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ON THE DEVELOPMENT OF THE TUSSER, *ANTHERAEA PERNYI* GUÉRIN-MENEVILLE, WITH SPECIAL REFERENCE TO THE COMPARATIVE EMBRYOLOGY OF INSECTS

By

Saburo Saito

(With eleven plates and four text-figures)

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Introduction

In spite of the far advanced knowledge of the embryology of vertebrate animals as well as of echinoderm and coelenterate animals among invertebrates, the corresponding knowledge concerning insects is still unsatisfactory owing chiefly to the technical difficulties in the study. On the other hand the lack of fundamental knowledge makes the matter conflict the more as the more new reports appear. At the present time most investigators try only to find a morphological parallelism between the insect embryo and that of the other animals without explaining any particular feature in insect development. It is generally understood that for the basis of the morphology of tracheata the study of Onychophora including the Peripatidae which is found only in the tropical regions in the world seems to be most important. However the occurrence of the animal is particularly rare and is far beyond our use.

It is only desired to prosecute the study with some insect which stands comparatively low in the phylogenetical order and will allow sufficient investigation without great technical difficulties. Fortunately we have at hand the tusser¹⁾ of Lepidoptera being cultured for silk-production. Above all the size of the egg, and the larva as well, is unique and offers many advantages for the embryological study.

The author intends first of all to investigate the general feature of development of this worm and then to discuss the fundamental significance of the phenomena in the animal development.

In the previous report²⁾ on the development of the worm the external changes were fully investigated and at present the internal structure will be examined.

In the meantime the rather fragmental data of insect development hitherto reported are in need of revision.

The work was begun in the spring of 1927 at the suggestion of the late Dr. S. HATTA, Professor Emeritus of the Hokkaido Imperial Uni-

1) The English name "tusser worm" for *Antheraea pernyi* as used in the previous paper will be corrected to "tusser". This was suggested by Dr. Y. TANAKA to whom the writer wishes to express his heartiest thanks.

2) S. SAITO:—Jour. Fac. Agr., Hokkaido Imp. Univ., Vol. 33, 1934.

versity. It gives the present writer a great pleasure to acknowledge deep indebtedness to him. It is also a great pleasure to express appreciation for the kindness of Prof. T. INUKAI in offering encouragement during the work and in giving useful criticisms and advice in preparing this paper.

I. Method of Study

The eggs of the tusser are in many respects extremely suitable for embryological research and there is an almost limitless supply as material. However, in preparation for microscopical study they are not easily sectioned as the accumulated yolk makes them brittle by the hardening fluids. In the present study the whole egg was necessarily sectioned including the embryo and yolk. After many unsuccessful attempts the author recommends the following method as giving favourable results. The eggs after deposition are killed and fixed in ALLEN's modified solution of BOUIN's fluid which is heated at 30–40°C for about four hours. They are left in the fluid for about ten hours until cooled. They are washed thoroughly with 30 per cent alcohol and placed in 50 per cent alcohol for about two days. Then they are transferred into 70 per cent alcohol for preservation. After remaining in the alcohol for about a week, the chorion of the egg is removed by tearing it open with a needle at the broad pole and gently pushing out its contents. The eggs after the chorion is removed are stained with borax carmine for about twelve hours *in toto*. Then they are dehydrated in absolute alcohol, and imbedded in paraffin in the ordinary method after passing through xylol and xylol-paraffin. They are sectioned serially in ten microns thickness with a GROOT's microtome, and mounted in order with egg albumin. The sections which were clarified with xylol, are mounted in Canada balsam as usual.

In the egg of the tusser, the polarity of the embryo is readily distinguishable even at the early stage of development as its cephalic end is located at the end where the micropyle is found. But as the egg is flattened ellipsoid in shape there is no exact way to forecast the dorsal and ventral sides of the future embryo. However, in the egg which is bilaterally symmetrical in shape along the axis through the micropyle, the germ band appears along the right lateral edge and so in most cases the dorsoventral sides of the egg can be guessed. But as stated in the previous paper, with the progress of the development, the germ band with its adherent amnion, moves back from the right serosa and soon

comes to lie in the middle of the yolk. Then the germ band bends back, and with the consumption of the yolk it is shifted finally to the left side serosa so as to occupy the opposite side. However the ventral surface of the embryo is directed constantly toward the right side of the egg. Consequently supposing the micropyle end is the front side, then the left side of the egg must be the dorsal and the right side must be the ventral. Care was taken not to injure the embryo during the preparation with caution as to the position of it according to the stage of development.

II. Observation

A. DEVELOPMENT OF THE EMBRYO FROM SEGMENTATION TO FORMATION OF THE ENVELOPS

In the previous paper mainly the early development of the animal including the segmentation and formation of the blastoderm and the later changes of the external structure of the embryo were described. Hereafter only a brief sketch of the previous work will be given for the sake of the easy understanding of the matter.

At the time of deposition in the eggs when stained with borax carmine, there are several nuclei surrounded by the cytoplasm which appears as a delicate pink cloud among the greyish yolk spheres. These nuclei soon divide rapidly and give rise to many smaller nuclei which are scattered in the yolk around the micropyle pole. The nuclei are surrounded also by a small mass of cytoplasm, and protoplasmic continuity seems to exist between these nuclei at an early period. When the cleavage nuclei become from 60 to 80 in number, they commence to migrate to the surface of the egg (Pl. I, Fig. 1). They continue the division and as they migrate to the surface from within they arrange spherically keeping a nearly equal distance from each other through the yolk. This is the first stage of the delamination.

The sphere swells further to reach to the egg surface, first at the micropyle end and at last at the opposite end. The nuclei which have arranged at the surface make a layer of cubical cells (Pl. I, Fig. 2). This layer of cubical cells is converted very soon into the one-cell layered epithelium, that is, the blastoderm (Fig. 2, b1) is differentiated. As stated in the previous paper, in the early stage of the blastula, the cells forming the caudal side of the eggs are larger at first than those of the anterior side. This difference disappears soon and in the section at

any level the blastoderm appears equal in thickness.

In the succeeding stage, in addition to the rapid increase of the blastomeres there occurs an uneven thickening of the blastoderm. This is very much likely brought about by condensation of the cells on one side. Thus the blastoderm becomes exceedingly thin on the dorsal side of the egg, the nuclei becoming scattered and flattened while it is thickened on the ventral side, where the cell elements come together thickly crowded to assume a spherical shape. By a further condensation from both the cephalic and caudal ends towards the center on the ventral side, the ventral plate becomes clearly visible on that side (Pl. I, Fig. 3).

The yolk cells or vitellophags are the cleavage nuclei which remained in the yolk at the time of the formation of the blastoderm. They are spherical or spheroidal in shape and surrounded by stellate cytoplasm which extends radially with long pseudopodial continuations for a considerable distance between the yolk polyhedra (Pl. II, Fig. 6).

When the ventral plate is differentiated fully out of the blastoderm, the antero-lateral sides of the plate fold out a little to make a semilunar ridge (Pl. I, Fig. 3). This fold is the beginning of the amniotic fold, which subsequently envelopes the head of the embryo. The amniotic fold of the caudal portion which appears very soon, grows much more rapidly than the cephalic fold.

The folds from all sides at last meet and fuse, that is, coalesce to give rise to the double membrane covering the ventral plate; the inner one is the amnion which is continuous directly with the edge of the ventral plate and the outer one is the serosa which is continuous with the remainder of the blastoderm.

The serosa now covers the entire egg, and within it is the amnion enclosing the ventral plate (Pl. II, Fig. 7c, am, se). Both membranes show the same appearance in section. In the later stage they are brought together above the ventral plate so closely in contact with each another that they appear as a sheet of single membrane (Pl. II, Fig. 7b, am). JOHANNSEN (1929) observed the belated formation of the amnion in *Diacrisia*, and came to the conclusion that the amnion does not begin to develop until the serosa wholly covers the egg surface, which is characteristic to the Lepidoptera. This is probably a misinterpretation of the fact owing to the extreme thinness of the amnion. On the contrary BRUCE (1887) observed on the bagworm (*Thyridopteryx* ?) that the amnion is formed simultaneously with the serosa from the

amniotic fold. JOHANNSEN was not right as he inclined to believe that the margin of the amnion is formed by the proliferation of cells around the germ band or the ventral plate and the middle portion is formed by the delamination of cells from the inner face of the serosa or by the cells liberated from the ventral face of the germ band. There is little room to doubt that the entire amnion is formed out of the blastodermic cells and mainly by the proliferation of cells from the margin of the germ band.

BOBRETZKY (1878) gave a special account of the connection between the amnion and the serosa in his description on the development of *Pieris crataegi*. According to him in this species the amniotic fold begins to develop in the same manner as the present species but, on the contrary when the fold has partly formed, only serosa continues to grow, while the amnion delays to extend.

When the amniotic folds close, the ventral plate underneath is condensed from the lateral side and becomes a three-cell layered plate. The contraction of the ventral plate from both the cephalic and caudal ends towards the center ceases after the completion of the amnion and serosa, while the lateral contraction continues in progress until a considerably later stage.

The thickening of the ventral plate caused by the contraction is particularly conspicuous at the lateral margin. The multiplication of cells occurs actively at the corner parts of the plate, so that they elevate from the general surface. The elevated parts are the origin of the procephalic and caudal lobes.

As the development advances the ventral plate extends caudally pushing its way through the yolk accompanying the amnion and then turns around the caudal pole of the egg alongside the serous membrane (Pl. I, Fig. 4a). After that the caudal end sometimes extends headwards in the center of the yolk and sometimes along the margin of the egg so that the amnion and serosa become closely contracted. A cross section of the egg at this stage shows that the thin amnion is separated from the serosa by a layer of yolk which is continued to the great central yolk mass. At the end of the blastoderm stage when the amnion and serosa grow over the ventral plate, the yolk mass commences to segment (Pl. I, Fig. 2). The segmentation occurs first at the peripheral part and then progresses into the center until finally the whole yolk is divided into numerous rounded masses of nearly equal size, each containing one to three vitellophags.

B. DIFFERENTIATION OF THREE GERM LAYERS

At about ten hours after the establishment of the ventral plate at the ordinary room temperature there occurs an invagination near the anterior margin of the ventral plate, and in about 125 hours old embryos it is seen clearly becoming somewhat deeper. At this time, at the anterior part of the ventral plate, where the invagination has appeared, transverse lines indicating some rudiments of the body segments become visible (Pl. I, Fig. 5). The invagination increases in depth and then extends elongating along the length of the ventral plate to become a groove at about 24 hours after its first appearance, which divides the plate into two symmetrical halves. Anteriorly the groove ends rather abruptly but posteriorly it bifurcates (Pl. III, Fig. 10g, Pl. IV, Fig. 11e, pg), into two grooves which run for a short distance becoming gradually shallow to fade at the general level of the plate.

In sections the groove appears much broader. The floor of the groove is somewhat thin, and the cells in it lose the former columnar shape, becoming more polygonal in outline, and more irregular in their arrangement (Pl. I, Figs. 4, 5, Pl. II, Figs. 7, 8, Pl. III, Figs. 9, 10, Pl. IV, Figs. 11, 12). On the other hand, the lateral parts of the ventral plate are somewhat thickened, and the cells are small in size, columnar in shape and arranged regularly (Pl. II, Fig. 8a). This fact indicates the active increase of the cell at the lateral portions.

The ridges of the groove grow upward. This is most conspicuous at the anterior portion while posteriorly the groove becomes gradually broader and its ridges become obscure (Pl. I, Fig. 4, Pl. II, Fig. 7, Pl. III, Fig. 10). It is clear, therefore, that the differentiation proceeds from anterior portion in a backward direction.

As the ridges of the groove become more raised, subsequently the groove becomes deeper, and at last the groove closes in such a way that a tubular canal is formed at the pre-thoracic portion. At this time the groove extends over the entire length of the ventral plate (Pl. III, Fig. 10, Pl. IV, Fig. 11).

The process of closure of the groove proceeds both anteriorly and posteriorly from the first fused point just behind the procephalic segment (Pl. III, Fig. 10). The terminal of thus formed canal both posterior and anterior remains open (Pl. III, Figs. 9b, 10a, Pl. IV, Figs. 11, 12). While the closure of the groove at the anterior portion takes place so as to give rise to a complete tube (Pl. III, Fig. 10), at the

posterior part it occurs incompletely (Pl. IV, Fig. 13) as explained above.

The invaginated groove has long been termed a primitive groove compared to the primitive groove of higher vertebrate animals as it becomes a differentiation center of the later development. From the time of invagination the mere uniformly structured cell aggregation of the ventral plate gives rise at the floor of the groove to the polygonal shaped cell mass and at its marginal ridges to the epithelial structure. This is the first stage of tissue differentiation.

The floor cells are the so-called inner layer which becomes the mesoderm of insects and the other epithelial structure is sometimes called the outer layer which serves as the origin of the ectoderm.

