages is shown in Figs. 131-135. In the young worm just before creeping out from the egg membrane, a narrow canal of the stomodaeum comes to be formed. The canal is filled with cytoplasm. The stomodaeum becomes engulfed by the body cavity (Fig. 139). In the young worm at the age of one day the stomodaeum is provided with fine cilia and its anterior portion comes near the ventral side of the rhynchodaeum (Fig. 142). At the age of 3 days the stomodaeum opens anteriorly into the rhynchodaeum and posteriorly into the midgut.

Brain and lateral nerves. In the early stages of development the differentiation of the brain and the lateral nerves is not detectable histologically. The nervous system of the adult worm is formed inside the mesodermal layer of the body, which afterward differentiates to the muscle layers of the body wall. The brain is composed of two pairs of ganglia, one dorsal and the other ventral, and two commissures, dorsal and ventral (Figs. 135 and 136, *dc, vc, dg, vg*). The ventral commissure is situated on the stomodaeum. The lateral nerve derived from the ventral ganglion runs posteriorly along the lateral side of the body (Fig. 137 and 141, *ln*).

Proboscis. The rudiment of the epidermis of the proboscis comes to appear in gastrular stages (Figs. 128 and 130, *rpr.*). It protrudes internally and is saccular in shape (Fig. 132, *epr*). The mesoderm cells surrounding the epidermis of the proboscis are arranged in a row (Fig. 134, *rmp*). The proboscis enters the body cavity and is secondarily connected with the rhynchodaeum by a pore as is shown in Figs. 133-135, 139 and 142. The base of the central stylet and two lateral pouches of the accessory



Oerstedia dorsalis var. *albolineata* Iwata. Figs. 137 and 138. Transverse sections of the embryo through the lateral nerves (137) and the midgut (138). ×215. Fig. 139. Sagittal section of the embryo just before creeping out from the egg membrane. ×215. Figs. 140 and 141. Transverse sections of the young worm immediately after liberation from the egg membrane, showing the brain (140) and the lateral nerves (141). ×215. Fig. 142. Sagittal section of the young worm at the age of one day. ×215. *sco*: cerebral sense organ. *bm*: blastocoel filled with mesoderm cells. *dc*: dorsal commissure of the brain. *dg*: dorsal ganglion. *epr*: epidermis of the proboscis. *ic*: intestinal caecum. *ln*: lateral nerve. *mg*: midgut. *oc*: ocellus. *orh*: opening of the rhynchodaeum. *pos*: provisional opening of the stomodaeum. *rhy*: rhynchodaeum. *rmp*: rudiment of the muscle layers of the proboscis and of the proboscis sheath. *st*: stomodaeum. *vc*: ventral commissure of the brain. *vg*: ventral ganglion.

stylets are found in the young worm at the age of 3 days.

Rhynchodaeum. The rhynchodaeum originates from a small number of small cells situated under the polar bodies (Fig. 126-128, *rhy*). The sagittal section of the rhynchodaeum in later stages is shown in Figs. 129-135 (*rhy*). In the young worm just before creeping out from the egg membrane, the rhynchodaeum elongates posteriorly in the body and is divided into three parts, of which the posterior part is blue in color with Heidenhain's azan (Fig. 139, *rhy*). The posterior part becomes wide largely in the young worm at the age of one day (Fig. 142). The proboscis and the oesophagus are connected with the rhynchodaeum at the age of 3 days.

Cerebral sense organs and ocelli. The young worm just escaped from the egg membrane is provided with the cerebral sense organs in front of the brain and the ocelli which are epidermal in situation (Fig. 140, *cso, oc*).

Formation of the mesodermal organs.

Circular and longitudinal muscle layers of the body wall. The mesoderm cells are recognizable in a later stage of the gastrula (Fig. 132, *ms*). The sagittal section of the mesodermal layer of the body in later stages is shown in Figs. 133-135. The mesodermal layer occupies a narrow space filled with the nuclei of the mesoderm cells. The differentiation of the mesoderm cells into the circular and the longitudinal muscle layers has not been observed in the present study.

