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A STUDY ON THE DEVELOPMENT OF THE TUSSER WORM, *ANTHERAEA PERNYI* GUÉR.

BY

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(With five Plates)

INTRODUCTION

So far as the writer is aware, knowledge of the embryology of insects in general is very poor though the study of the other animal groups in this field has had tolerable advancement recently. Apart from CHOLODKOWSKY'S work (1889) on the development of Coleoptera and WHEELER'S (1889) on Orthoptera, most works on the embryology of insects so far published have been confined to Lepidoptera. More particularly, with a few exceptions such as BOBRETZKY (1878), EASTHAM (1828, 1930) and JOHANNSEN (1929) who worked on the common butterflies (*Pieris*, *Diacrisia* and *Porthesia*), almost all other workers such as DOHRN (1876), TICHOMIROFF (1879), GRABER (1891), TOYAMA (1896, 1902), etc. rather specialized on the development of the silkworm, *Bombyx mori*, one of the most convenient species of domesticated silkspinnners for our use.

Fortunately there is still another silk spinner, the tusser worm, which is confined to a small place in middle Japan known as Ariakemura, a village in Nagano Prefecture where because of the high value of the silk produced by the worm it is cultured in half wild state. Years ago however an attempt was made to transplant the insect to Gumma Prefecture, in the neighbourhood of its original habitat, but it was unsuccessful. Ariakemura is accordingly the only locality in Japan, where the insect prospers. At the same time it seems true that the worm rather prefers a dry place as it is also adapted to China, Manchuria and Korea.

In its original habitat the spinner is of a bivoltine species but in Sapporo where the writer cultured it having brought it as cocoon, it becomes univoltine, simply because the cold season sets in before the eggs have hatched out. In addition the process of development is retarded greatly in this city as compared with that in the original locality. In

Sapporo from the time of deposition of eggs until the hatching it takes more than one month since the eggs deposited on the 10th of May 1927, for example, hatched out on the 16th of June. In 1929 on the other hand even one and a half months was required. In Ariakemura, on the contrary, only from six to seven days elapse from the deposition of eggs to the emersion of the larvae. This difference which is by no means small, is doubtless due mainly to the atmospheric temperature which at that season of the year in Sapporo registers 12.6°C. on the average, while in Ariakemura it shows 21.0° C.

Now among insects it is unique that the eggs of the tusser worm are extraordinarily large in size, measuring 3-4 × 3 mm. in diameter. This affords a great advantage for the study of embryology at any rate. Another convenience is that almost a complete series of stages of development can be obtained by raising the animal in Sapporo. Furthermore, contrary to the common silk worm, the tusser worm should supply a desirable material for the general study of insect development free from any possible modification which might be effected under domestication. This is the reason why the writer has undertaken the present study with this worm in the hope that much light might be thrown upon insect embryology.

The problem was first suggested by Dr. S. HATTA, Professor Emeritus of Hokkaido Imperial University, under whose guidance and direction the present study has been completed. The writer appreciates the courteousness of Dr. M. YAMANOUCHI who encouraged him to undertake the present work and retired from the field through he had started a similar study before him. The author wishes also to express his thanks to Dr. T. INUKAI for his constant help and encouragement during the work.

I. Materials and Methods

The material employed in the present work consisted entirely of the eggs of the tusser worm, *Antheraea pernyi* GUÉR. In May 1927 some cocoons were obtained in Ariakemura and shipped by rail to Sapporo. Yet they were kept alive as normal. Then they were placed carefully in a row in a wooden box. Soon after their arrival at Sapporo the moths emerged from the cocoons at which time copulation took place. It was then observed that out of 200 cocoons, only 70 were female, i. e. about 1/3 of the whole. In 1929 again, 100 cocoons were obtained also from the same locality. In this case the ratio of male moths to the female was approximately equal being 43 to 45.

Generally a female deposits from 200 to 250 eggs, the average being

239. The eggs used as the material in the present work were counted, therefore, to about 25000 in number. As development proceeded quickly in the early stages, the killing of the eggs at first was done immediately after deposition four times per day, namely at 6 a. m., 12 noon, 6 p. m. and at mid-night, but later they were killed only twice a day, i. e. at 8 a. m. and 8 p. m.

The reagents employed as fixatives consisted of 0.5 % chromic acid, nitro-acetic acid diluted with 10 volumes of water, saturated solution of corrosive sublimate, or ALLEN's modified solution of BOUIN's fluid. They were used at a temperature of 70-80° C. and reacted until cooled which lasted usually for 10 hours. After being taken out of reagent, the eggs were thoroughly washed with running water and placed in 50 % alcohol. After two days they were put into 79% alcohol in which they were preserved until used. Of the four reagents above mentioned, ALLEN's modified solution proved most favorable.

In the hardened eggs the egg-shell or chorion was not closely attached to the egg content as in the living state, but was separated from it by a space, so that the shell was removed from the egg by means of a needle without trouble. For the sake of convenience in treating the material a part of the blastoderm, the so-called ventral plate, was cut off from the remaining part of the egg, as the latter consists mostly of the yolk.

The ventral plate thus prepared was stained *in toto* with staining fluids which consisted of either alcoholic picro- or borax-carmin solution. When the latter was employed, the objects were placed in acidulated alcohol to remove the superfluous staining. The objects were then transferred into alcohols of higher grades and if not sectioned, they were infiltrated by use of xylol and embedded in Canada balsam as the finished prepare.

The material which were cut into serial sections, were also first stained *in toto*, borax-carmin being preferable especially in this case. After dehydration by absolute alcohol the material was put in xylol and then in xylol-paraffin. The procedure was carried out in thermostat until the material was imbedded in paraffin as usual. Complete serial sections, 10 microns in thickness, were made with a GROOT's microtome and mounted in order with egg albumin. The sections were clarified with xylol and mounted in Canada balsam.

All the figures illustrating the foregoing process have been drawn by means of the Camera Lucida of ABBE's system.

The observations have been made first of all on the superficial features of the embryo. The internal changes which occur during the development

were next examined by means of the serial section.

II. Structure of the Egg

The just deposited egg is brown in colour and depressed ellipsoid in shape, being 3-4 mm. across the long axis, and 3 mm. in transverse diameter, with a thickness of 2 mm. One pole of the ellipsoid is somewhat pointed, whilst the other is rounded or rather obtuse. The head of the future embryo is directed toward the acute pole, accordingly the other pole is occupied by the posterior end of the body. The whole egg is coated with a thick elastic chorion, the outside of which it is covered again with a waxy substance of brown colour¹⁾. Hence if the latter is taken off and the egg is immersed in alcohol, the chorion becomes semitransparent.

The egg has two investing membranes, the inner of which is very thin, transparent and structureless i. e. the vitelline membrane which adheres directly to the yolk and the outer is the chorion which is thick, semitransparent and tough. Though according to the study of the fly's egg by HENKING (1892) and emmet's egg by BLOCHMANN (1884)²⁾ the chorion is composed of two layers, "Endchorion and Exochorion", such a structure was not recognizable in the tusser worm egg. It is single layered and by reflected light whitish in colour.

The inner surface of the chorion is smooth, but the outer is possessed of elevations or protuberances which appear sometimes round and sometimes oval in shape under low magnifications, these being, strictly speaking, hexagonal in outline as closer examination proves. The outgrowths in question are scattered all over the outer surface of the chorion, existing closer together towards the acute pole of the egg, in the centre of which there is a micropyle. According to TOYAMA and ISHIWATA (1896) in *Bombyx* there are visible numerous minute pores for the respiration of the egg, to give off carbon dioxide and to take in the atmospheric air. However, the writer has failed to find the occurrence of such pores in this case.

Directly inside the vitelline membrane, there is found an extremely thin layer of protoplasm, which consists of the peripheral layer of the formative cytoplasm. It is called the centrolecithal ovum. It is evident that the protoplasm furthermore fills up the space between the food yolk granules which occupy the central part of the egg forming a scanty reticulum, although it does not always appear in the sections. The yolk

1) This is not the proper of the chorion, but is due to that of the yolk shining through the chorion which is dark yellow when dried.