With further development the boundary of the two layers becomes clearer (Pl. IV, Figs. 11a-11e).

Examining precisely the developmental change of the ventral plate there is found some fundamental difference between the primitive groove of the higher vertebrates, for example, of the chick and that of the insects. In so far as the differentiation of ectoderm and mesoderm from the region of the groove is concerned they agree without any objection. However the differentiation in insects is brought about chiefly *in situ* or at least the ectodermal development appears earlier and surpasses the mesoderm all the time. In the higher vertebrates, on the contrary, from the undifferentiated layer, the ectoderm and mesoderm develop simultaneously and the latter gains strength ever after.

It is not correct to call the anterior and posterior pores of the ectodermal tube by the name "blastopore" as many investigators have done since long ago. They are not comparable to the blastopore of *Amphibia* but rather resemble the neuropore of *Amphioxus*. The pores in question have no relation to the mesoderm nor to the endoderm formation in insects.

The relation of these two layers is understood from the diagrams representing the successive stages (Pl. II, Fig. 7d, Pl. IV, Fig. 13), in which the floor of the groove to be enclosed in the closing ridges is shown by the meshes and the region already covered with the ridges is represented by the spotted part.

The differentiation of the ectoderm proceeds posteriorly covering the mesoderm from both sides until at last the ectoderm extends the whole length of the ventral plate. There remains at the hindmost part a pore which is falsely called the posterior blastopore. The so-called

blastopores disappear sooner or later.

At the present the embryo is composed of two layers (Pl. V, Fig. 15), the outer layer or the ectoderm (etc) and the inner one or the mesoderm (mes). In his observations on the closure of the ectoderm of *Xiphidium*, WHEELER (1893) states that it proceeds simultaneously from its anterior end backwards and from its posterior end forwards, so that the last portion of the groove to disappear corresponds to that part of the germ band which later becomes the thoracic or baso-abdominal region.

The mesoderm has at first the form of a cord attached closely to the inner surface of the ectoderm. Then it spreads laterally with the same thickness underneath the ectoderm through the entire length of the embryo beginning from the anterior portion.

With the segmentation of the ectoderm, the mesoderm is also divided, though incompletely, at the place where the ectoderm is constricted. The process of segmentation begins at the middle portion of the ventral plate, at where the ectodermal groove was closed at first, and proceeds forwards and at the same time backwards as has already been observed by the present author (1934), TICHOMIROFF (1870) and TOYAMA (1902).

On the closed ectoderm there is a median longitudinal furrow which is called the neural furrow. The embryo increases the body length and bends strongly dorsally, sinking in the yolk. Then there exists the yolk granules between the amniotic and serous envelopes (Figs. 14, 15), and so the embryo is found completely imbedded in the yolk. At present two germ layers turn around at both terminals to make there a pouch; the anterior end consists of two symmetrical pouches while the posterior end is made of a single one (Fig. 15a, h).

In the following stage the wide procephalic lobes, the mandibular, the first and second maxillal, the three thoracic and some abdominal segments are incompletely constricted first in the outer layer, and then the inner layer also assumes a more segmental character corresponding to the outer constriction.

Soon after the closure of the so-called blastopore the stomo- and proctodaeums invaginate definitely. At this time almost all the body segments are constricted off and from them the cephalic appendages begin to appear. Now the mesoderm of the entire body comes to have the metameric structure, and moreover by the neural furrow the hitherto unpaired mesoderm is split again longitudinally into two lateral bands. The lateral constriction of the mesoderm, representing the body segment

is called the somite which in a strict sense differs from that of the vertebrates. At the bottoms of the stomo- and procto-daeums, that is, at the oral and anal ends the mesoderm cells aggregate compactly.

As the rudiments of the appendages grow, a part of the mesoderm enters into them (Pl. XI, Fig. 33), and makes the origin of muscle-cells. In the lateral overspreading of the mesoderm there appears a lumen (Pl. V, Fig. 15, Pl. XI, Fig. 33a), so as to split it into two layers which correspond to the splanchnic and the somatic layers of *Doryphola* according to WHEELER (1889). He describes how the cells composing the lateral streak are clearly of two layers, the upper consisting of cylindrical cells while the lower layer consists of flattened cells arranged regularly, but in this case as above mentioned the two layers have a similar appearance.

Now let attention be direct to the development of the endoderm. As soon as the closure of the blastopore is completed, the stomodaeum appears as a depression of the ectoderm at the median anterior ventral part of the bilobed pouch at the place where the anterior blastopore had formerly existed. With the development of the body the stomodaeal pouch extends dorso-posteriorly of the embryo (Pl. V, Fig. 14, Pl. VII, Fig. 23, Pl. XI, Fig. 32a).

Figs. 24a, b and c show serial sections through the anterior part of an embryo about 200 hours old. The stomodaeum (st) apparently has the paired external swellings around the depression; these are the rudiments of the antennae (at) and those of the mandibles (md).

The proctodaeum invagination appears later than the stomodaeum, as has already been observed by many other authors. Even after the stomodaeal depression grows to a considerable depth, the proctodaeum has not yet been seen. Generally the latter comes into view a day or more after the appearance of the stomodaeum. The posterior end of an embryo about 200 hours of age consists of thick ectoderm and the irregular mesoderm. First at about ten days of age, the proctodaeum is seen only as a faint depression at the posterior end of the body (Pl. VI, Fig. 16). Different from the stomodaeal depression, it is directed somewhat toward the ventral side of the embryo in V-shape, the blind end of which clearly is formed only with a thick epithelium. The depression deepens gradually forwards along the ventral wall of the embryo and terminates still in a V-shaped blind tube for a considerable time (Pl. VI, Figs. 19, 20).

Then there are observed the cells proliferating from the bottoms

of the stomo- and procto-daeums. These cells are the origin of the endoderm which gives rise to the wall of the mid-gut in the later stage. The rudiments of the endoderm from both ends are visible at first at the postero-lateral angles of the stomodaeal wall and also at the antero-lateral angles of the proctodaeum (Pl. XI, Fig. 32). These cells at the stomodaeum correspond to the "vordere Epithellamelle" of VOELATZKOW (1889) and HEYMONS (1894) who observed them in Coleoptera and Orthoptera. However they illustrated these cell masses as the continuation of the ectoderm, but the writer is of a different opinion as will be explained under the special heading about it.

From the thirteenth day the body-length begins gradually to shorten. This shortening is associated with the broadening of all the segments, growth of the appendages and many internal changes. The stomodaeum has now become deeper than in the preceding stage and its distal end commences to widen. At that time the wall of the stomodaeum consists of thick epithelium, except the bottom which becomes quite thin, constituting the so-called "Grenzlamelle" of HEYMONS (1894). The cell masses project into the yolk from the thick lateral wall of stomodaeum. These make a distinct structure which becomes the rudiment of the endoderm as already referred to (Pl. V, Fig. 15). At a slightly further advanced stage, the endoderm rudiments attach directly to the lateral mesoderm. In a more advanced embryo the endoderm elongates to become the ribbon-like bands (Pl. IX, Fig. 29). On the other hand, at the blind thick epithelial end of the proctodaeum which has invaginated in a little later stage than the stomodaeum, there occurs likewise an active proliferation of cells. This is the origin of the posterior endoderm rudiment (Pl. XI, Fig. 32b).

With the development of the embryo this proliferated cell-mass from the terminal end of the proctodaeum assumes at first the form of a short lamella which grows forwards (Pl. V, Fig. 15). In an older individual two lateral stripes grow out from this posterior endoderm origin and the stripes become attached to the lateral bands of the mesoderm just in the same way as the anterior endoderm.

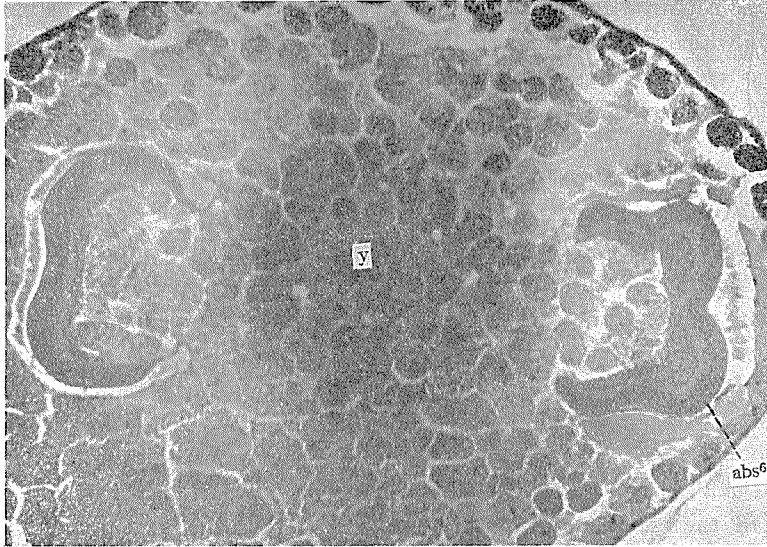
The endoderm cells do not differ in structure from the stomo- and procto-daeums. The same condition is described by SCHWALTZE (1899) in the lepidopterous genus *Lasiocampa*, by TOYAMA (1902) in the silkworm, *Bombyx*, by JOHANNSEN (1929) in *Diacrisia*, by DEEGENER (1900) in *Hydrophilus* (Coleoptera), as well as by a number of workers with species of other orders. From this the view that the epithelial layer

of the mid-gut arises from the ectoderm has been established by them. In the present study it is clear that this tissue seems to arise from cells in the lateral angles of the stomodaeal and also proctodaeal invaginations, but the writer would like to interpret this fact as follows; by the invagination of the procto- and stomodaeums, the cradles of the proper endoderm which are situated as a general rule on both the ends of the ventral plate are moved towards the special angles of the fore- and hind-guts. This inference is based on the general facts which have been observed in the different species of insects upon endoderm development. It is seen indeed that the tips of the invaginations become free from the mesoderm cells of the original lower layer before the embryo is 184 hours old and that later the cell source from which the mid-gut epithelium arises, appears continuous to the tissue of the invaginations.

Some of the earlier writers, namely prior to 1900, as TICHOMIROFF (1879, 1891), have described the origin of the endoderm of the yolk-cell. In some cases it is evident that during the early development these cells increase in number, but as the present writer has mentioned previously, they are destined to degenerate. In other instances, some of these early investigators maintain that the endoderm is derived from the splanchnic layer of the mesoderm by delamination. This is also impossible.

C. FATE OF THE GERM LAYERS

Having now examined the germ layer formation, let us turn our attention to the differentiation of the layer. After the completion of the so-called gastrulation in which the mesoderm formation occurs by means of invagination the embryo attains its maximum body length. The caudal end lies on the dorsal side of the egg, pointed forward. In a transverse section through the posterior part of the egg, the embryo is cut twice (Text-fig. 1). All the body segments are formed clearly and the appendages of the cephalic part appear from them as blunt projections. At this stage a few large, polygonal cells are clearly seen among the ectodermal cells at the interior portion of the ectoderm of each segment in the thorax and abdomen (Pl. VI, Fig. 21, Pl. XI, Fig. 33). These cells become the neuroblast, and give rise upon further differentiation to the ventral nerve cord. In a section passing through the second abdominal segment (Fig. 21) the neuroblasts (neb) appear three in number on either side, arranging themselves side by side in the deepest portions of the ectoderm. The rapid proliferation of the new



Text-fig. 1. A cross section of the posterior part of an egg, in which the embryo has developed to the maximum body length, showing that the posterior termination is situated to the dorsal side (left side of the figure) of the egg. The sixth abdominal (right side) and the anal segments are seen in the same section. $\times 60$. abs^6 , the sixth abdominal segment; y, yolk.

neuroblasts occurs at right-angles to the general surface of the ectoderm so as to form a column of neuroblasts. As the result the ectoderm is raised up thickening at that part, and consequently a furrow (nf) deepens in the center. As WHEELER (1893) suggested, the proliferated daughter cells probably do not divide to increase but aggregate to convert directly into ganglion cells. Only the original neuroblast seems to retain the power of production.

In a further advanced embryo in which the anlage of all appendages has been almost formed, the columnar arrangement of the neuroblasts is no longer visible since the individual cells have been by that time converted into definite ganglionic elements. On the outer periphery of the ganglia, however, colossal neuroblasts are still to be found.

At the anterior part the brain develops from the neuroblast in manner similar to the case of the ventral chain of ganglia as just explained. That is to say, at the time when the procephalic lobe was constricted from the other body part and the rudiments of the antennae

grew as a pair with faint evagination at both lateral sides of the stomodaeum which has been invaginated considerably at this juncture, a group of the enlarged cells or neuroblasts are to be found in the dorsal ectodermal wall of the procephalic lobe (Pl. V, Fig. 15h). EASTHAM (1930) stated in his study on *Pieris*, that the brain is constituted of three pairs of ganglia, viz., the procerebral ganglion occupying a large area between the front border of the embryo and the antennal part, the deutocerebrum in front of the base of the stomodaeum and the tritocerebrum behind the antenna. From this construction he concluded that the part of the head in front of the mandibular segment is formed clearly from three segments. In *Antheraea*, the arrangement of the ganglia is not distinct. The only fact seen is that the neuroblasts are dispersed over a comparatively large area.