Muscle layers of the proboscis and proboscis sheath. The mesodermal layer surrounding the epidermis of the proboscis is divided into two layers, of which the outer one becomes the muscle layers of the proboscis whilst the inner one forms those of the proboscis sheath (Fig. 142).

Formation of the endodermal organs.

Intestine. The midgut is differentiated from the endodermal cells invaginated by the polar ingression (Figs. 127-133, *mg*). A narrow canal of the midgut comes to appear in the embryo in Fig. 134. The middgut opens anteriorly into the oesophagus in the young worm at the age of 3 days. The intestinal caecum lies under the oesophagus (Fig. 142, *ic*). The formation of the anus has not been observed in the present study.

Summary of results

Notes on the development of the nemerteans.

Breeding season. Many of the nemerteans found at Akkeshi breed during the summer season especially in late July. Besides the nemerteans used in the present study it was ascertained that the breading season of *Tetrastemma stigmatum* and *Cerebratulus marginatus* are early in June and July respectively, while the gonads of *Amphiporus lactifloreus* and *Paranemertes peregrina* do not ripen in summer.

Cleavage and gastrulation. In general the eggs are round in shape and opaque with rich yolk. In the heteronemerteans which go through the pilidium stage in their life history, discharged eggs are irregular in shape and transparent with poor yolk. In *Lineus torquatus, L. alborostratus,* and *Tubulanus punctatus* the eggs are embedded in loose mucus secreted from the epidermis of the females. In *Procephalothrix filiformis* and *Oerstedia dorsalis* the secreted jelly substance coagulates, forming egg masses, while in *Procephalothrix simulus* loose jelly wraps the eggs. In *Emplectonema gracile* and *Micrura akkeshiensis* eggs are separately deposited, surrounded by a glutinous membrane which seems to be a product from the gonadal wall. The egg membrane is elevated from the egg surface. It must be studied further whether or not the egg membrane is raised due to insemination. The spermatozoon is a nematosperm. The acrosome of *Micrura akkeshiensis* is sickle-shaped, while that of *Lineus torquatus* is pointed.

As to the early development *Procephalothrix simulus, Tubulanus punctatus, Lineus torquatus, Micrura akkeshiensis, Emplectonema gracile* and *Oerstedia dorsalis* are alike in the fact that the egg shows the spiral type of cleavage (Coe, 1899; Wilson, 1900; Wilson, 1903; Nusbaum and Oxner, 1913; Ikeda, 1915; Delsman, 1915; Hammarsten, 1918; Reisinger, 1926), and in the 8-cell stage the micromeres are larger than the macromeres (Wilson, 1903; Delsman, 1915; Hammarsten, 1918; Reisinger, 1926). In all the nemerteans used in preparing the present paper the blastopore is situated at the boundary between the stomodaeum and the midgut (Coe, 1899; Nusbaum and Oxner, 1913; Hammarsten, 1918; Reisinger, 1926; Smith, 1935). In *Procephalothrix simulus* and *Oerstedia dorsalis* it is certain that the polar ingression takes place in the gastrula such as was ascertained by Delsman (1915) for *Emplectonema gracile,* by Hammarsten (1918) for *Malacobdella grossa,* and by Reisinger (1926) for *Stichostemma graecense.*

In *Procephalothrix simulus* the right-left and the antero-posterior axes of the future adult worm are determained in the blastula by the appearance of a pair of large blastomeres which differentiate into the nervous system. The fact is ascertained in *Procephalothrix filiformis* and *Tubulanus punctatus,* because the rudiments of the nervous system come to appear in the gastrula. In the pilidium the antero-posterior axis of the adult worm runs at right angles to the egg axis running through the animal and vegetal poles (Salensky, 1912), while in the case of *Micrura akkeshiensis* the antero-posterior axis of the adult runs opposite to that of the larva or the egg axis. In *Tubulanus, Procephalothrix, Emplectonema* and *Oerstedia,* the body becomes elongated posteriorly and becomes elliptical in shape as a result of the rapid cell-division of one lateral side of the embryo. Accordingly the vegetal pole comes near the animal pole where the apical plate is formed. In contrast to the posterior extension of the midgut, the stomodaeum shifts gradually nearer to the apical plate. In the adult larva the distance between the apical plate and the mouth in *Tubulanus punctatus* is shorter than that in *Procephalothrix simulus* and *P. filiformis.*