2) The original paper by BLOCHMANN (1884) was not accessible to the writer who learned of it only through HIRSCHLER's (2924) accounts.

granules are spherical in shape and are much smaller at the periphery than in the centre of the egg. Besides the granules of the yolk there are transparent fat globules of various sizes and a translucent albuminous substance which appears as polygonal masses by fixation.

The yolk in the fresh state crushed between the slide and the cover glass contains also plenty of oil globules of different size, strongly refracting the light and a greater number of spherical albuminous masses.

In the hardened eggs the yolk makes a network with meshes of various size. The structure is often seen in sections of eggs hardened in saturated sublimate or picrosulphuric acid. The yolk granules however undergo gradual changes into a liquified structure in the course of embryonal development.

III. Segmentation and Formation of Blastoderm

Since the segmentation of the egg in this worm, as in the other insects, is of a superficial type, i. e. the process goes on within the yolk, it is naturally not possible to observe the early development from the outside. The eggs remain unchanged as seen externally until the ventral plate is first differentiated from the blastoderm.

The first segmentation nucleus of the egg from 12 to 24 hours after oviposition lies in the periphery near the micropylar pole with an acute end. The nucleus is embedded in the mass of the peripheral cytoplasm. Changes occurring in the nucleus are seen rarely owing to the difficulty of satisfactory fixation due to the yolk mass which does not allow the application of ordinary methods for preservation of the cytological details¹⁾.

The cleavage nucleus (Fig. 1b) is round in shape with a large nucleolus (no) surrounded by a thick layer of cytoplasm (cy), which in the egg hardened in picrosulphuric acid often appears as astral radiations. The outer layer of the cytoplasm generally disappears by degrees into the surrounding yolk substance.

The nucleus continues to divide until about 60 to 80 nuclei are formed. Each of these nuclei thus formed is surrounded by the astral radiated cytoplasm and scattered irregularly in the yolk. However the distribution of the nuclei is first limited to approximately the upper $\frac{1}{3}$ acute part of the egg.

The nuclei which now appear amoeboid in shape commence to migrate to the surface of the egg (Fig. 1a). First, the nuclei (n) take a spherical

1) The chorion is tough and apparently impervious to fixing fluids in short time, and so, it was necessary to use the heat-method.

arrangement at the acute pole of the egg, each having a long cytoplasmic tail which is directed toward the centre of the sphere. This sphere of nuclei expands gradually until it reaches the egg surface, first at the micropylar pole, then, by enlarging the inscribed circle, to the posterior part. In consequence of the asymmetrical swelling of the sphere it happens that the micropylar pole is first occupied by the nuclear layer at the right side, then on the left side and finally at the opposite pole.

On reaching the periphery, the nuclei assume a conical form and then become gradually flattened. The multiplication of the nuclei after reaching the surface occurs very rapidly. In addition to this it is remarkable that the nuclei at the surface at the same time are continuously reinforced by others migrating from the yolk. The latter is nothing else than the descent of the cleavage nuclei which remained in the yolk yet continue to divide while the other ones are moving to the periphery. With the increase of the nuclei at the periphery there appears a distinct boundary between the cells. In this manner, the epithelial one cell layered tissue which is distinctly demarcated from the inner yolk mass is formed (Fig. 2).

The egg next enters the blastoderm stage, the blastoderm being at the periphery while the yolk and its cellular elements remain in the central part.

Fig. 2 is a cross section through the centre of an egg in the blastoderm stage. Examining carefully one sees that the blastoderm (bl) exhibits an epithelial structure only at one side of the egg, while at the opposite side it shows a rather loose structure in which the round cells arrange as in a chain.

It is marked that since the first division of the cleavage nucleus the nuclei have undergone a steady diminution in size. The cleavage nuclei which no longer take part in the formation of blastoderm remain in the yolk and become the yolk cell or vitellophag (Fig. 1c) which retains the original amoeboid structure until a later stage. However it is clear that there is originally no difference between the blastomere and the yolk cell. In fact, nothing indicates an early differentiation of cells destined either to remain in the yolk or to form the blastoderm. Later the yolk cell is large in size as compared with the blastomere (Fig. 1b), with a large spherical nucleus (n) which is provided with fine chromatic granules but without a nucleolus, surrounded by a thin amoeboid cytoplasmic coat (cy). The vitellophags increase in number simply serving as a kind of nutritive cells connected with the liquifaction of the yolk. They are sometimes binucleated being destined to degenerate sooner or later. In the meantime one

observes among vitellophags with amoeboid masses of cytoplasm, several nuclei which are somewhat smaller than normal and show no marked difference between the poly-nucleated, ordinary vitellophags which are of degenerating nature.

The yolk granules in the egg are distributed at first nearly homogeneously, but at the early blastodermic stage the yolk mass is divided into a number of distinct spheres from the peripheral part. Then, at the end of the blastoderm stage this yolk division extends to the entire yolk mass (Fig. 4), each sphere enclosing one, less commonly, two or more yolk cells, thus each yolk spherule (Fig. 1c) becomes a unit of one cell. The process is designated as the secondary segmentation of the yolk.

It is desirable to give here the approximate time required for the development so far described. Probably the first and second polar spindles are formed in the ovaries or during oviposition in this worm. The fusion of the female pronucleus with the male takes place during the first day after oviposition, and this is followed soon by the appearance of the cleavage nucleus in the acute end of the egg. Next the rapid division of the cleavage nucleus is carried on and the arrangement of the nuclei thus formed at the egg surface occurs by the end of the second day. During the third day of development the blastoderm is differentiated.

IV. Differentiation of the Ventral Plate

As soon as the blastoderm is completed by the rapid multiplication of the blastomere, *i. e.* the peripheral cells, and at the same time by the insertion on the inner segmentation nucleus, there occurs an unequal development in it. The blastoderm becomes exceedingly thin on the left surface where the cells exist much scattered, and contrary to this the blastoderm on the right side is thickened to become two or three layered epithelium (Fig. 4). Moreover a slight contraction both from the cephalic and caudal ends towards the centre of the thickened right side occurs and thus the germ band or "ventral plate" is formed. The aggregated mass of cells or ventral plate (vp) is distinctly marked from the remaining part of the blastoderm which now consists of rather larger and scattered cells. It occupies the area of two thirds of the centro-lateral surface in the egg as seen in the figures (Figs. 3 a, b and 4).

The exact mode by which the germ band is formed is only seen by a median cross section of an egg (Fig. 4). BOBRETZKY (1878) noted the same condition in Lepidoptera (*Pieris crataegi* and *Parthesia chrysorrhoea*)

and WOODWORTH (1889)¹⁾ likewise noted the fact in *Evanessa*. JOHANNSEN (1929) also recognized a similar process and so figured it, although he states that the completion of the germ band is due only to the partial increases of the cells, that is, the cells at the micropylar end and at the lower pole of the egg remain undivided, while the cells around the centre of the egg actively divide by mitosis. The thickening of the germ band is carried out by radial lengthening of cells on the ventro-lateral side of the egg, and then it becomes a two-cell layer, while the blastoderm at the dorsal side of the egg remains the same. KOWALEWSKY (1871) also states that in *Apis mellifica* the cells which constitute the germ band are originally smaller and flatter than those which form the extra-germ band.

At the transition point from the germ band into the extra-germ band there occurs the infolding of the blastoderm to make the so-called "amniotic folds" (Fig. 4, amf) around the germ band. The folds appear as a double fold of the blastoderm, and on further development the caudal and cephalic folds grow much more rapidly than the lateral. The amniotic fold gradually envelops the germ band, its cells becoming more flattened and scattered as it grows (Fig. 5). The fold and the blastoderm of the extragerm band continue to become thinner and thinner. As to the further development of the amniotic fold the matter will be treated again later.