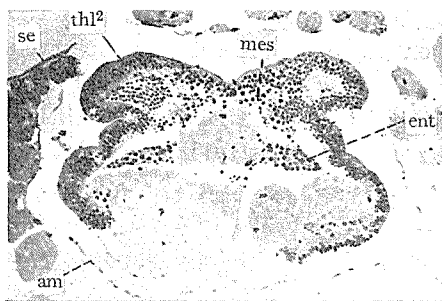
It has already been noted that the fore- and hind-guts are of ectodermal origin. They appear at first as simple invaginations at both ends of the embryo. With the advancement of the development both depressions deepen and the end-wall becomes thin, flattens and then somewhat reflects to form a short side pocket around the gut-canals (Pl. IX, Fig. 29).

After all the anlagen of the appendages have been developed in segmental arrangement the paired invaginations of the ectoderm arise in the cephalic part metameric corresponding to the segments. This ingrowth of the ectoderm develops into the chitinous beams which protect the cranium and support the attachment of the muscles of the mouth parts (Pl. X, Fig. 31a). The detailed observation on the endoskeleton may be given later. At this stage the paired invaginations, which give rise to the spiracular system, grow also at the thoracic and abdominal segments. They are at first simple blind tubes invaginated into the intersomitic part (Pl. VIII, Figs. 28c, e), and then the ending of each tube bifurcates, one extending dorsalwards along the ectoderm and the other passing towards the median line of the abdomen (Fig. 28c, astg). The invagination takes place in the first and second thoracic and in each of ten abdominal segments, but later the second thoracic one and the last two abdominal spiracles close before hatching, the latter especially closing in an early stage, without forming the spiracles. This result agrees with the findings in the embryo of *Bombyx* by TOYAMA (1902).

The development of the Malpighian tube has become a topic of discussion. TOYAMA (1902) states on *Bombyx* that "Malpighian vessels

arise as three separate pairs of outgrowths from the blind end of the proctodaeum." HATSCHEK (1677) on the same insect also observed three tubules on each side of the proctodaeum and said that "die drei malpighischen Drüsen jeder Seite münden durch ein gemeinschaftliches Anfangsstück in das blinde Ende des Hinterdarmes." DU BOIS (1933) also stated in his study on *Sciara* (Diptera) that this organ is derived from the ectoderm. While in 1932 HENSON asserted that this organ is of endodermal origin and that it connects with the proctodaeum by the so-called interstitial ring which is imagined to be originated from the ectoderm. So far as the observation in this worm goes they are ectodermal organs without doubt as TOYAMA and HATSCHEK ascertained. That is to say, from the antero-lateral wall of the proctodaeum, at first a dorsal pair, then a ventral pair and at last a lateral pair of Malpighian tubes appear (Pl. VI, Figs. 17-19, Pl. XI, Fig. 34). As the development of the embryo advances, the three outgrowths on each side of the proctodaeum connect with the gut by a common duct (Pl. VIII, Fig. 28d, Pl. XI, Fig. 35).

As above stated, in consequence of the formation of the neural furrow, the mesoderm is divided into four parts, the oral and anal groups and two longitudinal parallel bands containing many pairs of somites. It is noted that each somite in the embryo when about 200 hours old, differentiates into the splanchno- and somato-pleures between which a coelomic cavity develops (Pl. V, Fig. 15, Pl. VII, Fig. 26, Pl. XI, Fig. 33). By the further outgrowth of the appendage, the element of each somite is divided again into two parts (Fig. 33a), namely the main part around the coelomic cavity (ca) and a small group of mesodermal cells which enters into the ectodermal swelling of the appendages. The latter are nothing but the anlage of the muscle of the appendage. At this stage one can find some peculiar cells among the somatic mesoderm of abdominal segments. They are fairly larger than the surrounding mesodermal cells in size, and much paler in colour. These are known sometimes as the primitive germcells, of which the differentiation has been already studied by TOYAMA (1902) in *Bombyx*, by HEYMONS (1912) in *Phyllodromia* and by WHEELER (1897) in *Xiphidium*. According to WHEELER in *Xiphidium* the clusters of the primordial germcells occur constantly only in the first to the sixth abdominal segments. In *Antheraea* they are differentiated mostly from the cells of the somites in all abdominal segments with the single exception of the anal segment, and therefore, they are metameric in origin.



Text-fig. 2. A cross section through the second thoracic segment, showing the endoderm rudiment which appears as two triangular cell-masses at the two sides. $\times 60$.

am, amnion; ent, endoderm;
mes, mesoderm; se, serosa;
thl², second thoracic leg.

The main part of the somite is formed at first by cells uniformly arranged radially in transverse section. As development proceeds the somatopleure develops into the muscle of the body wall, while the splanchnopleure still retains its uniformate columnar arrangement of cells. With the formation of the endoderm-strand the latter comes in contact with the endoderm. The coelomic cavity enlarges very widely (Pl. VIII, Fig. 28, Pl. IX, Fig. 30).

The heart rudiment appears as cell-groups loosely scattered at the dorsal terminations of the splanchnopleures on both sides (Pl. VIII, Fig. 28), at the stage of the complete formation of the endoderm strand. As the body wall grows dorsally, this heart rudiment moves dorsalwards along the body wall, and meets from both sides in the middorsal line. Then there appears a canal within it to make a longitudinal tube above the alimentary canal (Pl. X, Fig. 31).

The blood-cell differentiation occurs in a very early stage, in which the plate-like mesoderm has just splitted into two lateral strips. At first the cells are liberated from the inner surface of the whole mesoderm and migrate into the yolk (Pl. V, Figs. 15d, g). Later they assume the spherical form normal to the insect blood-cells.

Next, as to the formation of the mid-gut, there is no doubt that it is of endodermal origin. As already pointed out there occurs a proliferation of the endodermal cells from the bottom of either the stomodaeum or of the proctodaeum. The cast out cells from both ends are connected to become two parallel endoderm strands (Text-fig. 2). EASTHAM (1930) in *Pieris* observed an endodermal cell-mass before the occurrence of the invaginations of the stomo- and procto-daeums, and he states that this cell-mass has been proliferated inwardly from the germ band, somewhat differently from the formation of the mesoderm. In this case there are no particular cells other than the mesodermal cells before the appearance of the stomo- and proctodaeums. The cells

constituting the two endodermal strands increase in number and cover the yolk at first from under and then extend upwardly to form a complete tube around it (Compare Pl. X, Figs. 31e, f with Pl. X, Fig. 31c).

D. DEVELOPMENT OF THE ORGANS

The histological differentiation of the germ layers in insects is still largely ignored. So far as the writer is aware, only the studies on *Pieris* by EASTHAM (1930) and on *Carausius* by LEUZINGER, WIESMANN and LEHMANN (1926) have touched upon the problem a little precisely.

1. Nervous System

The development of the nervous system in insects has been investigated by a number of prominent workers. But their studies are limited to its early development and moreover their materials belong mostly to Orthoptera, Dermaptera, Coleoptera, Diptera and Hymenoptera. Among the works in Lepidoptera, *Bombyx* by HATSCHEK (1877) and *Pieris* by EASTHAM (1930) are to be referable.

The early development of the ganglion beginning from the segregation of the neuroblasts out of the dermatoblasts and the successive increase of the neuroblasts has already been described. Now the further formation of the nervous system will be traced. The nervous system differentiates into four principal parts: 1) the brain or supraoesophageal ganglion, situated in the head above the oesophagus, 2) the suboesophageal ganglion also situated in the head, but below the oesophagus, 3) the ventral nerve-cord running ventrally through the thorax and abdomen, and swollen into paired ganglia at the posterior body part, 4) the sympathetic nerve system innervating the mouth and oesophagus parts.

The early development of the brain has already been described. It is formed from the neuroblasts distributed in a large area. From first there are no clear boundaries in the brain. However judging from the cellular arrangement and the distribution of the nervous fibres one can see three parts in it though not very clear. The protocerebrum, sometimes called as the ganglion of the first head segment, forms the foremost and greater portion of the brain and then follows the deutocerebrum, namely the ganglion of the second head segment, which sends off nerves to the antennae. The hindmost is the tritocerebrum, the ganglion of the third or intercalary segment, from which the nerve cord to the sympathetic ganglion is sent off. The development of the above nerve cord will be studied at a future opportunity. At about 350 hours the

brain develops into a symmetrically fused ganglion (Pl. IX, Fig. 30a shows its paired structure, but Pl. X, Fig. 31a shows the fused structure).

In this animal the well-developed sympathetic nervous system is present as in the larva of the common silk-worm, *Bombyx mori* (TANAKA, 1928). However, the present author has observed with difficulty the development of the frontal ganglion of the dorsal sympathetic system.

The suboesophageal ganglion (Pl. IX, Fig. 30a) is a large ganglion situated in the ventroposterior portion of the brain, and is connected with the brain by the short stout circumoesophageal commissures (Pl. X, Fig. 31a). It is also of paired origin, but with the progress of the development both sides fuse into a ganglion (Pl. X, Fig. 31c shows the paired structure). Through the section it is clearly seen that the nerve-cell are densely distributed ventrally under the nerve-fibre. This ganglion gives off three pairs of nerves: the mandibular nerves from its extreme upper anterior corner, the maxillary nerves from its lower anterior corner, at level slightly behind the mandibular nerve, and the labial nerves which arise lateroventrally from the broadest part of the ganglion, farther back than the maxillary nerve. The suboesophageal ganglion narrows posteriorly, and finally gives origin to the two exceedingly slender ventral nerve cords, running parallel very close together backwards into the prothorax.

The ventral nerve cord is also of paired origin. At the thoracic part two slender nerve-cords are seen distinctly, but at the abdomen the cords come in contact with each other so closely as to unite into a single cord. At the prothorax, there is a pair of ganglia called the prothoracic ganglion. However, these paired ganglia become a single pentagonal ganglion by fusing in a later stage. From this ganglion the first crural nerves to the fore legs and to the prothoracic muscles arise. Behind this ganglion there exists the mesothorax. The ventral cords now diverge posteriorly slightly from one another. Then come a pair of large mesothoracic ganglia (Fig. 31c) which are situated at the level of the connection between the fore- and hind-guts. These fuse with one another too, in a later stage and give off the second crural nerves to the second thoracic legs and also to the muscles of the mesothorax. The ganglion in the third thoracic segment is best developed, situated closely behind the second thoracic ganglion and it gives off nerves to the third thoracic legs and to the muscles.

The abdominal ganglia are eight in number, one each located in

the anterior portion of the abdominal segments from the first to the eighth (Pl. X, Figs. 31e, f). The first seven abdominal ganglia are all closely similar in size and structure. Each gives off a pair of strong branching nerves to the segmental muscles of its own segment (Fig. 31f, aga^{5,6}). The eighth abdominal ganglion (Fig. 31f, aga⁸) is much larger than any of the others. It gives off three pairs of strong nerves to the segmental muscles of the posterior three abdominal segments (Fig. 31f, aga⁸ shows two pairs of these nerves).

At the early stage of the formation of all ganglia, the thoracic and abdominal ganglia are closely similar in structure. They are immense in size as compared to the size of the animal, and in addition the nerve-cords are relatively short. The nerve-cells in all thoracic and abdominal ganglia are found to be located mostly ventrally.

At first all the ganglia behind the brain are arranged in one continuous series to the anus. As the development advances and the embryo stretches in length, the foremost mandibular and the following two maxillary ganglia, and also the last three abdominal ganglia still hold the same situation but the other ganglia are separated. Thus the suboesophageal ganglion is formed from the mandibular and two maxillary ganglia, and similarly the large last abdominal ganglion is made from the three posterior abdominal ganglia.

The above findings agree in the main with the accounts of other insects so far studied only in the early stage by others.

2. *Respiratory System*

The respiratory system develops at about the one week stage as a series of invaginations of the lateral ectoderm into the intersomitic part. The anlagen appear at first in the first and second thoracic and first ten abdominal segments, but the second thoracic and the last two posterior invaginations of abdomen stop their development and begin to retrograde before hatching. The opening of each remaining invagination develops into the stigma.

The first prothoracic stigma is situated at the base of the first thoracic legs. The abdominal stigmata lie at the lateral portion of the first eight abdominal segments. They are of an elongated oval form and lie obliquely to the body axis.

The tracheal system in the embryonic stage consists of three parts of main trunks in each body segment, the main stigmatal trunks and the dorsal and ventral transverse tracheae. The tracheal system arises

as three horns of the stigmatal invagination in their early development (Pl. VIII, Fig. 28g, astg⁸ shows the development of the dorsal and ventral transverse tracheae).