As shown in the present paper it is certain that the so-called body cavity of the nemerteans is derived from the blastocoel in which the mesoderm cells appear as scattered elements without forming the somatic and splanchnic layers known in the annelids (Hammarsten, 1918; Reisinger, 1926). Nusbaum and Oxner (1913) and Salensky (1912 and 1914) hold the view that the bands of the mesoderm cells form the somatic and splanchnic layers with the coelom which is derived from the blastocoel between them. To ascertain the formation of the mesoderm cells further study on the early stage of the development is needed. In the Desor larva, however, Nusbaum and Oxner (1913) detected 4d-blastomere as a source of the mesoderm by studying the cell lineage. Salensky (1914) also recognized two mesoblasts in *Prosorochmus viviparus.* On the contrary, Hammarsten (1918) and Reisinger (1926) found that the mesoblasts are composed of four cells in number which are derived from micromeres of the second quartet (2a-2d). Lebedinsky (1897) and Smith (1935) also recognized four mesoblasts as is shown in the development of *Micrura akkeshiensis* and *Procephalothrix filiformis.*

Larva. The nemerteans commonly pass through a swimming larval stage during development. A small number of them creep out from the egg membrane and are viviparous. In the palaeonemerteans the larvae were elliptic in shape and were provided with an apical and a caudal tuft (Smith, 1935). In the heteronemerteans the pilidium larvae are common as larval forms. The Desor larva of *Lineus ruber* creeps out from the egg membrane, while the Iwata larva of *Micrura akkeshiensis* is elliptic in shape and is provided with only an apical tuft. The pilidium larva takes food during development, while the Desor and Iwata larva do not take food until the young worms complete metamorphosis. In the Enopla the larvae of many species are elliptic in shape and are provided with a long anterior and a short posterior tuft (Coe, 1943; Lebedinsky, 1897; Hammarsten, 1918). A small number of them develop directly inside the egg membrane (Reisinger, 1926; Reinhard, 1941) and are viviparous (Salensky, 1914; Coe, 1904).

Epidermis. In *Tubulanus, Procephalothrix, Emplectonema* and *Oer-*

stedia, the adult epidermis originates from the larval epidermis. The secondary origin of the adult epidermis of *Micrura akkeshiensis,* which belongs to the Heteronemertea, agrees with that of the pilidium described by Salensky (1912) and of *Lineus ruber* (Nusbaum and Oxner, 1913). It must be noted that the larval epidermis of *Tubulanus punctatus* swells up in the outer part to form a cavity. It may be found that the swelled layer of the epidermis is a provisional organ of the larva which will be broken down when the young worms begin to creep on sea bottom. According to Delsman's description of the development of *Emplectonema gracile,* it has been learned that the young worms break down provisional epidermis of the larva. In the present study the adult epidermis of *Emplectonema gracile* becomes complete in form in an early stage of development.

Oesophagus. In *Tubulanus, Procephalothrix* and *Micrura,* the oesophagus of the adult worms is originated from the ectodermal stomodaeum (Coe, 1899; Nusbaum and Oxner, 1913; Hammarsten, 1918; Reisinger, 1926; Smith, 1935). The ectodermal origin of the oesophagus of *Emplectonema* and *Oerstedia* was ascertained in histological sectioning of the embryo. In *Tubulanus* and *Procephalothrix* the mouth of the adult worm is epidermal in origin, while in *Micrura* it is formed in the anterior portion of the stomodaeum as in the pilidium described by Salensky (1912). In *Emplectonema* and *Oerstedia* the stomodaeum is generally formed by invagination from the level of the epidermis and finally separates from the latter followed by formation of a communication with the ectodermal rhynchodaeum (Hammarsten, 1918; Reisinger, 1926).