V. Changes of the External Structure

Here, for the sake of convenience to make the matter clear to the understanding let us turn our attention to the external change of the embryo, discerning three stages from the time the formation of the ventral plate until hatching.

STAGE I

By this stage the ventral plate has differentiated from the blastoderm, caused by thickening as mentioned above. So far as the writer is aware, WHEELER (1889) was the first to observe the earliest stage of the development of the ventral plate in the insect (*Xiphidium*). According to him before formation of the ventral plate the cells of blastoderm are specially concentrated at four different points, two on the median line and two others across it, which are destined to be converted into two procephalic lobes, a caudal end and an indusium respectively. A similar structure is said

1) The writer could not see the original papers by WOODWORTH (1889) and by KOWALEWSKY (1871), but the former was cited from JOHANNSEN'S accounts and the latter from BOBRETZKY'S paper respectively.

to be seen prior to the formation of the ventral plate in some Crustacea (*Astacus*, *Homarus*). The condition, however, is very different in the present form.

The youngest stage with which the present writer has dealt is at 120 hours of age somewhat trapezoidal shape in outline distinctly marked from the other parts of the blastoderm. The anterior edge of the trapezoid plate, as it may be called, represents the cephalic end of the future embryo, while its basal part becomes the caudal end. In the following development the total plate is constricted segmentally along its median line which corresponds to the median plane of the embryo. Next, another transverse constriction arises anteriorly and the earliest beginning of the two cephalic lobes is formed (Fig. 6). At this stage the ventral plate has become entirely thickened and looks rather more solid than flat.

STAGE II

Next comes the stage (Fig. 7) in which the ventral plate is remarkably decreased in breadth at the anterior edge or cephalic end and is elongated along the longitudinal axis making a posterior protuberation in the middle part of the plate. The plate elevates at the four corners simultaneously; the anterior elevations make the cephalic lobes (cl), behind which there appear anterior lateral grooves. The longitudinal groove between them has also become very distinct and this is nothing else than "the primitive groove" (pg) which becomes more and more striking stage by stage (Figs. 7-11). The ridges bordering the above mentioned groove may for convenience be called "the primitive ridges". The groove and the ridge extend backwards to fade out at a short distance posteriorly.

Later the posterior pair of elevations of the ventral plate gives rise simply to the hindmost segments of the worm known as "the caudal lobes" (Cd.). The embryo at the instant consists, therefore, of the paired cephalic and caudal lobes and the unsegmented intermediate part (us).

In an embryo of advanced stage (Fig. 8), the intermediate part constricts off a segment (s^1) at its anteriormost part. As later changes show (Figs. 9-11), the other segments are added one after another behind this one, and so the segment must be referred to as "the first body segment".

Further segmentation of the ventral plate is brought about simply by shallow constriction which becomes deeper as the stage advances being more distinct also from the side towards the median line, as clearly seen with transmitted light (Fig. 10, s^1 , s^2). This process is exhibited more obviously in the later stages (Figs. 13, 14).

At the next stage (Figs. 9, 10) the so-called primitive groove which shows the main axis of the future embryo, extends posteriorly with distinct lateral primitive ridges on both sides as before (pg). It is a narrow longitudinal groove which suddenly widens posteriorly and at the same time becomes shallow gradually to be brought up at last to the general level of the ventral plate. The hindmost part of the ventral plate in this stage becomes very distinct and makes the caudal lobes (Cd). As the whole ventral plate diminishes in breadth the embryo now assumes the form of a shield (Fig. 11). The approaching of the primitive ridges toward each other to close the groove takes place at first in the part of the second segmental constriction and extends gradually from that point either backwards or forwards (Fig. 12, pg), so that it remains open both in the anterior and posterior parts until a later stage (Figs. 12, 13, ap, bp). The closure is followed by the coalescence of the ridges which have been brought in contact at the free surface. As a consequence of the process an internal canal remains which represents the early state of "the archenteron". The hindmost part of the primitive groove remains wide open for a while being often designated as "the blastopore" (Fig. 13, bp). In a strict sense however the blastopore should be the primitive groove itself as will be explained later.

The so-called amnion fold which arises as the folding of the blastoderm around the ventral plate, is detected as early as Stage I (Fig. 6), in which the ventral plate is just formed.

The groove is visible in the one week (168 hours) old individual as a mere furrow but in a 176 hours old embryo (Fig. 16) it is obliterated without leaving any trace. The anterior extremity of the groove opposite to the blastopore (Figs. 13, 15, ap) is closed at about the same time as the latter (Fig. 16).

At the next stage (Figs. 15, 16) a median longitudinal furrow appearing on the segments thus formed can be seen. This is "the neural groove" (nf). There is apparently no connection between the neural groove and the primitive groove. At this stage one can see externally about 8 body segments but internally when seen by transmitted light there are 12 (Fig. 14).

STAGE III

This stage (Figs. 17, 18) is represented by the 184 hours (7-8 days) old embryo and is sharply marked from the preceding by two features. First, all the body segments have completely developed by this stage

including three cephalic, three thoracic, and ten abdominal (s^{7-16}) ones. Besides this the cephalic and caudal lobes (cl, Cd) are also striking. Second, from the segment the anlage of the cephalic and thoracic appendages are beginning to appear. In addition to this the stomodaeum (st) which is continuous with the archenteron becomes obvious at the anterior extremity of the body between two cephalic lobes. It may be seen that the inner angle of the cephalic lobe produces the anlage of the antenna (at), while its anterior edge is giving rise to the labium (lb). At this stage the thoracic appendages are built powerfully (thl¹⁻³) and are easily distinguished from the cephalic appendages (md, mx¹, mx²) which are still rudimentary. On the abdominal segments (s^{7-16}) the neural furrow (nf) is still conspicuous whereas that on the thorax has already faded. The caudal lobes which were formerly very striking (Figs. 7-16), become the terminal segment of the body, which is brought about by the obliteration of the median furrow separating the lobes.

In an older individual (Figs. 19, 20) of this stage, not only the cephalic and thoracic appendages are again divided transversally into two segments, but also all the abdominal appendages (abl¹⁻¹⁰) become very clear. The neural furrow fades at this stage except for some individuals. The lobular structure of the caudal lobes is entirely lost, they occupying merely the hind extremity of the body.

The cephalic lobes which embrace the stomodaeum (st) from both sides, are rounded in outline at first (Figs. 17, 18) but soon widen transversely (Fig. 20).

At this stage there is about to be revealed a developmental fact which is very characteristic and interesting. First of all one recognizes the embryonic body grows so that it attains its maximum length (Figs. 21, 22). However the body length shrinks and shortens thenceforth in the next advanced stage (Figs. 23-25). Secondly during the advancement all the abdominal segments carry a pair of appendages (abl¹⁻¹⁰), except for the hindmost segment which has been derived from the caudal lobes. The latter is first perforated by the proctodaeum (pr), and then later a pair of appendages (cal) are formed on it. All the segments may now be recognized as homonomous so far as the external morphology is concerned. In the meantime the cephalic and thoracic appendages lengthen further to be segmented while the abdominal ones remain unsegmented. Heteromerity comes in, however, very soon, some segments losing their appendages and some moreover coalescing to other segments, as later history shows.

The stomodaeum has undergone changes in respect to its aperture

which is diminished in width. The antennae (Fig. 24, at) are segmented into two, while the labium (lb) grows in dimension.

Now the striking feature acquired by the embryo in the present stage (348 hours, 14 days) in the reduction of the body length (Figs. 25, a, b, c) being shortened by about 20 % in total length and on the contrary, the increase of thickness as compared to the measurements of the preceding stage (Figs. 21, 22).

In addition to this, on the six abdominal segments — the first, the second and from the seventh to the tenth — the appendages have disappeared (Figs. 25, a, b, c). Therefore as the persistent appendages which are retained throughout life (Figs. 24-27, abl³⁻⁶), all the cephalic and thoracic affixes and four abdominals are enumerated. These on the abdominals develop further to be segmented into two articulations.