The main stigmatal trunks (Pl. X, Fig. 31f, stg) are the strongest of the three, connecting directly with the stigmata. They run along each side of the body, under the stigmata from the eighth abdominal segment as far as the first thoracic segment. The ventral transverse tracheae which run ventrally and connect the stigmata of the two sides, are smaller than the main stigmatal trunk. The dorsal transverse tracheae are distinct in an early stage (Pl. VIII, Fig. 28c), but they do not exceed in size the ventral tracheae in the later stage.

BÜTSCHLI (1870) was the first who worked on the tracheae of the bee. According to him the tracheae arise as paired invaginations of the ectoderm arranged segmentally and these invaginations develop into tracheae in all the thoracic and abdominal segments except the last two pairs. In the embryo of the Lepidoptera, according to HATSCHEK (1877), there are fourteen pairs of stigmata, belonging to the fourteen body segments behind the mouth. However TICHOMIROFF (1879) pointed out the error of HATSCHEK who missed one foremost post-oral segment. In the *Antheraea*, the twelve spiracular invaginations develop at first, of which the meta-thoracic spiracle and the last two posterior pairs of the abdominal spiracles cease to develop in the early stage. The above study on the tusser confirmed the findings by TOYAMA (1902) on *Bombyx*.

3. Circulatory System

The circulatory system of the embryo in the stage of about 20 days is composed of the dorsal vessel and the blood cells. The dorsal vessel in the finished condition is a tube running dorsally along the alimentary canal. The origin of the dorsal vessel has already been described above. It ends at the ninth abdominal segment, where it is widest (Pl. X, Fig. 31e). Forwardly it narrows sharply to a slender tube at the first thoracic segment and penetrates into the head, opening into the haemocoel below the brain (Text-fig. 4 shows the anterior limit of the dorsal vessel which opens at the portion between the two hemispheres of the brain). It closely contacts with the dorsal ectoderm (hypodermis) at the thoracic and abdominal regions at which the hypodermis is slightly invaginated along the line of contact (Pl. X, Figs. 31c-f). The broad dorsal vessel is nothing else than the heart, and the narrow anterior

portion is the aorta. The heart proper seemingly is not different from the aorta in the embryonic stage, except for its great width. However the aorta originates from the antennal mesoderm: the antennal mesoderm divides into two parts, of which the outer enters into the ectodermal evagination for the appendage and the inner passes dorsally into the part between the dorsal hypodermis and the stomodaeum. As in the formation of the heart, the antennary mesodermal cells of the splanchnic mesoblasts form a tube above the stomodaeum and give rise to the aorta.

It has already been described how the blood cells are differentiated from the cells liberated from the inner surface of the whole mesoderm into the yolk. They are all one kind and regularly almost spherical in form with spherical nuclei. CHOLODKOVSKY (1891) was quite uncertain concerning the origin of the blood formation in *Blatta*, pointing out in some place the derivation of the blood from the yolk cells, while in other case asserting a mesodermal origin. The origin of the blood cell from the yolk cells which are destined to degenerate is out of the question.

As to the antennal mesoderm origin of the aorta, the above described study agrees with those of most researchers, such as WIESMANN (1926) on *Carausius* and EASTHAM (1930) on *Pieris*, etc. According to EASTHAM the aorta has also an opening to the body cavity in front of the heart instead of connecting directly with it. But the present author failed to find such a construction in *Antheraea*.

4. Excretory System

The principal organ of excretion consists of the Malpighian tube and the fat-bodies. For the sake of convenience, the development of the latter organ will be discussed later together with the differentiation of the muscle. It was explained already that the Malpighian tube is of ectodermal origin. It is a paired organ each part consisting of three tubules which open with a common duct in the proctodaeum at the antero-lateral side. The structure of a tubule is very simple, being a slender, elongated blind tube, penetrating freely into the haemocoel. At the beginning of the development they extend posteriorly along the ventro-lateral wall of the hind-gut (Pl. VIII, Figs. 28d, e, Pl. IX, Figs. 30g, h). The embryo at this stage increases in body length and the formation of the mid-gut is brought about. On the other hand the contraction of the segments by telescoping occurs at the posterior body region. This, on the contrary pushes the hind-gut towards the posterior part of the body so as to cause the Malpighian tubules to direct anteriorly.

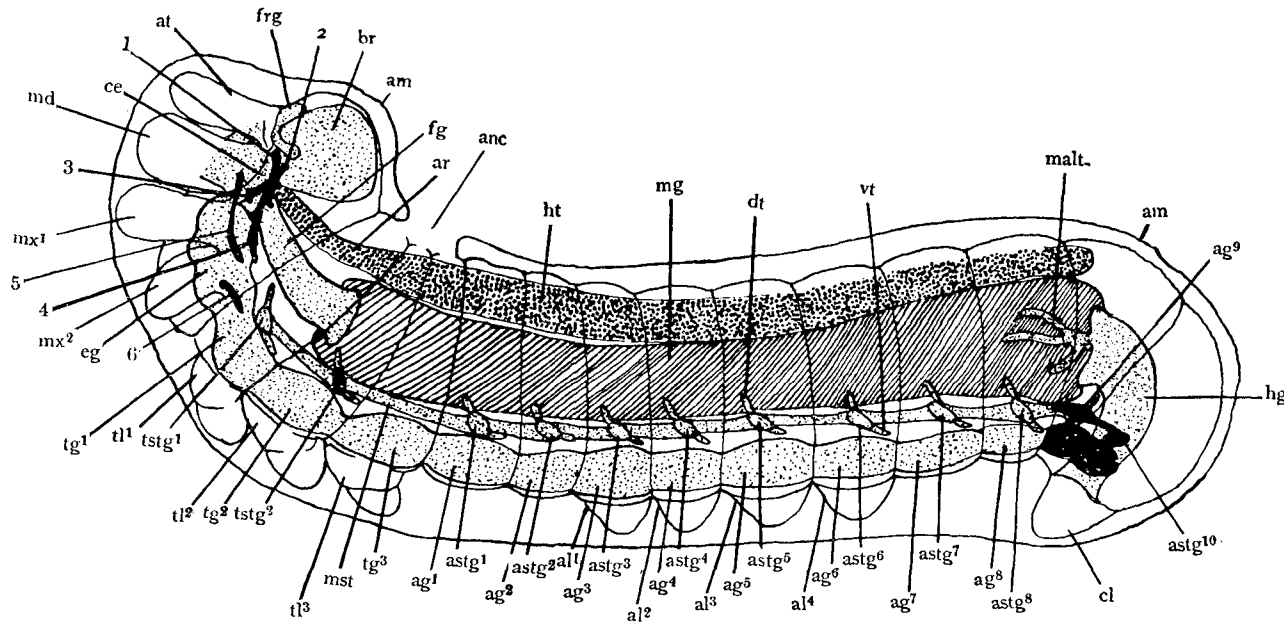
Then they extend anteriorly and the common duct swells out to become the so-called urinary bladder. The ventral tubule runs forward alongside of the gut to the region of the third abdominal segment, the lateral one attains to the fourth abdominal segment and the dorsal one remains slightly shorter than the lateral. Each tubule turns back running further than its starting point. The tubules are all of approximately the same diameter throughout, and uniform in structure. Each is shown in section to be composed of a ring of five or six cells which are in general appearance and in staining affinity much the same as those of the tracheal system.

NAKATA (1935) recently published a paper on the development of the Malpighian tube in *Bombyx* in the Lepidoptera. The above findings agree in the main with his results, but only in a minor detail does his account differ a little. According to his description, the Malpighian tube develops on each side as three separate outgrowths of the ventrolateral wall of the blind end of the proctodaeum, and just before the revolution of the embryo, the proximal ends of three tubules at each side gather together to form a common tube. In *Antheraea* however the appearance of the common duct occurs in a much earlier stage.

5. Alimentary Canal

The early development of the alimentary canal has already been mentioned. In the later stage the fore-gut narrows suddenly in diameter at the pharynx, and passes between the circumoesophageal commissures (Pl. X, Fig. 31a). Immediately behind the circumoesophageal commissure and at the anterior part of the second thoracic segment (Pl. X, Fig. 31c), it continues to the mid-gut. The wall of the fore-gut is composed of a single layer of small epithelial cells.

The mid-gut is of large diameter becoming gradually larger as it extends posteriorly and it runs almost straight. Its posterior end is still completely closed as the anterior blind end of the hind-gut inserts there. The wall is very thick, consisting of a layer of epithelial cells of rather large size covered externally with a thick muscle layer (Pl. X, Figs. 31e-f) derived from the splanchnopleure of mesoderm. The point of junction with the hind-gut removes posteriorly with the advance of development, as the mid-gut always increases in length while the growth of the hind-gut ceases in an early stage. In the about two weeks old embryo the junction is situated at the sixth abdominal segment (Pl. IX, Figs. 29, 30g), but it removes posteriorly to the ninth segment on the



Text-fig. 3. A diagram showing the various organs in the stage just before the revolution of the embryo in the egg. The spotted organs are of ectodermal origin, the meshed portions are derived from the mesoderm and the shaded parts show the organs originating from the endoderm. The second thoracic (tstg²) and the ninth and the tenth abdominal stigmata (astg¹⁰) are smeared to show that they degenerate with the advance of the development. The black ninth (ag⁹) and tenth abdominal ganglia fuse with the eighth ganglion in the future.

ag¹⁻⁹, first to ninth abdominal ganglia; al¹⁻⁴, first to fourth abdominal legs; am, amnion; anc, umbilical canal; ar, aorta; astg¹⁻¹⁰, first to tenth abdominal stigmata; at, antenna; br, brain; ce, circumoesophageal commissure; cl, caudal leg; dt, dorsal trunk of the respiratory system; eg, subesophageal ganglion; fg, fore-gut; frg, frontal ganglion; hg, hind-gut; ht, heart; malt, Malpighian tube; md, mandible; mg, mid-gut; mst, main stigmatal trunk; mx^{1,2}, first and second maxillae; tg¹⁻³, first to third thoracic ganglia; tl¹⁻³, first to third thoracic legs; tstg^{1,2}, first and second thoracic stigmata; vt, ventral trunk of the respiratory system; 1-6, first to sixth invaginations at the head, which develop to the endoskeletons and glands in this region.

twentieth day (Pl. X, Fig. 31f).

The hind-gut is widened anteriorly and receives there the Malpighian tubes. It runs straight from the end of the mid-gut to the anus which is situated between the caudal legs. The hind-gut wall is lined with a single layer of small cells. The Malpighian tubes open at the anterior end of hind-gut in a pair of common ducts. In the early stage of development there is no direct connection between the fore-gut and mid-gut or between the mid-gut and hind-gut. They are separated from each other by a single membrane which will be broken soon after taking up of food. SELVATICO (1881) stated apropos in *Bombyx mori* that the mid-gut is enclosed by its own wall attached anterior to the fore-gut wall and posterior to the hind-gut wall. Therefore according to SELVATICO there are two separating membranes between the guts. However other observers who have investigated this or other species of Lepidoptera have not agreed with this.

6. Development of the Head

The morphological constitution of the head part of an insect is generally very complicated and there is no other way than by embryological studies to settle the question. This proposes a very interesting morphological subject. In 1902 TOYAMA described in detail the development of the endoskeleton and the various organs in the head on the common silk-worm, *Bombyx*, but he has not touched upon the question proper. In 1930 EASTHAM on *Pieris* attempted to settle this question by the embryological observation of every organ in the head with a certain degree of success. In this case the study of segmental structure of the embryo is of primary importance. The appendages, mesoblastic somites and neuromeres serve as the indicators of segments.

At first the mesoderm-cells at the anterior end make a large mass differing from that at the hinder region, of which the first three pairs of mesoblastic somites behind this large mass are those of the mandibular, maxillar and labial segments. The mesodermic cell-mass differentiates gradually into somitic structure. At first it is divided into an anterior small mass and the posterior large one. Then the posterior mass splits further into three somites, while the anterior makes an irregular somite which enters later into the labral evagination and becomes the muscle cells. By this time the stomodaeum develops perforating the posterior mass. Of the three posterior head somites the last one is situated just in front of the somite of the mandible; the anteriormost one which is

the largest, becomes a clear compact somite at the base of the antenna while the middle one becomes insignificant.

Working on *Pieris* EASTHAM (1930) stated that the middle one represents the anlage of the premandibular appendage which is the temporary foetal organ. In *Antheraea* the present writer failed to observe this appendage.

WIESMANN (1926) said in the study of the problem in *Carausius morosus* Br. in conjunction with H. LEUZINGER and F. E. LEHMANN that in the head all segments are formed from clear somites out of the cephalic mesoderm. EASTHAM (1930) who is the most recent worker on this question, finds in *Pieris* that the cephalic mesoderm is first divided into an anterior and a posterior part, and then the anterior part passes into the labral lobes, while the posterior remains just on the dorsal surface of the epipharynx instead of surrounding the stomodaeum. As to the origin of the head mesoderm the just-named author says that it develops from the proliferated mass of cells called the anterior mesenteron rudiment which has direct connection with the stomodaeum.