Brain and lateral nerves. As to the formation of the nervous system Salensky (1912 and 1914), Nusbaum and Oxner (1913), Hammarsten (1918), Reisinger (1926), and Smith (1935) agree in that the dorsal and ventral ganglia arise from a single pair of the rudiments of ectodermal origin and the lateral nerves arise as posterior outgrowths of the ventral ganglia. In *Tubulanus* and *Procephalothrix* the blastomeres, which are expected to be differentiated into the brain and lateral nerves of the adult worm, come to appear in the blastular stage of development, while in *Micrura* the rudiments of them are derived from the head blastodiscs of the larva. In *Emplectonema* and *Oerstedia,* the origin and formation of the brain and lateral nerves were not ascertained histologically, though it is certain that the rudiments of them come to appear in the early stage of development. In *Procephalothrix* the brain and lateral nerves of the adult worm are formed in the blastocoel in which the mesoderm cells are scattered. In *Tubulanus* and *Micrura* they are formed in the epidermis of the larva. In *Emplectonema* and *Oerstedia* they are formed in the blastocoel inside the mesoderm cells which become muscle layers of the body wall.

Proboscis. In the larvae of *Tubulanus* and *Procephalothrix,* the proboscis is not formed completely. For its completion the larva must take food, such as flagellates during the swimming larval stage of development. In *Micrura* the proboscis of the young metamorphosed worm is thought to be used for capturing pray. In *Emplectonema* and *Oerstedia* the proboscis is used for that purpose when the larva or the young worm takes food. In *Emplectonema* the proboscidial apparatus is completely formed in the larval stage of development, while in *Oerstedia* the proboscis is formed completely in the young worm at the age of 3 days in the present study. The epidermis of the proboscis is ectodermal in origin, while the muscle layers of that organ and its sheath are of mesodermal origin (Lebedinsky, 1897; Salensky, 1912 and 1914; Nusbaum and Oxner, 1913; Hammarsten, 1918; Reisinger, 1926).

The meso-endodermal organs. The muscle layers present in the body wall, proboscis and proboscis sheath are all of mesodermal origin, while the intestine is formed from the endoderm (Nusbaum and Oxner, 1913; Hammarsten, 1918; Salensky, 1912 and 1914; Reisinger, 1926).

Discussion on the phylogeny of the Nemertea

The nemerteans have hitherto been divided into the following four orders on the morphological basis: Palaeonemertea, Heteronemertea, Hoplonemertea and Bdellonemertea. Main morphological differences lie in the relative position of the nervous system in muscle layers and in whether or not the proboscis is armed with stylets. The Palaeonemertea can be divided into two groups: one with the nervous system external to muscle layers as in the following families, Tubulanidae, Hubrechitidae, and Carinomidae, and the other with the nervous system in the inner longitudinal muscle layer of the body wall as in the Cephalothricidae. The Hoplonemertea and Bdellonemertea agree with each other in having the nervous system inside muscle layers. In the case of the family Carinomida it must be noted that the lateral nerves lie anteriorly outside muscle layers, while they are posteriorly situated in the inner longitudinal muscle layer.

On the other hand, the nemerteans can be divided into two groups from the embryological view-point; namely those showing direct and those indicating indirect development. The latter group is represented only by the Heteronemertea.

In the present report the following species, *Procephalothrix simulus, P. filiformis, Emplectonema gracile* and *Oerstedia dorsalis* belong to the first group whilst the following species, *Lineus torquatus, L. alborostratus* and *Micrura akkeshiensis* to the second group. So far as the writer's observations go, the direct development is divided into two types, the first one was observed in the Palaeonemertea which produces no proboscis in the larval stage of development, while the second covering the Hoplonemertea and Bdellonemertea does produce the proboscis in this stage, which is connected with the rhynchodaeum. The indirect development can be divided further into the following three types: those creatures with pilidium larva, those with Desor's larva and that type seen in *Micrura akkeshiensis*.