As to the cephalic and thoracic appendages, there is little change, all the thoracic ones being segmented now into three, the two posterior cephalic (mx¹, mx²) into two, while the first cephalic (md) remains one-segmented as before. The antennae are also bi-segmented. On the other hand, the first and second cephalic appendages have grown remarkably in length but the last cephalic (mx²) is, contrarily, shortened as the development advances, as seen in the figures. The labium (lb) grows stage by stage so as to reduce the dimension of the entrance of the stomodaeum gradually.

Besides the shortening of the body length, it is to be noted that in advanced stages the tenth abdominal segment is coalesced with the last caudal segment (Fig. 25). It happens then that the ninth segment is again fused with it (Fig. 27). At the anterior part of the embryo the paired rudiment of the labium is now lost to a great extent to be converted ultimately into an unpaired median structure bordering the stomodaeum in front (Figs. 25-27, lb). Of the eight remaining abdominal segments, four — the first, the second, the seventh and the eighth — are free from appendages and smooth on their surface (Fig. 27). By the growth of the embryo in body thickness the appendages on a segment which attach closely together in the early stage separate from one another leaving a wide space between them. The anlage of the stigma (stg) is first seen both on the side of thoracic and abdominal segments at the stage in which the obliteration of the abdominal appendages takes place (Fig. 24). The umbilical canal (amc) is very much reduced in circumference. The external form of the larva is now completed.

VI. Revolution of the Embryo

Here attention must be directed to the position of the embryo in the egg which differs greatly from the other kind of animals. At about 100 hours of development, the ventral plate extends longitudinally so as to cover one lateral side of the yolk surface.

As is well known, at the stage when the origin of appendages appears the dorsal surface of the embryo is directed toward the centre of the egg and accordingly the embryonic body shows a heavy dorsal bend (Fig. 28, a). As the embryonic membranes develop there occurs the invasion of the yolk granules between the amnion and serosa; this causes the embryo to sink deep into the yolk. At the same time a reduction of the body length takes place, and then the embryo is gradually removed toward the centre of the egg so that the dorsal bend is a little decreased in degree.

As the embryo which is situated at present along the longitudinal axis of the egg, grows in length in this position, the anterior half of the body bends ventrally, so that the body takes an S shape (Fig. 28, b). The further growth of the embryo, and the rapid consumption of the yolk lead the embryo, particularly the dorsal part, to remove to the opposite side to the place where the ventral plate was first differentiated out of the blastoderm, commencing at the posterior end and extending progressively anteriorly. Now the body begins to bend ventrally and at last shortly before the hatching, the dorsal wall of the embryo comes directly under the bottom of the micropyle. Thus a revolution in the position of the embryo has been brought about (Fig. 38, c). It would appear, from the above, that the revolution is caused simply by the mechanical locomotion of the embryo resulting from the growth of the body and the decrease of the amount of yolk.

GENERAL CONCLUSION AND COMPARISONS

Taking into consideration the differentiation of various parts of the body revealed in every stage as described above, attention should be given to generalize on the whole development of the external form. The most anterior and the most posterior segments, namely the cephalic and caudal lobes respectively, are the first to differentiate from the ventral plate and this paired origin is in contrast to the remaining body segments which are formed one after another as unpaired metameres. The hindmost body segment is formed first at stage III (184 hours), whereas the foremost is already constricted off at stage I (136 hours). The part lying between these two

extreme segments differentiates into the body segments as just stated, the differentiation going on from the front backwards. When fully established, the body segments number sixteen, of which the anterior six constitute the three cephalic and three thoracic, while the remaining ten posterior segments, represent the abdominal segments. In addition to these there come the cephalic and caudal lobes, so that the whole body is composed of eighteen metameres. These eighteen segments are homonomous, as is clearly seen at the time when the embryo attains its maximum body length. Each segment has a pair of appendages. However further structural changes soon set in. On the first, the second, the seventh, the eighth, the ninth and the tenth abdominal segments the appendages disappear, while those on the other segments transform into the limbs characteristic to the body regions to which they belong. As already incidentally touched upon, the mandibles are differentiated from the first body segment, the second and the third body segments give rise to the first and the second maxillae, and the following three — from the fourth to sixth — constitute the thoracic segments which produce the three pairs of thoracic limbs. The four abdominal segments produce the persistent limbs. A pair of limbs on the terminal segment is produced from the caudal lobes. From the cephalic lobes, the antennae are protruded. Whether the labium which is formed in pairs like the cephalic lobes, represents a segment or not, the writer cannot determine at present.

It would appear, from the above observation, that the body segments develop homonomously under the domination of the palingenesis of the insect. Coenogenetical influence, however, causes heteromerity which is the case in later stages.

Most of papers hitherto published dealing with insect embryology attempted to elucidate the development of the meso- and ento-derm (Untersblatt) and only a few of them described the external change of the embryo.

One can enumerate N. BOBRETZKY (1878), K. TOYAMA (1902), O. A. JOHANNSEN (1929) and L. EASTHAM (1928, 1930) who contributed to our knowledge on the embryology of the Lepidoptera.

N. BOBRETZKY (1878) working principally on the formation of the blastoderm of *Porthesia* observed two bodies in the interior of the egg at the earliest stage, each consisting of a nucleus enclosed in a thin protoplasmic layer with stellate prolongation. By a continuous division of the nuclei, they become scattered through the interior of the ovum, some of them passing to the surface simultaneously. At the surface the protoplasm around

each nucleus contracts itself to become a rounded cell body, distinctly cut off from the adjacent yolk and thus the blastoderm is formed.

S. SCHWARTZE (1899) who made a careful study of the Chinese silk worm, *Lasiocampa fasciatella* MÉN. var. *excellens*, agrees with the present writer in respect to the fate of the vitellophags.

The observation of K. TOYAMA (1902) on *Bombyx mori* begins from the stage of "the ventral plate". He observed the procephalic lobe extending laterally, sixteen outer segments and the neural furrow making their appearance just as in the tusser worm. The cephalic and thoracic appendages, the stomodial and proctodial depression become distinct shortly after the appearance of the neural furrow.

In 1928 L. EASTHAM worked on *Pieris* and ascertained what K. TOYAMA (1902) observed in the development process of *Bombyx*.

Incidentally the same author in 1930 published an organogenetic study of *Pieris rapae* and made many valuable observations on the development of the mesodermic somite, the nervous system, the alimentary canal, the heart, the aorta and the trachial system.

The Coleoptera have been favorite objects of investigation. W. M. WHEELER (1889) has given an excellent account of the oviposition, the development of the egg, the formation of the blastoderm, the germ layer and embryonic envelopes in his study of *Doryphora descemlineata* SAY. V. GRABER (1891) on *Hydrophilus* and H. STRINDBERG (1913) were excellent. However there was no precise observation beyond the old literatures.

Many studies have also been made to elucidate the embryology of the Diptera. During 1863 there appeared A. WEISMANN's work on *Musca*. W. NOACK (1901) has been the most recent writer on the embryology of this animal¹⁾, giving a clear account of the fertilization, the pole-cell, the yolk cell and the formation of the blastoderm, the meso- and ento-derm.²⁾

Many important observation have been contributed to the embryology of Hymenoptera. As early as 1870, O. BÜTSCHLI attempted observations on the *Apis*. His figures 17 and 18 show distinctly that there is a pair of appendages on each body segment, and he gives an account of the formation of the mesenteron³⁾. O. DICKEL (1904) working on the same

1) *Calliphora erythrocephala*, *Lucilia illustris* (M.) and *Lucilia regina* (M.) were employed as his materials.

2) His account of the germ layer differentiation is confirmatory of the view of O. Bütschli (1883) and R. Ritter (1895). The gastrulation furrow remains wide open in front for a time and the anterior border to the furrow is formed by a layer from which proliferate cells which serve as anterior entoderm rudiment.

3) He stated that the mesenteron originated from the nuclei from a protoplasmic matrix and the mesenteron epithelium from the yolk cell.

material (*Apis*), says that the blastomeres arrange in two parallel rows at the early stage of the formation of the blastoderm (His figure 7).