It has been mentioned that the tubular ingrowths of ectoderm appear at the intersomitic parts of the head and become the origin of the endoskeleton. At first they are the simple ectodermal invaginations behind each appendage of the head. The first invagination arises at the posterior part of the antenna (Pl. VII, Fig. 28a), and goes backwards as far as the posterior end of the pharynx to meet the prolongation of the invagination from the antero-lateral part of the second maxilla. From that junction of the invaginations a transverse process appears across the anterior end of the oesophagus, and above the suboesophageal ganglion to connect the two sides. The second invagination arises at the outer part of the first one and terminates on the body-wall making a curved canal. The third one starts from behind the mandible and goes backwards along the stomodaeum for a short distance. Then it turns outwards to meet the second under the brain (Pl. X, Fig. 31a shows this connection). The fourth is the mandibular gland which later becomes the salivary gland. The fourth invagination arises behind the mandible, and goes straight back parallel with the oesophagus. It has a free terminal at the level of the posterior end of the fore-gut in the second thoracic segment (Fig. 31c). The fifth invagination arises between the first and second maxillae and goes forward to join the posterior prolongation of the first invagination (Pl. X, Fig. 31a). The sixth opens

on the labium (Fig. 31a) going inward and backward; it dichotomizes immediately at the point where the circumoesophageal commissure from the suboesophageal ganglion joins with the brain. These dichotomized tubes pass backward through the outer part of the salivary glands and turn abruptly forward at the anterior border of the first thoracic segment, but they return after a while to the original direction (Fig. 31b). They go back with the windings on both lateral parts of the gut. The sixth converts into the silk-gland after the fusion of the two sides at the bases of the both second maxillae.

The finding on the development of the internal skeleton and the derivated glands in the head agrees in the main with those of TOYAMA (1902) on *Bombyx* and of EASTHAM (1930) on *Pieris*. The present writer failed to find the maxillary gland which is called the labial or hypostigmatic gland by TOYAMA. Although there is general agreement among the investigators, in the study of the number and the fate of the invaginations, there is considerable divergence of opinion regarding the proper position of development of each invagination. The writer supports EASTHAM who is of the opinion that the differences among the workers may be due to the findings on different stages, or otherwise, they overlooked the changing situation of the invagination during the development.

7. Muscular System

Soon after the external segmentation of the embryo appears, the somite formation occurs in the mesoderm. In each somite the cells arrange in radial order and then the coelomic cavity appears in the center. On the other hand, the out-growth of limb-buds is formed externally. At the first stage the coelomic cavity is a thick-walled lengthened ellipsoid hollow in transverse section (Pl. V, Fig. 15g), while in the sagittal section, the cavity extends antero-interiorly far into the intersegmental regions. There is no connection yet between the adjoining ones (Fig. 15h). For the sake of convenience it is proposed to distinguish the extended part as sub-somitic against the proper somitic mesoderm in each somite. In the work on *Pieris*, EASTHAM (1930) discriminates one other portion, the median mesoderm which lies above the neural groove continuous along the whole length, forming a connecting link between the somites of both sides. According to him this median mass becomes a formative center of blood-cells. In *Antheraea*, the mesoderm corresponding to his median mesoderm does not exist. At least,

there is no connection between the somites of the two sides as is shown in the figures (Pl. V, Figs. 15d-f), and moreover, the blood-cells in this case originate from the inner surface of the mesoderm as has already been mentioned.

As the limb-bud develops, a great part of the subsomitic mesoderm passes into the ectodermal hollow of the limb and gives rise to the muscle (Fig. 15g). Later the mesoderm cells which have entered into the ectodermal hollow of the limb arrange themselves in the curved bands which pass outwards from the inner part of the limb to the body wall, and supply the anlage of muscles to that part. A part of the somitic mesoderm gives rise to the ventro-longitudinal muscles of the body wall (Pl. VIII, Fig. 28b).

The somitic mesoderm, as development proceeds, loses the radial arrangement of the cells at its ventral wall, and forms a flowing mass of irregularly shaped cells (Pl. VIII, Fig. 28b). These cells become the primordia of the fat body. After the differentiation of the cells of the ventral wall of the somatic part of the somites into the fat body, the somitic mesoderm in transverse section shows Y-shaped (Fig. 28b). The outer layer of the remaining part of the somite differentiates into the dorso-lateral and dorso-longitudinal muscle of the body-wall. On the other hand, the inner or splanchnic part of the mesoderm, with the development of the endoderm strand, takes nearly a horizontal position so as to cover the endoderm (Pl. V, Fig. 15c), and gives rise later to the visceral muscles. However, as described already, the dorsal terminal part of the splanchnic part takes part in the formation of the heart.

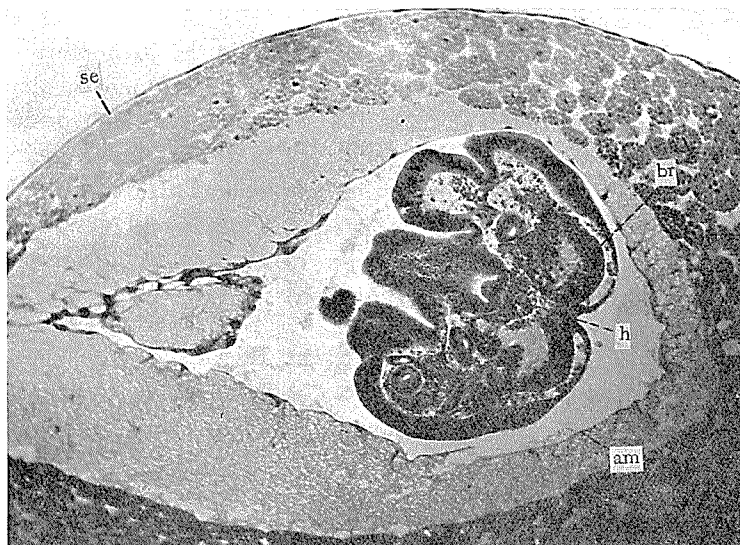
E. POSITION OF THE EMBRYO IN THE EGG

The revolution of the embryo in the egg during the development has been described briefly in the previous paper, and at present special attention is given to explain the relation of the developing body form to the position in the egg.

The first sign of the tussek-embryo is a slight thickening of the blastoderm of the ventral surface of the egg. This thickening or precursor of the ventral plate is caused by the partial cell-proliferation of the blastoderm. This plate becomes a very distinct and somewhat trapezoid-shaped patch occupying almost the entire ventral surface of the egg. By the upgrowth of its antero-lateral parts, there occurs the differentiation of the procephalic lobes in the ventral plate. Then the primitive groove extends backwards along the mid-longitudinal line of

the plate. At the time when the two procephalic lobes are very distinctly formed in the early blastoderm the ventral plate sinks into the yolk by the formation of the amnion and serosa. Then the elongated blastoderm begins very soon to segment making slight transverse constrictions, indicating the future body-segments. It is noted that, at this time the embryo has so strong a dorsal curvature that the posterior end lies on the dorsal side of the egg opposite to the anterior part of blastoderm (the ventral plate). Then on both sides of the median line in the embryo, each segment swells up to form the first anlage of the appendage. At first, three pairs of swellings become distinct at the anterior part, and thus the rudiments of the appendages of the mouth part are made. Next, three very similar swellings make their appearance, destined to become the future thoracic legs. At the same time, from the cephalic lobes a pair of swellings from the ventral part arise to form the antennae, and just in front of them another paired swelling, that of the labrum anlage, appears. The abdominal appendages also begin to develop and both the stomodaeal and the proctodaeal invaginations take place clearly. On the other hand, following the constriction of the body segment there occurs an important inner change, namely the somites are formed definitely. At this time the embryo attains to the maximum body-length (Pl. V, Fig. 14). Then the reduction of the body-length takes place by the telescoping of the body-segments and the fusion of some abdominal segments. During this process, the embryo-head is situated constantly near the micropyle, and consequently, the whole embryo assumes the position again at the ventral side of the egg, bringing back the posterior body end to the posterior pole of the egg as before (Pl. VIII, Fig. 27). The dorsal flexure of the embryo decreases, and the space between the serosa and amnion is gradually narrowed, in which many yolk granules were found at the previous stage. Naturally the proctodaeum changes its orientation to indicate the direct rear of the embryo (Compare Pl. V, Fig. 14 with Pl. VII, Fig. 27).

All the appendages are formed definitely and the dorsal body-wall in the posterior portion is formed completely by the fusion of the growing lateral walls. The still existing dorsal curvature decreases by degrees, and at last the terminal segment bends ventrally (Pl. IX, Fig. 29). As the development proceeds, the amount of the yolk decreases in the neighbourhood of the embryo, and this is accompanied by the gradual transfer of the embryo towards the center of the egg (Text-fig. 4). The terminal abdominal segment now turns forward from



Text-fig. 4. A cross section of an embryo removed into the yolk preparing for the revolution. Compare this figure with the figures in Plates VIII and X. $\times 60$. am, amnion; br, brain; h, heart; se, serosa.

behind, growing forward. The whole embryo becomes finally to bend ventrally, and removes gradually towards the opposite side of the egg with the consumption of the yolk. Thus the embryo at the final stage takes a quite opposite position in the egg (Pl. X). During this development the embryo lies constantly on the same geometrical plane containing the egg axis, while the position of the head is fixed. The development of the dorsal body wall begins from both the body-ends and proceeds towards the central thoracic region (Pl. X).

At last the dorsal wall of the second thoracic segment remains open. The yolk outside of the embryo-body is in direct connection with the mid-gut through this opening. Thus sometimes the opening is erroneously called the umbilical canal.

Among the studies on the revolution of the embryo of the Lepidoptera, there is a very recent work by JOHANNSEN (1929) on *Diacrisia virginica*. According to him the revolution in his case occurs in a more complicated way; the rotation takes place about the longitudinal axis of the embryo until the ventral surface is directed towards the center of the egg. Formerly GRABER (1890) made an interesting discovery that the Phytophagous Hymenoptera closely resembles the Lepidoptera

in the displacement of the embryo and in the amputation of the envelopes. The revolution of *Xiphidium* embryo in the Orthoptera studied by WHEELER (1896) differs greatly; the germ band moves along the margin making an arc till it takes the completely opposite position on the ventral surface of the egg, then it rests, and next turns back to the original position passing through the same route. He compared these movements to the single vibration of a pendulum and designated the ascending movement as anatrepsis, the descending as katatrepsis, the intervening resting stage as the diapause.

F. EMBRYO JUST BEFORE HATCHING

The embryo just before the hatching is about 0.6 cm. in body length, occupying the entire inside of the egg, bending at the seventh body-segment. It appears to have the same construction as the finished larva, consisting of a head and thirteen post-cephalic segments, of which the last is the largest while the second and third are the broadest. From the third post-cephalic segment the body becomes thin slightly towards the caudal extremity.

The head is brownish yellow in colour, bordered by a faint constriction with the next segment. It is rather large in comparison with the body size, furnished with some bristles. Although the mouth parts are still rudimentary, they are complete in constitution. One pair of antennary papillae is found on the anterior convex surface of the head. On the ventral surface of the head there is the relatively large opening of the mouth, on the anterior border of which there are lobe-like labrum and on the posterior the labium. The weak mandibles lie on both sides of the mouth opening. The maxillae are represented by cylindrical lobes.

The segments behind the head are sepial yellow in colour, and each segment is ornamented with white warts which are furnished with tufts of hairs. The next table shows the position of the warts and the number of their bristles.

The epithelium of the pharynx and oesophagus which is formed by the invagination of the ectoderm, is continuous with the external hypodermis and is covered with a fine chitinous lining or intima, which corresponds to the cuticle of the hypodermis. The epithelium of the hind-gut possesses also a fine internal chitinous intima. The mouth and the hind-gut are connected by the mid-gut or mesenteron formed from the endoderm. The boundary between the mid-gut and the hind-

	Thoracic segments			Abdominal segments									
	I	II	III	1	2	3	4	5	6	7	8	9	10
Subdorsal line	9-11	7-9	5-6	6-9	8-11	7-9	5-7	5-8	5-7	9-11	5-6	4-5	7-11
Supraspiracular line	—	4	5-6	5-6	5	5-6	6-7	3-5	3-4	3-4	3-4	5-6	—
Spiracular line	8-11	—	—	—	—	—	—	—	—	—	—	—	—
Subspiracular line	—	8-9	8-11	5-7	7-8	7-8	6-8	7-9	7-8	7-8	6-7	7-9	—
Basal line	3-4	2-3	2-3	—	—	—	—	—	—	—	—	—	—

gut is marked by the entry of the Malpighian tubes. The relative length of these three parts of the alimentary canal at the last stage differs more or less with the individuals, but generally the mid-gut extends from the second thoracic to the ninth abdominal segment.