Judging from the above data, both morphological and embryological, the Heteronemertea is well established among the nemerteans, while the Hoplonemertea and Bdellonemertea are identical with each other in the characters above mentioned. The Palaeonemertea can be divided into two groups only from the morphological difference. But these differences can be enumerated as follows: the Cephalothricidae are characterized by having the head sharply pointed, the mouth situated far behind the brain, the nervous system lying in the inner longitudinal muscle layer of the body wall, the body musculature composed of two layers (outer circular and inner longitudinal), and in wanting the cerebral sense organs and dorsal blood vessel; the other three families have the body musculature composed of two layers (outer circular and inner longitudinal) or of three layers (outer circular, inner longitudinal and inner circular) and the nervous system external to the body musculature.

Out of these differences, the relative position of the nervous system seems to be the most important justification for dividing the order into two; palaeonemerteans including most of species belonging to the order in the former sense and those represented by Cephalothricidae of which the nervous system lies in the inner longitudinal muscle layer.

Though the Hoplonemertea and the Bdellonemertea are closely related in main structure and main process of development, there can be found still some distinguishable differences, for example the armature of the proboscis is absent in the latter. But these morphological differences seem to be secondarily derived as the result of adaptation to the parasitic life. Bürger (1895) pointed out that the Bdellonemertea is nothing but a family in the Hoplonemertea. Accordingly the present writer proposes a new system of the Nemertea in the following lines.

A) Class Anopla

The mouth is posterior to the brain. The proboscis is not armed with stylets.

1. Order Archinemertea *new order*[*, **]

The head is sharply pointed. The mouth is situated far behind the brain. The body musculature consists of 2 layers, outer circular and inner longitudinal. The cerebral sense organs are absent. The dorsal vessel is wanting. The nervous system lies in the inner longitudinal muscle layer. The excretory system consists of many isolated nephridia.

2. Order Palaeonemertea

The body musculature consists of 2 layers, outer circular and inner longitudinal, or 3 layers, outer circular, longitudinal, and inner circular. The nervous system is external to the body musculature.

3. Order Heteronemertea

The body musculature consists of 2 layers, outer circular and inner longitudinal, and 1 additional layer (outer longitudinal) provided with a well developed cutis. The outer circular muscle layer is rarely present. The nervous system is lying on the circular muscle layer.

B) Class Enopla

The mouth is located anterior to the brain. The proboscis (except in the Bdellonemertea) is armed with one or more sharply pointed stylets.

1. Order Hoplonemertea

The body musculature consists of 2 layers, outer circular and inner longitudinal. The nervous system is internal to the body musculature.

a) Suborder Monostyliferoidea

The armature of the proboscis consists of a single central stylet lying on an elliptical base. The pouches of the accessory stylets usually 2 or more in number.

b) Suborder Polystyliferoidea

The armature of the proboscis consists of many small stylets lying on a sickle-shaped base. The pouches of the accessory stylets are more than 2 in number.

c) Suborder Bdellonemertoidea

The armature of the proboscis is absent. The adult worms are ectoparasitic in pelecypods.

In the development of *Micrura akkeshiensis* the circular and inner

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[*] The name of the new order is designated at the suggestion of Prof. T. Uchida.

[**] Bürger (1895) described the order Mesonemertini for the Cephalothricidean group in which the genus *Carinoma* is included. In this case the order was thought to be derived from the Protonemertini in characteristics.

longitudinal muscle layers of the body wall are thought to be products of mesoderm cells, while the brain and lateral nerves are differentiated in the ectodermal layer of the blastodisc which later differentiates into the epidermis and outer longitudinal muscle layer of the adult worm. These facts show that the nemertean body structure is basically composed of