Orthoptera have long been the objects of morphological study, as they are easily obtainable at all seasons of the year and are of very convenient size for dissection. W. PATTEN (1884) published a preliminary note on the development of *Blatta germanica* (LINN.), and reported an undoubted gastrular furrow starting posteriorly and ending in the region where the mouth will subsequently develop. According to him the groove so formed closes from behind forwardly and in this way a longitudinal mesoderm is enclosed between the ectoderm and the yolk. In his study of the embryology of *Blatta germanica*, N. A. CHOLODKOWSKY (1888, 1890) maintains that there is a distinct blastopole groove running the length of the ventral plate. During the year 1889 W. M. WHEELER's work appeared on the embryology of *Blatta germanica* by the suggestion of W. PATTEN, containing the embryology of *Doryphora descemlineata*, carefully written with figures. He said that all the nuclei, formerly found in the yolk, probably rise to the surface to form the blastoderm and reinforce it in the formation of the blastoderm.

In short, in spite of all the works above mentioned, there has not been any work which has given a definitive account of the external change of the insect embryo.

Concerning the formation of the blastoderm most authors who worked on the other groups of insects agree with the present writer.

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Plate VII

- Fig. 1a. Longitudinal section of an egg at the segmentation stage.
The nuclei (n) have reached the surface at one pole. vim,
vitelline membrane; pp, periplasm; y, yolk. × 25.
- Fig. 1b. One segmentation nucleus. cy, cytoplasm; n, nucleus; no,
nucleolus. × 460.
- Fig. 1c. One yolk spherule with a vitellophag in the centre.
× 460.
- Fig. 2. Cross section of an egg at the blastoderm stage, in which
the epithelial tissue has formed completely at one side,
while at the other side it is still loose. bl, blastoderm;
yc, yolk-cell. × 20.
- Figs. 3, a, b. Lateral and dorsal (Fig. 3b) views of a ventral plate
which is differentiated from the other part of the blasto-
derm. exgb, extra-garband; vp, ventral plate. × 18.
- Fig. 4. Cross section of an egg shown in the above figure, show-
ing the 'growth' of the amnion. amf, amnion-fold; vp,
ventral plate. × 20.
- Fig. 5. Cross section of an egg showing the completion of the
envelopes. am, amnion; se, serous membrane. × 20.

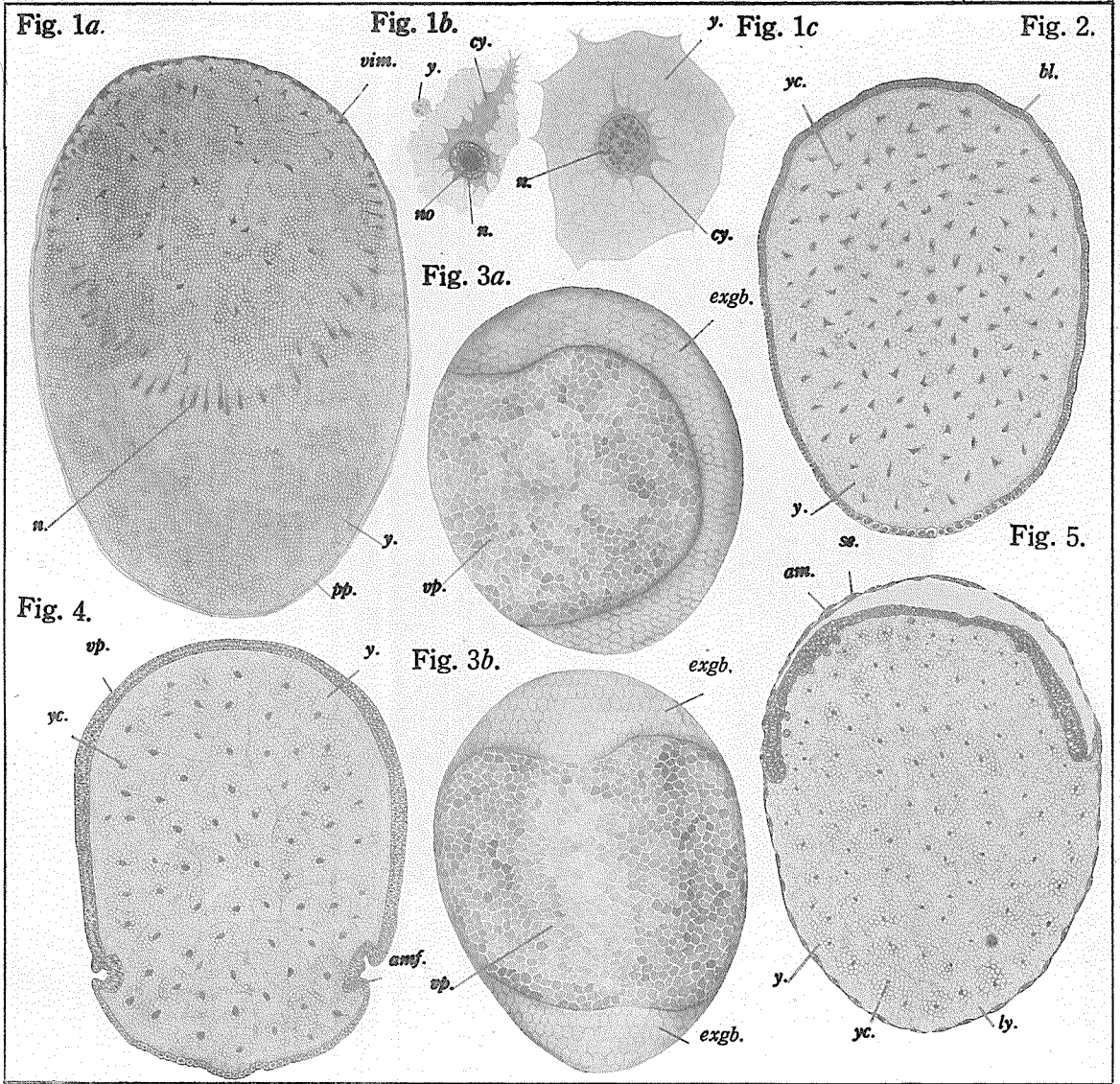


Plate VIII

- Fig. 6. Surface view of an egg 120 hours old, the trapezoidal ventral plate which is provided with a notch (an, pn) at its upper and lower margin. × 40.
- Fig. 7. Surface view of an egg 126 hours old, with a pair of globular cephalic lobes (cl) and the first traces of the primitive groove (pg), the caudal lobes (Cd) are about to appear. × 40.
- Fig. 8. Surface view of an egg 136 hours old, in which the caudal lobes (Cd) and the primitive groove (pg) have become striking; the first body segment (s^1) is marked off. us, unsegmented part. × 40.
- Figs. 9-11. Surface views of the ventral plate 142 hours of age, a little further advanced than the stage of Fig. 8. The primitive groove (pg) is narrowed anteriorly, and widened posteriorly, the body segments are differentiated from the second (s^2) to the fourth (s^4). ap, anterior widening of the primitive groove. × 40.
- Fig. 12. Surface of a ventral plate from an egg 160 hours old, increased in length. The primitive groove (pg) is about to close, commencing at about the second body segment. bp, blastopore. × 40.

Fig. 6.

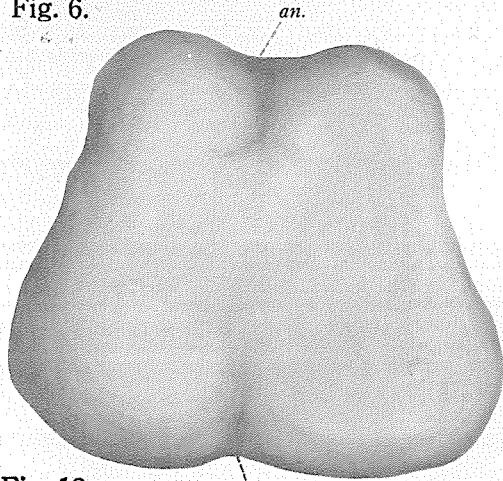


Fig. 7.