The salivary glands exist close to the pharynx which has become distinct, and open to the mouth cavity at the bases of the two mandibles. The Malpighian tubules at the last stage are very simple in structure. They are a slender and very elongated tube, lying closely to the surface of the mid-gut. The posterior end is blind and attains to the middle part of the hind-gut. The whole tubules are bathed with blood.

The nervous system of this stage assumes the constitution of that of the larval stage, but it is relatively of immense size considering the size of the embryo. The nerve cords do not appear between the successive ganglia, especially, the abdominal ganglia touch each other like a row of flat beads. However, at the thoracic region, the nerve cords develop and at the same time ganglia become relatively small in comparison with those of the early stage.

Of the circulatory system the differentiation of the heart and the aorta becomes clear; that is, the anterior part of the dorsal vessel in the region before the prethoracic segment becomes a very slender and straight tube giving rise to the aorta, while the other part of the vessel remains the same in size to become the heart. The blood, the lymph-like fluid, increases in amount and in it the discoid-shaped or sometimes ovoid corpuscles are scattered and floating.

In the respiratory system, the paired longitudinal tracheal trunks becomes more marked than the other tracheae, and from them a number of branches arise at each body segment. These branches divide again and again into the fine tracheae known as capillaries. A trachea as seen in transverse section is formed of two layers, an outer ectotrachea

and an inner endotrachea. The former is a cellular layer corresponding to the hypodermis, consisting of flattened and hexagonal cells, possessing oval nuclei which often bulge out from the contour of the smaller tracheae. The latter is a chitinous non-cellular layer corresponding to the cuticle.

In brief, the embryo just before hatching has the complete organization of the larva, apart from the incompleteness in the development of the nerve-cords at the abdominal region and the large sized embryonic head.

III. Discussion

A. GENERAL ASPECTS OF INSECT EMBRYOLOGY

Having described precisely the development of the tusser in the above, those observations should be compared with those obtained by the other investigators in the field of insect comparative embryology.

As to the blastoderm formation, the manner of arrangement of the segmentation nucleus has been observed by almost every embryologist. KÖLLIKER (1842) observed a blastoderm formed surrounding the egg yolk in *Chironomus*, *Simulia* and *Donacia*, stating that the nucleated cells of the peripheral layer increase in number, and a structure of a single cell-layer in thickness finally is completed over the egg-surface. BURMEISTER (1848), studying on *Palingenia*, found a cell-layer of unequal thickness on the surface of the egg. In ZADDACH's excellent paper (1854) on the development of the Phryganids, he described the "Keimhautblastem" forming in a peculiar way, that is, a number of "Flecke" appears in the fine granulated peripheral layer, which are the first blastoderm cells; these are nucleated and surround the egg in a continuous single layer. He said also that these blastoderm cells cover the ventral surface of the egg with the advance of development, but they disappear gradually at the dorsal surface. HUXLEY (1857) gives a figure in a paper on an *Aphis*'s egg showing a peripheral layer of large nuclei on one side of the egg, which are according to him the nuclei of the blastoderm cells from the early nucleus of the egg (first segmentation nucleus). BOBRETZKY (1878) working on *Porthesia* (Lepidoptera) observed two bodies in the egg at the earliest stage, each surrounded by a stellate prolongation of cytoplasm. They begin to divide, but the preponderating amount of yolk prevents the egg from segmenting at the same time. By a continuous division, the nuclei increase in number

and they are scattered in the interior of the ovum. Then some of them pass to the surface simultaneously and at the egg surface the protoplasm surrounding each nucleus contracts to become a rounded cell body, distinctly separated from the yolk.

Studying the development of the Diptera, WEISMANN (1863) noticed the clear peripheral cell-layer covering the egg as observed by others, and called it the "Keimhautblastem." According to him the blastoderm cells appear first by a process of free cell formation in the yolk. The nuclei appear first, and then are surrounded by a small amount of protoplasm to form the cells, which are round in shape at first, and become prism-like later. Then the cells are brought to the surface of the egg. According to him another cell layer, the so-called "inner Keimhautblastem" is formed inside of the above blastoderm, perhaps supplying nourishment. However it degenerates gradually and finally disappears. The writer believes that WEISMANN's "inner Keimhautblastem" is nothing but the germ-cells which have been observed by many recent investigators. KUPFFER (1886) working on the development of *Chironomus* agrees with WEISMANN regarding the free cell formation in the yolk. A little different type was observed by KOWALEVSKY (1886) in *Apis*, in which the development commences with the appearance of a number of protoplasmic prominences, each forming a cell already at the margin of the yolk, separated from each other by short intervals. Shortly after a second group of cell bodies appears and intervenes between the first. DU BOIS (1933) observed that the egg of *Sciara* in Diptera shows a typical blastoderm stage at about eight hours after the oviposition, a single layer of small cylinder-shaped cells surrounding the yolk mass.

METSCHNIKOFF (1866) found on *Simulia* that the blastoderm covers the entire egg with the same thickness excepting the posterior pole which is much thicker. This appears as the result of lengthening of the posterior blastoderm cells. In *Cecidomyia* (Diptera) according to the same author, a single nucleus was first noticed within the egg, which soon divides repeatedly. The nuclei thus formed pass to the periphery where cells are first formed collecting the protoplasm. The cells are at first spherical, but later become cylindrical. In the study on the viviparous aphids he also found a single nucleus in the egg at the first stage.

Regarding the above, METSCHNIKOFF was of different opinion from WEISMANN who insisted on the free cell formation in the yolk at the

first stage of development. After METSCHNIKOFF the cleavage nuclei were formed and later they gave rise to the blastoderm cells at the periphery of the egg.

It has long been a question whether the division of the segmentation nucleus occurs by mitosis or by amitosis. WHEELER (1889) found the mitotic division (his fig. 23) in the egg of *Phyllodromia* (*Blatta germanica*), and HEYMONS (1897) confirmed this in the study on the same material. Moreover, HEYMONS described the mitotic type in the *Forficula* and *Gryllus*. On the contrary, STRINDBERG (1915) showed the amitotic division in the egg of *Eutermes* of which the nucleus takes the lobe-form. Nevertheless he mentioned the mitotic division in the study on *emmet* (1913). SCHWARTZE (1899) and SCHWANGART (1904) asserted the amitotic view in *Lepidoptera*, and KAHLE (1908) also in *Miaster*. HIRSCHLER mentioned in the work on *Aphis* (1912), that at least the fertilized egg in summer presents the mitotic division, and he later (1924) comes again to the same conclusion from his study on *Musca*. In 1929 JOHANNSEN on *Diacrisia virginica* (*Lepidoptera*) noted that there are no nuclei undergoing mitosis in the increase of the yolk cells.

In the blastoderm formation of the tusser, at first the nuclei surrounded by amoebiform cytoplasm increase to 60-80 in number by successive divisions, and assume a spherical arrangement at the micropylar part. Then by the expansion of this sphere, the nuclei reach the egg surface. There they multiply and form a cover of nuclei with the constant intervention of the segmented nuclei. Here the complete cells are visible having a distinct boundary between them. Accordingly the present writer can not accept WEISMANN's free cell formation theory in the segmentation. On this matter further considerations will be offered in the conclusion. In the tusser at least, amitotic division is seen insofar as the division of the vitellophages is concerned, but the increase of the blastomere is carried out only by mitosis.

The place of the first appearance of the blastoderm differs according to the materials. HEIDER (1885, 1889) who worked on *Hydrophilus* observed that it first begins to differentiate in the middle part of the egg and then extends to both poles. A similar result was obtained by WHEELER (1889) on *Doryphora*, by SALING (1907) on *Tenebrio* and also by LECAILLON (1897) on *Lina*, *Clythra*, *Gasterophysa* and *Agelestica*. In the study on *Musca* (*Diptera*), WEISMANN (1863) describes the "Keimhautblastem" as appearing first at the anterior pole of the egg and spreading over the entire surface. NUSBAUM (1888) studying with

Meloe (Coleoptera), on the other hand, observed the blastoderm being formed on the hind pole of the egg at first and then extending to the whole surface. HIRSCHLER (1909) described the other mode on *Donacia* (Coleoptera); "Zuerst entwickelt es sich an der Ventralseite in der Aequatorialzone, später verbreitet gleichzeitig auch in den seitlichen und dorsalen Partien der Aequatorialzone, zuletzt aber kommt es zum Vorschein an der Dorsalseite in der Nähe bei der Pole". In Orthoptera HEYMONS (1897) wrote on *Gryllus* and *Periplaneta* as follows:—"....beginnt seine Bildung an hinteren Pole und setzt sich von dort nach vorn fort. Die Epithelzellen liegen anfangs in weiten Abständen voneinander und senden pseudopodienartige Fortsätze aus, die sich auf der Oberfläche des Eies ausbreiten." Moreover, he has stated on *Gryllotalpa* "....nur an der Ventralseite des Eies und lässt die ganze Dorsalseite des Eies anbedecken,," GANIN (1869) observed in Hymenoptera that the blastoderm cells arise first at the posterior pole and then spread. In this case, the cells at the pole are the largest, while at the dorsal the smallest. According to CARRIÈRE (1890) who worked on *Chalicodoma* belonging to Hymenoptera, the blastoderm covers at first the median part, then the anterior pole and at last the posterior pole. On *Formica* of the hymenopteron insects, STRINDBERG (1915) confirms CARRIÈRE's observation, while he obtained the following result on *Vespa* (Hymenoptera) (1915); "zuerst am micropylaren Pole erscheinen und erst hernach in den übrigen Teilen der Eioberfläche auftauchen." HOFFMANN (1913) mentioned briefly as follows on *Xenos* in Strepsiptera; "....nicht an der ganzen Eioberfläche, indem ein Eipol umbedeckt von ihm bleibt...."

As to Lepidoptera insects, in 1924, JOHANNSEN was of a different opinion studying on *Diacrisia*. He describes how the cells destined to form the serosa of the embryo occupy the dorsal peripheral part a little earlier than those which are to form the blastoderm on the ventral side. In *Antheraea*, as mentioned above, the blastoderm is formed at first at micropylar pole, next it extends towards the ventral side, then towards the dorsal side and at last reaches to the obtuse pole.

Considering from the above it may be that the type of the blastoderm formation differs not only with the different orders, but also with the species. However it is quite clear that the type seems to be determined by the form of the egg. Generally in the egg of spherical or fairly oval shape, the blastoderm is formed on the entire surface simultaneously, but in the rather long egg there exists a developing center of the

formation, from where it gradually extends to the other parts. It is interesting to note that the center above mentioned becomes the head part of the future embryo.

The confirmation on the origin of the so-called yolk cell of *Antheraea* coincides with most of those of other insects. It was that in the blastoderm formation all nuclei do not reach to the surface of the egg, but a small number remain in the yolk to become the yolk cells. DU BOIS (1933) insisted upon a different origin of this cell in *Sciara*, in which the germ-cells differentiate from the somatic cells in the blastodermic stage. According to him, all the nuclei of the somatic group reach to the surface of the egg, and then the several nuclei separate themselves from there, penetrating into the yolk. These become the yolk cells. No other, similar observation has yet been made.

Considering the origin of the mesoderm which has long been disputed by many investigators on the insects in almost every order, the present remarks will be confined mainly to the Lepidoptera with reference to the other orders when necessary. So far as the writer is aware, KOWALEVSKY (1871) is the first to make observations on the formation of the mesoderm of Lepidoptera. He mentioned that the mesoderm in *Sphinx populi* was formed by the overgrowth of both lateral portions on the middle portion of the ventral plate; this is a similar mode to that observed at the post-thoracic part of our material. BOBRETZKY (1878) states in his study on *Pieris crataegi* and *Porthesia chrysorrhoea* that the formation occurs by an inward growth of cells from the bottom of the primitive groove, that is, by a proliferation of individual cells. According to him the mesoderm formation in the Lepidoptera takes place later than in the other orders, appearing after the formation of the embryonic envelopes, the amnion and serosa. The above statement is also valid in our case of *Antheraea* insofar as the time of formation is concerned, but the proliferation of individual cells is out of the question in such higher insects as his material. TICHOMIROFF (1882, 1891) observed the formation of mesoderm by true invagination at the anterior end, and by the inward growth of cells at the median part of the primitive furrow. In 1887 BRUCE also shows the inward growth on *Thyridopteryx* at the beginning of the mesoderm formation. In 1884, PATTEN described, on the Phryganid *Neophylax*, how a middle portion of the blastoderm which is destined to develop into the mesoderm is first differentiated *in situ* from the lateral part and this latter encloses the former, overgrowing to form two layers, his upper and lower layers.