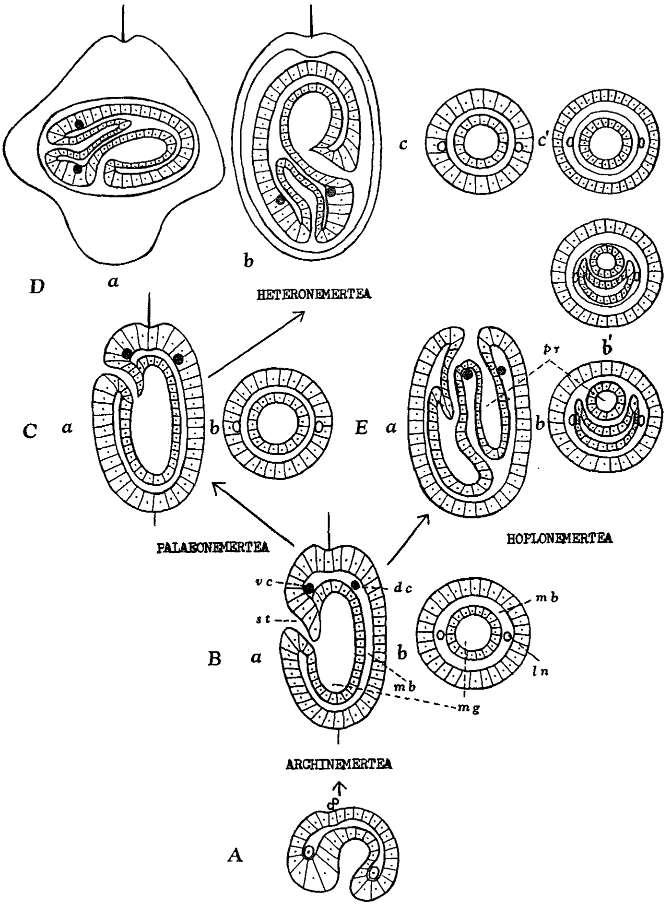


Fig. 143. Diagram showing the interrelationship of the nemerteans from their development. A, Gastrula. B, Sagittal section (a) and the transverse section (b) of the larva of the Archinemertea. C, Sagittal section (a) and the transverse section (b) of the larva of the Palaeonemertea. D, Sagittal section of the Pilidium (a) and that of Iwata's larva (b) and the transverse section of both larvae (c) and the adult worm (c′) of the Heteronemertea. E, Sagittal section (a) and the transverse section (b) of the larva and the transverse section of the adult worm (b′) of the Hoplonemertea. *dc*: dorsal commissure of the brain. *ln*: lateral nerve. *mb*: muscle layers of the body wall. *mg*: midgut. *pr*: proboscis. *st*: stomodaeum. *vc*: ventral commissure of the brain.

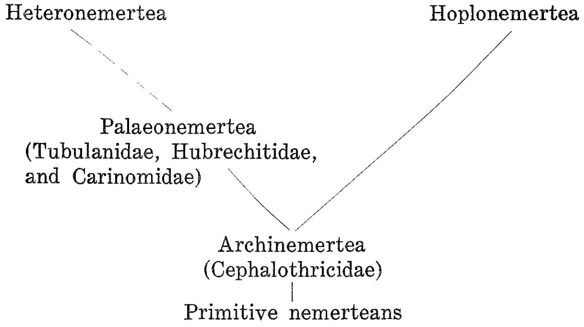
the outer circular and inner longitudinal muscle layers of the body wall as in the cases of Palaeo-Hoplo-Bdellonemertea. The Heteronemertea are closely related to the Palaeonemertea, as the nervous system in these groups is formed outside the mesodermal layers of the body wall. In comparing the development of *Micrura akkeshiensis* with that of *Tubulanus punctatus*, the Heteronemertea is more complicated in development and in organization of the adult worm than the Palaeonemertea, as the proboscis and cerebral sense organs of the former are completely formed during development and the outer longitudinal muscle layer of the body wall is differentiated in addition to the outer circular and inner longitudinal muscle layers.