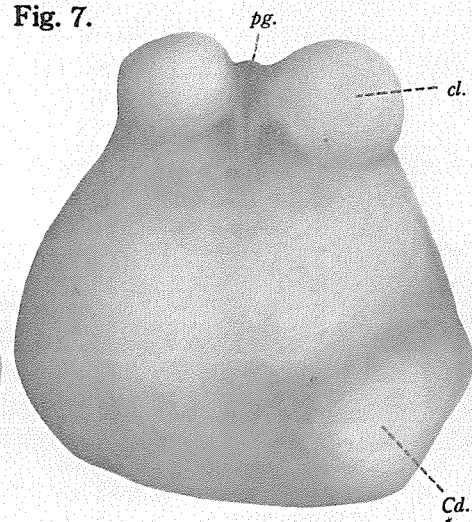


Fig. 8.

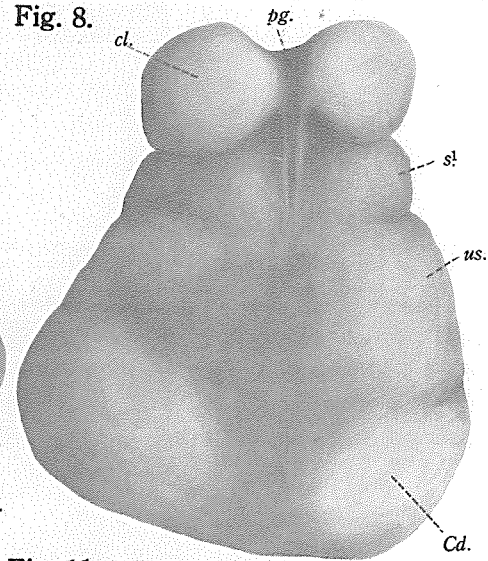


Fig. 12.

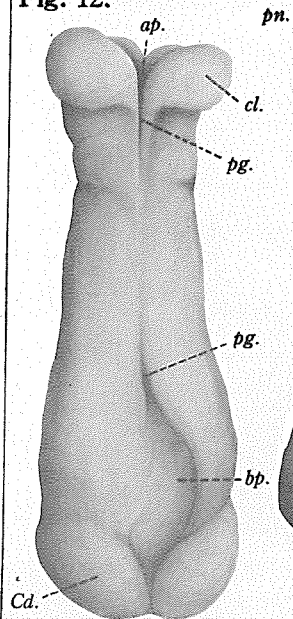


Fig. 9.

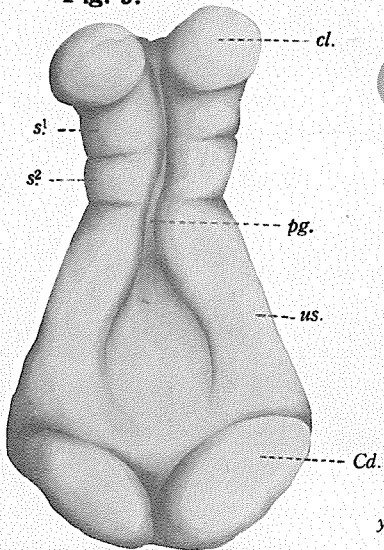


Fig. 10.

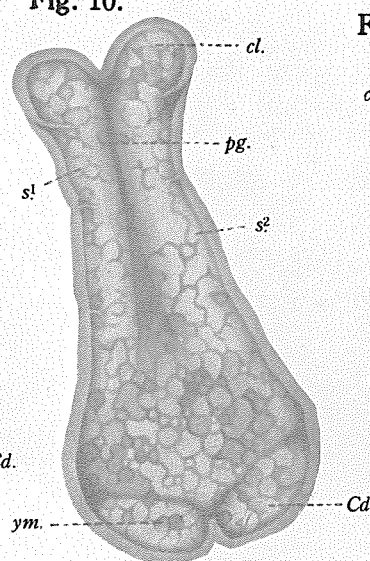


Fig. 11.

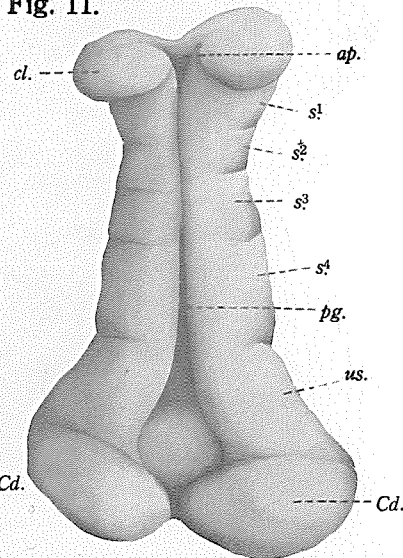


Plate IX

- Figs. 13, 14. Surface views of embryo taken from eggs older than the embryo shown in Fig. 12. The body segments (s^1 - s^2) are added, and the closure of the primitive groove (pg) has extended further posteriorly. × 40.
- Fig. 15. Surface view of an embryo 168 hours old, in which the blastopore (bp) is about to close. The neural groove (nf) is now obvious. × 40.
- Fig. 16. Surface of an embryo 176 hours old in which closure of the blastopore (bp) is almost completed. × 40.
- Fig. 17. Surface view of an embryo 184 hours old in which the stomodial depression (st), cephalic (md, mx¹, mx²), thoracic (thl¹⁻³) and anterior abdominal appendages have come into view. × 40.
- Figs. 18-20. Surface views of the embryo consecutively older, the youngest (Fig. 18) is a little older than the embryo represented in Fig. 17. at, antenna; lb, labium; abl¹⁻¹⁰, abdominal appendages. × 40.

Fig. 13.

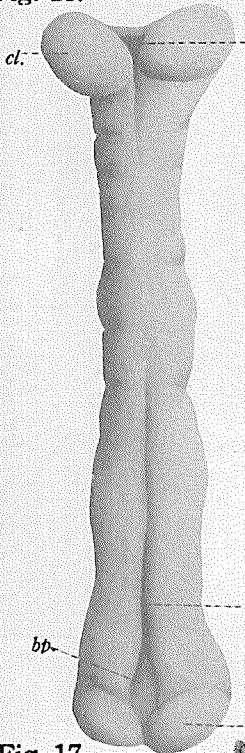


Fig. 14.

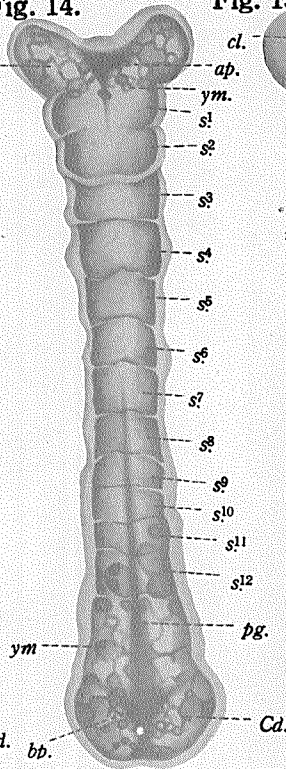


Fig. 15.

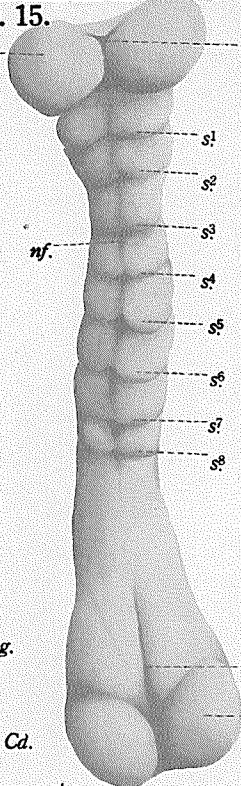


Fig. 16.