GRABER (1890) noticed on *Pieris* the overgrowth of lateral parts on the median part which become mesoderm invaginating toward the yolk. SCHWARTZE (1899) concludes that the process differs in different regions of the embryo, saying, "durch Einsenkung eines Rohres, bald durch Zellwucherung von Boden einer Rinne aus, bald durch seitliche Ueberschiebung". JOHANNSEN said his study on *Diacrisia* in 1929, that the gastrula tube meaning the primitive groove of the others, is not formed, but instead, there is formed a longitudinal cell cord as seen in the embryo of the honey bee. TOYAMA (1902) and SCHWANGART (1904) observed the different type of mesoderm formation in the different regions of the embryo. In 1927 and 1930 EASTHAM pointed out in his studies on *Pieris* that SCHWARTZE, TOYAMA and SCHWANGART had considered the different stage of the same development process as different types. He stated that the blastoderm of uniform thickness first proliferates loose cells from its median portion and then the middle part slightly invaginates. The writer can not accept the views of SCHWARTZE, TOYAMA and SCHWANGART nor that of EASTHAM. In the present writer's material, the formation of the mesoderm occurs by means of the invagination of the longitudinal median part of the blastoderm clearly at the small anterior part while indistinctly at the posterior.

Now regarding the formation of mesoderm we consider the other orders of insects for the comparison. According to STRINDBERG (1913), in a coleopterous embryo there commonly occurs a longitudinal invagination of the middle part of the germ band to form a tube, which is similar to that found in Lepidoptera. Then the invaginated elements break down into an irregular layer of cells between the ectoderm and the yolk and become the mesoderm. A similar invagination process was found by PATTEN (1884) in Trichoptera.

On the other hand, the process of overgrowth of the lateral portions on the median longitudinal part of the blastoderm instead of invagination is described by NELSON (1911) in honey bee in Hymenoptera; the two lateral parts become the upper layer (ectoderm) and the middle enclosed part the lower layer (mesoderm).

In Diptera, NOACK (1901) gives an account of the mesoderm formation by the invagination of the middle portion of the blastoderm. He called this invaginated tissue mesentoderm because later the endoderm is derived from the two ends of that tissue as the other authors, such as BÜTSCHLI (1888), VOELTZKOW (1888), RITTER (1890) etc. stated. DU BOIS (1932) described on *Sciara* (Diptera) the formation of the

mesoderm by means of true invagination; "along the median longitudinal line of the germinal streak (in *Antheraea* the formation of the so-called germinal streak does not occur), a groove appears, which rapidly grows deeper, while its two ridges draw nearer and finally join, forming a tube, but soon the cavity of the tube disappears by flattening." On the mode of mesentoderm formation, the writer will discuss thoroughly in the part treating endoderm formation.

In other insect orders, Orthoptera has been mostly studied with regard to the origin of the mesoderm. BRUCE (1887) was the first who described the median growth of the germ band as the origin of the mesentoderm in the Orthoptera, with rather unsatisfactory descriptions and figures. According to the description of the above author on *Stagnomantis carolina*, "When the union of the folds (so-called amniotic fold) is effected and the embryo is separated from the surface and is covered ventrally by the amnion, the under layer is formed, as in *Meloe* and *Thyridopteryx* as an ingrowth from the median line of the embryo." In 1888 and 1890 GRABER stated the mesoderm formation as having resulted from a true invagination in *Stenobothrus variabilis*. In his later paper (1890) on the same insect, the account was briefly repeated without any important addition. Neither KOROTNEFF in his study on *Gryllotalpa* (1885) nor AYERS in his study on *Oecanthus* (1884), succeeded in finding the invagination. WHEELER in 1893, in his "Contribution to insect embryology" devoted considerable space to a review of this question. His conclusion is that "in all the families of the order (Orthoptera), save the Phasmidae, an invaginate gastrula has been found, and there can be little doubt that the investigator who is so fortunate as to study embryo of this family will find in them essentially the same process of germ layer formation. The view is now pretty generally held that in the insects both mesoderm and endoderm arise from a median longitudinal furrow" (the mesoderm throughout nearly the entire length, the endoderm only in the oral and anal regions of the germ band). In 1894, HEYMONS concluded in the Orthoptera and Dermaptera, that there is no true invagination process but the under layer (mesoderm) arises from all parts of the embryonic area. Moreover he attained to the conclusion that the layer which is generally known as the mesentoderm is in reality only mesoderm, and the endoderm appears relatively late and arises from the ectodermal bottoms of the stomodaeal and proctodaeal invaginations. H. KNOWER (1900) in his embryological study of the termite, stated that the origin of the

mesoderm and endoderm is a collection of cells diffusing from the ectoderm by means of true gastrulation. His result agrees with HEYMON's conclusion insofar as the origin of the mesoderm is concerned, and on the other hand it agrees with WHEELER in the interpretation of the invaginated groove.

From the above it may be seen clearly that the different types of mesoderm formation so far observed in various insects belong eventually to the same category of developmental law which shows a considerable variety according to the phylogenetic order. Thus the ingrowing loose cells formed in Orthoptera show the first step of the primitive groove formation of Lepidoptera. Invagination is a common type of mesoderm formation in Trichoptera, Coleoptera and Lepidoptera, and furthermore the process of overgrowth of lateral parts on the median part is observed by most of the authors in the Lepidoptera and Hymenoptera. Again the invagination of Coleoptera, Trichoptera or Diptera is more complete in comparison with that of Lepidoptera, which is in turn better developed than in Hymenoptera and Orthoptera. Supposing that there is a tendency for the invagination in the higher group to be more complete than in the primitive ones, a group including Orthoptera and Hymenoptera assumes the lowest place in an ascending series of evolution, and the group of Coleoptera, Trichoptera and Diptera might be admitted to be the most advanced, while Lepidoptera is intermediate. This development series agrees approximately with the systematical order of the insects. In *Antheraea* the invagination takes place throughout the entire length of the blastoderm, but as mentioned above, it shows an incomplete process in the hinder part.

The origin of the lining membrane of the mid-gut that is the endoderm has not yet been clearly ascertained: some authors maintain the opinion that this originates from the yolk cells, some ones believe it coming from the endo-mesoderm while others think of it as of ectodermal origin. In short no conclusion has yet been established. The writer will discuss under a special heading whether this discrepancy is due to the error of observation or of interpretation, and also examine whether the developmental process differs so greatly in the various insects.

Here we will be content to give a short historical review of the matter. In Lepidoptera, BOBRETZKY (1878) observed that the lining membrane of the mid-gut originates from the yolk cells. TICHOMIROFF was the first who described minutely the formation from the endoderm in *Bombyx mori*. In his first paper (1879), he stated that it arises by

delamination from the ento-mesoderm, while in later papers (1882, 1891) he mentioned that it is originated from the secondary yolk cells derived from vitellophags by division. GRABER (1890) made many valuable observations on the bipolar origin of the endoderm from the indifferentiated blastoderm in *Bombyx*, *Pieris*, *Gasteropacha*, etc., and he was sure that the endoderm can not be derived from the yolk cells. SCHWARTZE (1899) made a very valuable observations on this problem and stated that the origin of endoderm is from stomodaeal and proctodaeal invaginations. This was confirmed by TOYAMA (1902) in *Bombyx*. In 1905 HIRSCHLER observed also the formation from the cells proliferated from the posterior ends of the stomodaeum and proctodaeum, and he stated that in addition to this the lower layer (according to him, the lower layer consists of a median longitudinal stripe of the endoderm and the mesoderm) takes part in the formation of the mid-gut. STRINDBERG (1915) in *Bombyx* and EASTHAM (1912, 1930) in *Pieris* find that the endodermal rudiments both behind the stomodaeum and in front of the proctodaeum give rise to the mid-gut. However the latter author corrects the statement of HIRSCHLER (1905) showing the participation of the median section of the mesoderm in the formation of the mid-gut, mentioning that "this is most probably incorrect since the median portion of the mesoderm almost universally gives rise to blood cells and the proliferation of cells for this purpose may easily have been wrongly interpreted."

Views derived from studies of Coleoptera respecting the formation of lining membrane of the mid-gut are to be divided into the following four main groups: 1) theory of the yolk-cell origin by TICHOMIROFF (1890): 2) theory of the formation by the cells proliferated from the blind ends of the stomodaeum and proctodaeum, backed by LECAILLON's observation on the leaf-beetle (1897, 1898), DEGENER on *Hydrophilus* (1900), SALING on *Tenebrio molitor* (1907), GRABER on *Doryphora* (1891) and MANSOUR on *Calandra orizae* (1927): 3) theory of the building originally from the true endoderm cells developed at anterior and posterior ends of the embryo. WHEELER in *Doryphora* (1889), HIRSCHLER in *Donacia crassipes* (1909) and HEIDER in *Hydrophilus piceus* L. (1885, 1889) belong to this school: 4) a theory which is a little modified view of the third, that is, in addition, the splanchnic layer of the mesoderm contributes to the formation of the mid-gut, as shown in the works of KOWALEVSKY on *Hydrophilus* (1871) and NUSBAUM on *Meloe proscarabaeus* (1888).

In Diptera, as already described in the part on mesoderm formation, NOACK (1901) described that the endoderm is formed together with the mesoderm by the invagination of the middle portion of the germ band, namely from the meso-endoderm. DU BOIS (1933) found in *Sciara* (Diptera) that the endoderm appears as a small mass of cells at both ends of the germinal groove at the time of the formation of the mesoderm.

As to the endoderm of Orthoptera, AYERS (1884) held the opinion that it is derived from the yolk cells in the study of *Oecanthus*, while a considerable number of observers such as KOROTNEFF studying on *Gryllotalpa* (1884), NUSBAUM (1888), CHOLODKOWSKY (1888, 1891) and WHEELER (1889, 1893) on *Blatta* noted the origin from the endomesoderm. HEYMONS (1895) asserts that the endoderm is derived from the stomodaeal and proctodaeal wall by means of cell proliferation.

From the above studies upon the endoderm origin of the mid-gut of insects, there are recognizable two theories in the rough; the first of which asserts that the whole body of the insect is effected only at the expense of two layers, ectoderm and mesoderm, and so insects furnish an unique exception to the general rule of development in the coelomates not possessing an endodermal germ layer, should it be granted that the yolk cells do not participate in the mid-gut formation (TICHOMIROFF's view). The second theory maintains, on the contrary, that all three germ layers are present. The latter theory is possibly divided again into four views. The first is the formation of endoderm by inward migration of cells from the blastoderm into the yolk (DEEGENER's view). The second maintains that the lower layer, which is produced by the invagination of the median part or by the overgrowth of the lateral parts on the median part, gives rise to the endoderm at both ends and to the mesoderm at the middle part (WHEELER's view). The third one shows a minor variation, that is, in addition to the above the median stripe of the endoderm exists from the first as a continuous tissue (HIRSCHLER's view). The fourth view asserts that the anterior and posterior rudiments of the endoderm arise at both ends of the mesoderm band, independent of the differentiation of the mesoderm (EASTHAM's view). The writer holds to the last view, though in spite of the different features observed in various degrees with the different materials, he thinks that the endoderm formation of all insects can belong eventually to the last view. However on account of the importance and interest, the writer will explain plainly the general criticism of

this problem together with consideration of the mesoderm formation.

In *Antheraea*, the endoderm first appears at the bottom of both the stomodaeal and the proctodaeal invaginations in the body length reducing stage. These two rudiments starting from the opposite positions extend, respectively separating into two, anteriorly and posteriorly to bring about two continuous bands running near the lateral walls of the body. These two bands of endoderm form the ventral wall of the mid-gut by inward growth, then they grow dorsally, and at last a complete mid-gut is formed. On the fate of the yolk cells or vitelloghags, as stated in the previous paper, the writer agrees with the statements of many recent writers. They take no part in the formation of the embryonic body but as a kind of nutritive cell perhaps responsible for the yolk liquefaction, and degenerate *in situ*.

NUSBAUM and FULINSKI (1909) and EASTHAM (1927, 1930) presented an excellent consideration on the development of the mid-gut classifying the mode of development of the mid-gut in insects into seven different types according to the time of development of the endoderm, the time of development of the stomodaeum and proctodaeum being taken as the standard. *Antheraea* belongs to the seventh type of the above authors as the growth area of the endoderm rudiment remains dormant until the stomodaeum and proctodaeum appear.

On this point the writer would like to call attention to the following important tendency in the mesoderm formation of the insect in which there seems to exist a parallelism with the endoderm formation; in the lower insect for example in Orthoptera as soon as the formation of the germ band is completed, the mesoderm develops, while in the higher insects like Lepidoptera, Coleoptera, Hymenoptera and Diptera after the formation of the germ band there is some interval before the mesoderm appears. This seems to show an evolutionary grade of development in different groups of insects.

The formation of two cellular envelopes, the amnion and serosa, from the extra-embryonal blastoderm, is considered to be one of the most characteristic feature of the insects. The envelopes are not, however, common to all insects. *Formica* of Apterygota studied by STRINDBERG lacks the amnion completely, and according to HOFFMANN (1913, 1914) also in *Xenos* of the same group there is no serosa. Apterygota is generally regarded as Insecta Anamnia being placed against the Pterygota which are characterised by the possession of an amnion (Insecta Amniota), and it is comparable to the Amniota and Anamnia

in Vertebrates.