As described before in this article the Palaeonemertea can be divided into two groups; one is represented by the Cephalothricidae and the other containing the families, Tubulanidae, Hubrechitidae and Carinomidae. There are some common characters of the larval forms, such as formation of the rudiment of the nervous system, and absence of the proboscis during larval stages of development. Therefore, these two groups seem to be nearly related. The main difference of these groups lies in the position of the nervous system in the blastocoel in the Cephalothricidae and outside the muscle layers in the other three families. As to the position of the nervous system, the Cephalothricidean group seems to be more primitive than the other families of the Palaeonemertea. Another primitive character in the Cephalothricidean group lies in the position of mouth of their larvae more posterior than in the other three families.

Procephalothrix belonging to the Archinemertea and *Emplectonema* and *Oerstedia* of the Hoplonemertea bear resemblances in development: the body axis of the worm, larval form, and the nervous system formed in the blastocoel. The latter differs from the former in having the nervous system inside the muscle layers but the differentiation is complicated in that the proboscis and the oesophagus are secondarily connected to the ectodermal rhynchodaeum.

The following characters are common in all nemerteans, so they can not be employed phylogenetically in a discussion of the interrelationships of nemertean groups: the ectoderm differentiates into the epidermis, the ocelli, the nervous system, the cerebral sense organs, and the oesophagus; the mesoderm differentiates into the muscle layers of the body wall, proboscis, and proboscis sheath; the endoderm differentiates into the intestine only.

The genealogical tree of the Nemertea is given below.



Summary

1) The development has been reported of 8 species of nemerteans: *Procephalothrix simulus, P. filiformis* and *Tubulanus punctatus* belonging to the Palaeonemertea, *Lineus torquatus, L. alborostratus* and *Micrura akkeshiensis* belonging to the Heteronemertea, and *Emplectonema gracile* and *Oerstedia dorsalis* var. *albolineata* belonging to the Hoplonemertea.

2) *Procephalothrix* and *Tubulanus* exhibit the direct type of development. The eggs show the spiral type of cleavage. The blastopore is situated between the midgut and the stomodaeum. The larva is elliptic in shape and is provided with ocelli and lateral tufts (*Procephalothrix*) also with the apical and caudal tuft (*Procephalothrix* and *Tubulanus*). The body becomes elongated by posterior extension of a lateral side of the embryo, which becomes to the dorsal side of the future adult worm. The oesophagus and the nervous system are ectodermal in origin. The muscle layers of the body wall originate from the mesoderm cells. The intestine is of endodermal origin.

3) *Lineus* and *Micrura* exhibit the indirect type of development. The eggs show the spiral type of cleavage. In *Lineus* the eggs differentiate into pilidium larvae, while in *Micrura* the egg differentiates into the Iwata larva modified from the Desor larva.

4) *Emplectonema* and *Oerstedia* exhibit the direct type of development. The eggs show the spiral type of cleavacge. The blastopore is situated between the midgut and the stomodaeum. The larva of *Emplectonema* is elliptic in shape and is provided with the apical and caudal tuft. In *Oerstedia* the young worm creeps out from the egg membrane. The body becomes elongated by posterior extension of a lateral side of the embryo, which becomes to the dorsal side of the future adult worm. The ectoderm differentiates into the oesophagus, nervous system, epidermis of the proboscis, cerebral sense organs, and rhynchodaeum. The muscle layers of the body wall, proboscis, and proboscis sheath originate from the mesoderm cells. The intestine is of endodermal origin. The proboscis and oeso-

phagus are connected secondarily with the rhynchodaeum.

5) From the morphological and embryological view-point the writer here proposes a new system of the nemerteans and sets up a new order Archinemertea for the family Cephalothricidae. The Hoplonemertea includes the Bdellonemertea as a suborder. From the phylogenetical view-point the Archinemertea comes to the base of the genealogical tree just differentiated from the primitive ancestor. From the Archinemertea arise two branches, one differentiated to the Hoplonemertea and the other developed first to the Palaeonemertea and then to the Heteronemertea.

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