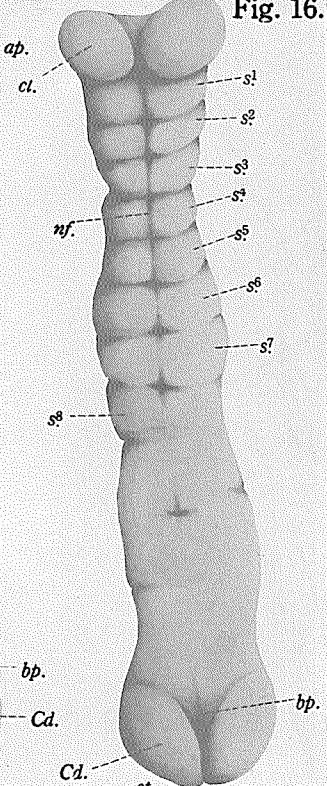


Fig. 17.

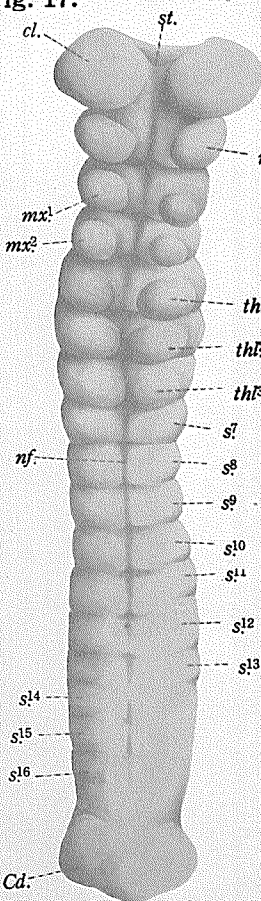


Fig. 18.

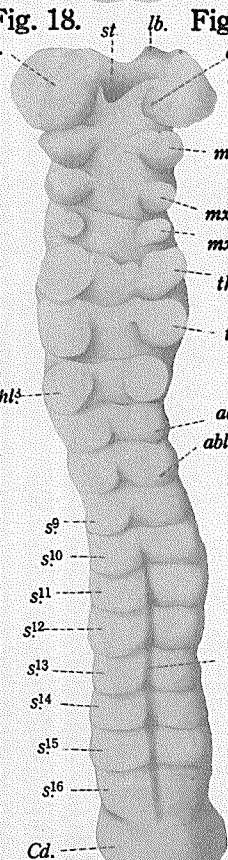


Fig. 19.

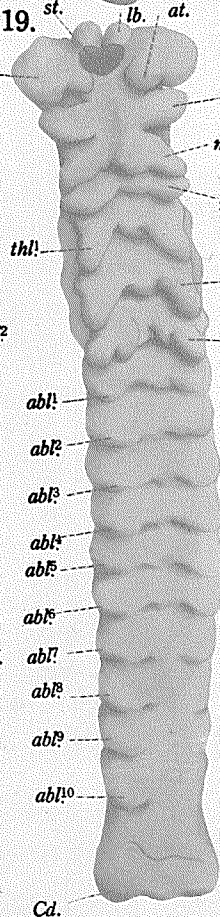


Fig. 20.

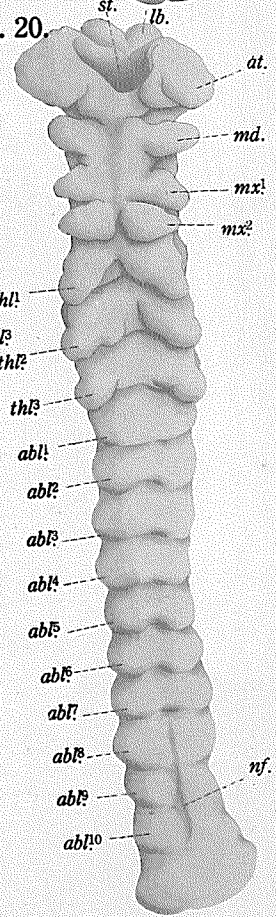


Plate X

- Figs. 21, 22. Surface views of an embryo 216 hours old in which the maximum growth in body length has been attained; all segments with a pair of appendages. pr, Proctodaeum; cal, caudal limb. × 40.
- Fig. 23. Surface view of an embryo 240 hours old; the diminution of the stomodial aperture (st); the abdominal appendages on the first, second, seventh to tenth segments have disappeared, while the caudal limbs (cal) appear on the caudal lobes. × 40.
- Figs. 24, a, b. Surface and lateral (Fig. 24b) view of an embryo 276 hours old; the body is diminished in length; and the stigmata (stg) have come into sight. amc, umbilical canal; ym, yolk-mass. × 40.
- Figs. 25, a, b, c. Surface, dorsal (Fig. 25b) and lateral (Fig. 25c) views of an embryo 348 hours old; the last abdominal segment is coalesced with terminal caudal segment. × 40.

Fig. 21.

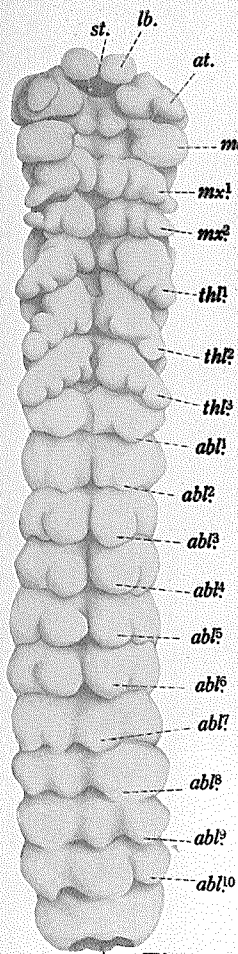


Fig. 22.

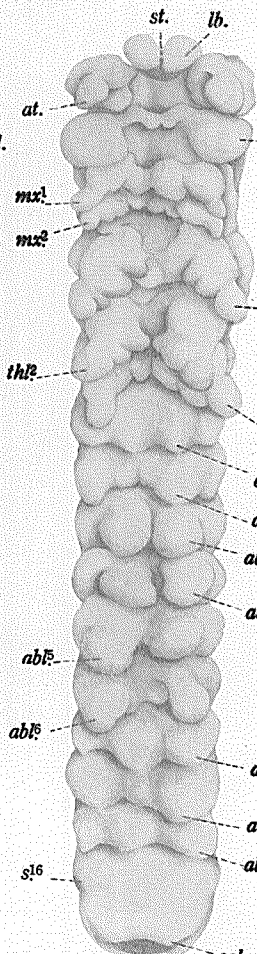


Fig. 23.

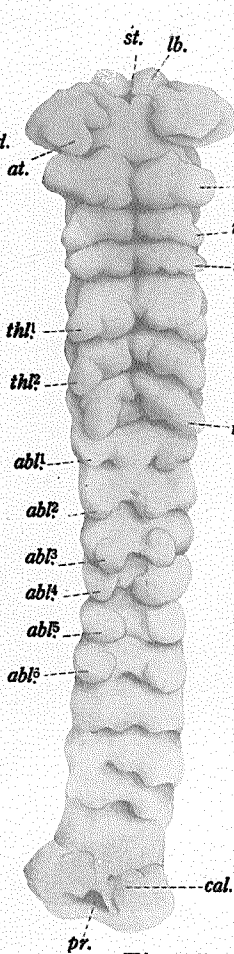


Fig. 24a.

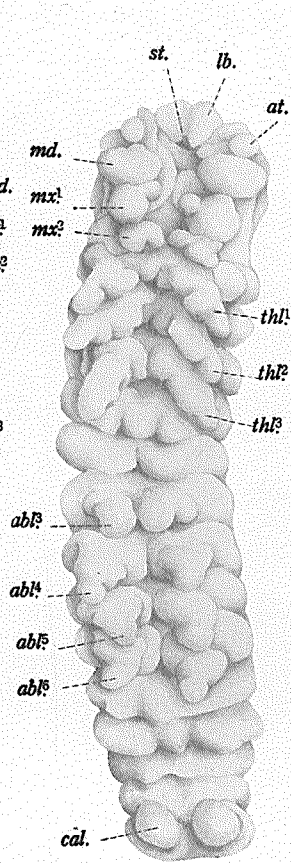


Fig. 24b.

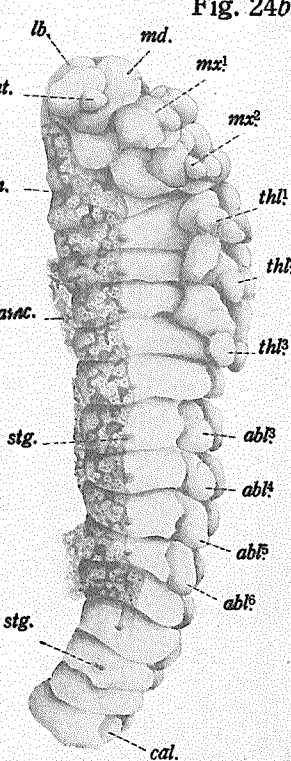


Fig. 25a.

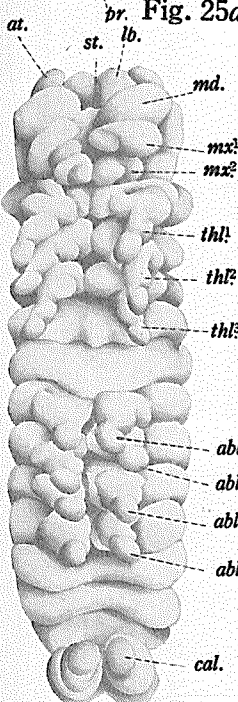


Fig. 25b.

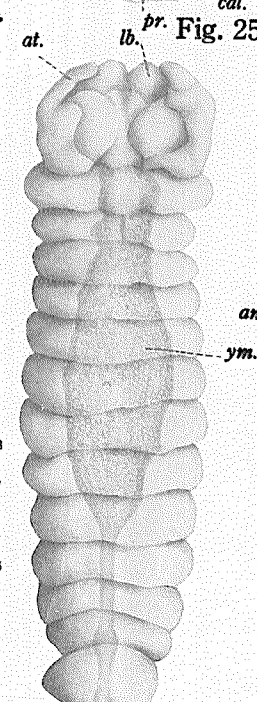


Fig. 25c.

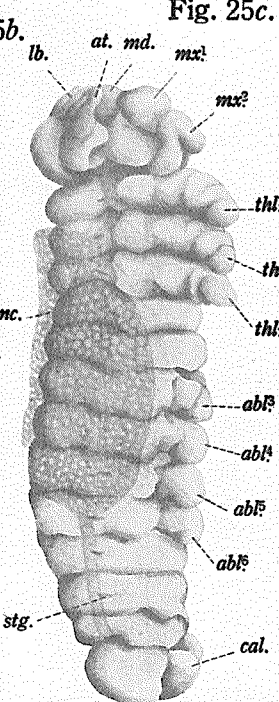


Fig. 25d.

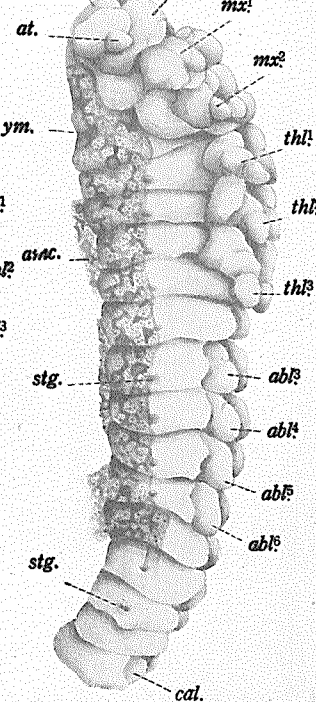


Plate XI

Figs. 26, a, b. Surface and lateral (Fig. 26b) views of an embryo
at the age of 420 hours. Two hind segments are fused.

× 40.

Fig. 27. Surface view of an embryo at about 20 days (526 hours)
which represents the oldest stage.

× 40.

Figs. 28, a, b, c. Three situations of the embryo in the revolution.

× 20.

Fig. 26a.

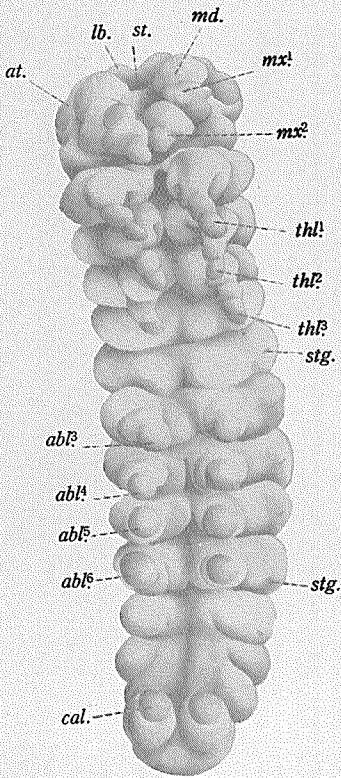


Fig. 26b.

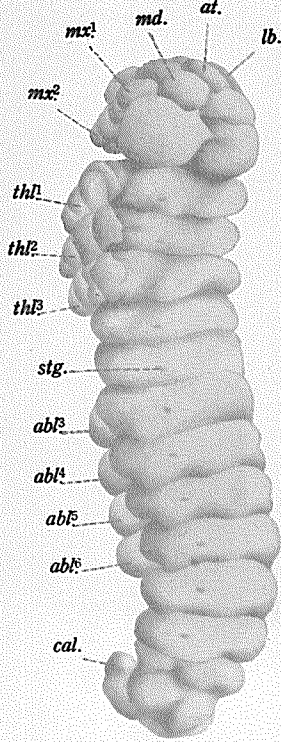


Fig. 27.

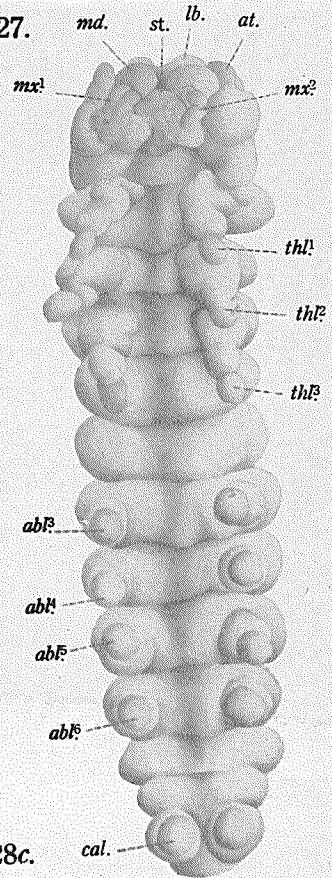


Fig. 28a.

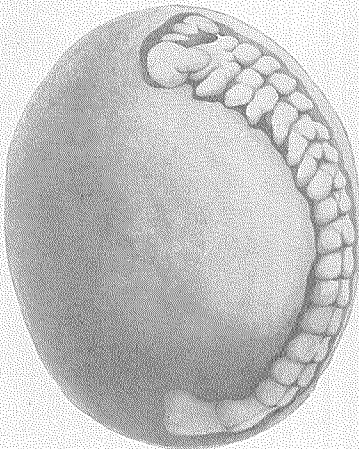


Fig. 28b.

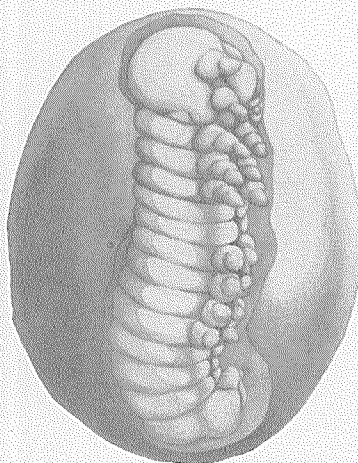


Fig. 28c.