The development of the embryonic envelopes was observed by several workers. In the study of the lepidopterous insects GANIN (1869), HATSCHEK (1877) and JOHANNSEN (1929) came to the conclusion that the cells destined to form the serosa appearing at the micropylar end and the lower pole of the egg are larger than the others destined to form the germ band. This shows the fact that the cells which form the serosa are already sharply differentiated in the blastula stage. According to JOHANNSEN, in the development of *Diacrisia*, after completion of the serosa, the amnion begins to develop. In 1897, HEYMONS in the study on *Lepisma saccharina*, called the entire extra-embryonal region a serosa before the development of the amniotic fold, and according to him the cells at the edges of the embryonic band are pulled out as a thin cellular membrane to become the amnion independent of the serosa.

Various theories, all more or less vague and intangible, have been proposed by different investigators to account for the amnion and serosa. BALFOUR (1881) regarded these membranes as probably derived from an early ecdysis. AYERS (1884) refuted BALFOUR's suggestion. KENNEL (1896) regards the embryonic membranes of insects as homologous with the so-called "amnion" in *Peripatus*, being perhaps the remainder of the trochophore of the annelid. EMERY (1887) suggests that the envelope may be homologous with the shell of the Entomostraca.

Many attempts have been made to explain the mechanism of the formation of the envelopes in insects. So far as the writer is aware, WHEELER (1889) is the first who has adapted RYDER's (1886) "mechanical explanation" in the formation of the insect's amnion. We agree with his idea which, concisely, is as follows; "There is the vesicular one-cell-layered blastoderm filled with yolk, and the ventral plate arising by the rapid proliferation. By the resistance of the yolk being less than the external resistance of the tightly fitting chorion the ventral plate is forced to invaginate. This invagination is favoured by the displacement of the yolk during its liquefaction and absorption by the growing of embryo. We may suppose that this invagination, which results the formation of the amnio-serosal fold, assumed a definite character in different groups of insects." On the other hand, WAGNER (1894) published his "comprehensive theory." According to him the uniform cells of the epithelium build up a certain homologous reciprocal relation between them and then certain cells of the epithelium are

altered in nature under the influence of some special forces breaking the reciprocal relation with neighbouring cells. Thus a separation of the cells of changed nature from the layer occurs, to make the envelope cells.

WILLEY published in 1899 a different theory to the effect that the extra-embryonal blastoderm (i.e., the serosa and amnion) is a secondary cellular membrane derived in a curious roundabout manner from a more primitive extra-embryonal trophic membrane, "the trophoblast" which is preserved in the embryo of *Peripatus novae britanniae*. Finally KNOWER (1900) concluded in his embryological study on the Termites, that the amnion is a derivative of the rudimentary embryonic ectoderm.

In the case of the tusser, the two envelopes are formed completely by the folding of the blastoderm fringing the embryo, though sometimes these two membranes show the appearance of a single sheet of membrane as the result of the close attachment with each other.

A considerable number of works have been contributed to our knowledge of the origin and early development of the germ cells of the Lepidoptera. The earliest worker, whose findings are worthy of consideration, was HEROLD (1815). He gave a remarkable description of the gross aspects on the development of both ovaries and testes of several species in Lepidoptera, principally of *Papilio (Pieris) brassica*, beginning from the late embryonic period to the adult stage. For us the chief value of his work lies in his discovery that the sex of the larva is already determined before hatching. He found the ovary consisting of four tubules with a common duct at its posterior end, and the testes of four small sacs with a duct extending from the side.

According to HEYMONS (1891), SÖCKOW (1828) distinguished in *Bombyx pini* (*Gastropacha pini*) the male from the female before hatching, thus confirming HEROLD's results. The above author described the rudiment of the germ gland as an outgrowth from the hind intestine (hind-gut). The reproductive organs were not found by MEYER (1849) in *Liparia auriflua* before the age of three weeks in the caterpillars, and he considered the young larvae as sexless. He was first, however, to make a finer histological examination of the developing germ glands.

BESSELS (1867) made a more accurate microscopical examination of the embryonic germ cells, and found on the contrary, that the differentiation of sex is recognizable in the early embryonic stage. In a late embryonic stage of *Zeuzera aesculi* he found the rudimentary germ cells embedded in the fat-body at each side in the eighth abdominal

segment. BRANDT (1878) did not study the early development of the germ cells, but described both ovaries and testes in embryo of *Pieris brassica* just before hatching, and he distinguished as HEROLD (1815) did, the female germ gland from the male germ gland by the duct which in the male is attached to the side of the small testicular sacs.

O. HERTWIG and R. HERTWIG (1881) observed the rudiments of the germ glands of *Zygoema minos* lying between the somatic and splanchnic layers of the mesoderm. TICHOMIROFF (1882) and SELVATICA (1882), according to GRABER (1891), observed the germ glands of *Bombyx mori* in comparatively early embryonic stages. The male germ glands of *Zygoema filipendulae* and other lepidopterous insects were described by SPICHARD (1886) as arising from the mesoderm. At the late embryonic stage each germ gland is made up of four "Urzellen der Geschlechtsanlage" and small cells lying between them, and the whole mass is surrounded by a flat-celled epithelium. As the four "Urzellen" increase by mitosis and the surrounding sheath grows inward, the four testicular follicles are established.

The earliest appearance of the primitive germ-glands of Lepidoptera was recorded by WOODWORTH (1889) on *Evanessa antiopa*. At the blastoderm stage, a group of cells is cut off by an infolding from the ventral plate near its posterior end; these cells remain in contact with the ventral plate at the place from where they are produced. Later stages show that these cells produce the generative organs.

In nine and a half days old embryo of *Pieris crataegi*, GRABER (1891) figured the "Anlagen der Samendrüsen" on both lateral and dorsal sides of the intestine, thus occupying a similar position as that of the *Bombyx mori* as stated by SELVATICA (1882). Each Anlage consists of a few large cells and is surrounded by a small celled epithelium, the whole being embedded in fat-body. In the silk-worm embryo, with the well developed appendages, TOYAMA (1902) found the rudiments of the germ glands, differentiating from the cells of the mesodermic somites. The most recent account of the origin of the germ cells in lepidopterous insects was presented by SCHWANGART (1905). He observed on *Eudromia versicolora* the first indication of a germ gland in two or four hours after blastoderm formation. A part of the blastoderm in the posterior region of the egg, but not at the posterior end, becomes several cell-layers thick, the inner cells being larger in size, richer in yolk, and their nuclei having one or two nucleoli, but less chromatin than the (overlying) blastoderm cells. These inner, "primitive germ cells" soon

became amoeboid, and during several hours before mesoderm formation, are separated into several cell groups which move forward through the yolk. Each group divides into two, half of the cells migrating to either side of the body, where they lay near the coelom from the fourth to sixth abdominal segments. Their later history was not observed.

So far as the writer is aware, there is no paper describing the origin of the germ gland of the Neuroptera. Generally the Coleoptera and Dermaptera have been studied in the literature on the embryology of insects, albeit by only a few authors, but most of them contain only fragmentary accounts of the origin and early development of the germ cells. There is wealth of literature regarding the early appearance of the germ cells in the Diptera and Hemiptera; especially there are many papers on the bee in Hymenoptera and on *Blatta germanica* in Orthoptera. From among these studies some important ones will be taken and an attempt made to make a brief summary on the development of the germ cells. HEYMONS (1894) said that the germ cells of insects (Dermaptera and Orthoptera) arise essentially in the same manner as the germ cells of annelids. His indication of metamerism of the germ cells agrees with the findings in *Japyx* by GRASSI (1884) and in *Xiphidium* and *Blatta* by WHEELER (1897); the former observed seven egg-tubes occurring in the consecutive seven anterior abdominal segments, and the latter described six. OUDEMANS (1887) on Thysanura figures seven egg-tubes, but without a clearly marked metameric arrangement.

The differentiation of the germ cells in the Diptera takes place in a very early stage; for instance, in *Sciara*, according to DU BOIS (1933) they are recognizable clearly at the posterior end of the egg at the time of the fifth cleavage, being markedly different in size and structure from the blastodermic (somatic) cells (His figs. 5, 13, 14). These first germ cells divide quickly, forming a bunch of germ cells until they reach to the number of twenty-two to twenty-eight. They begin then to migrate inward, going separately through the blastodermic cells, and afterward move along the mesodermic layer, which has already been formed, until they reach the definite place for the gonads in the fifth abdominal segment.

In *Antheraea*, the differentiation of the germ cells from the somatopleure of the mesoderm takes place at a considerably later stage in comparison with the other lepidopterous species, viz., *Bombyx* (SELVATICA, 1882), *Evanessa* (WOODWORTH, 1889) and *Pieris* (GRABER, 1891). The

anlage of the germ cells makes a metameric appearance in the abdominal segments except the anal one at the stage of the maximum body length, and so far as the writer's observation goes, there is no difference with the sex in the early stage.

The blood cells are said by DOHRN (1876) to have some relation to the yolk cells in *Bombyx mori*. AYERS (1884) also maintains the same view as DOHRN in his study on *Oecanthus*. WILL (1888) comes to the same opinion in his study on *Aphis*, saying that the cell-elements of the blood arise from endodermal yolk cells. CHOLODKOWSKY (1885) also holds the same opinion in regard to *Phyllodromia*. KOROTNEFF (1885) on the other hand states that the blood cells of *Gryllotalpa* at an early period are found almost everywhere between the yolk and the mesoderm; they are derived, according to him, from the cells of the somatic mesodermal layer. They lose their connection with the other parts of the mesoderm, and fall into the body cavity. PATTEN (1884) adopts a similar view in his researches on Phryganids. WHEELER (1889) again confirms the view that the blood cells are formed out of the mesoderm in *Doryphora*, though he differs somewhat from the above two observers as to the place of its origin. Among the above opinions KOROTNEFF's observation seems to be in best agreement with that of the present author in the tusser. Different views held by the other authors are presumably due to the mis-identification of the cell elements found in the yolk.

Neuroblast, and the cells of a similar character have been described and figured by a number of investigators in the development of Arthropoda. Perhaps the earliest observation of this kind was the monograph of REICHENBACH (1886) in *Astacus* (Cray-fish). He described the nerve cord in an early stage as consisting of two kinds of cells, a few large pale elements being arranged in a single layer and confined to the periphery and a much greater number of small and more deeply stained cells forming the bulk of the ganglia. KOROTNEFF (1885) was the first who studied the development of ganglionic cells in the insects. In his study on *Gryllotalpa* embryo, the nerve cord in an early stage is found to consist of two kinds of cells, a few large pale elements and a much greater number of small cells. NUSBAUM (1887) found huge succulent cells in the young nerve cord of the *Mysis* (Shizopoda) embryo. He compares them with the large cells of REICHENBACH and believes that they have a similar fate, and he added one fact that the karyokinetic figure is confined only to the large cells. GRABER (1889) figures and

describes a cross section through an abdominal ganglion of a *Melolontha* embryo, in which he finds three median cells and two symmetrical lateral ones. In a subsequent paper (1890) he describes a foliated condition of the ganglia in the nerve cord of *Stenobothrus*. So far as the neuroblasts are concerned, he did not add anything new to KOROTNEFF's account. In 1891 NUSBAUM, in his paper on the development of *Meloe* again figures neuroblasts. They frequently show mitosis. SCHWARTZE (1899) finds that in *Lasiocampa* the visceral sympathetic nervous system originates from evagination of the ectoderm of the dorsal side of the stomodaeum.

It is desirable to give here the general features of the development of the tusser, beginning from the segmentation. After the migration of the segmentation nuclei to the egg surface their differentiation into the blastomeres and vitellophags occurs. The latter takes no part directly in the formation of the embryo while the former makes a uniform layer of cells (the blastoderm) enclosing the whole yolk at the egg surface. Thus blastoderm is formed of a uniform structure, in which later a partial thickening takes place, differentiating into the ventral plate or the embryonic part separated from the extra-embryonal part. However further important structural changes soon set in. The median longitudinal element of the ventral plate invaginates and then the ventral plate becomes a structure of two layers consisting of upper and lower layers resulting from the coalescence of the ridges of this invaginated groove. On the other hand, at the extra-embryonal part an important change takes place; a fold appears around the embryonic part, which spreads over the latter, coalesces, and gives rise to double membranes, the amnion and serosa.

Now the embryo, which increases in body length and finishes the closure of the invaginated groove beginning from the middle part (gastrulation) sinks into the yolk and the stomodaeal and proctodaeal depressions appear at the two ends of the embryo. Then the mid-gut rudiments appear at the bottom of both the stomodaeal and proctodaeal depressions. Thus the embryo becomes at that time to consist of three kinds of cell-layers, ectoderm, mesoderm and endoderm. The further differentiation of these three layers in *Antheraea* follows exactly the classic schema in lepidopteran development, so the writer will be content only to give a brief account thereof. The initial endodermic masses grow quickly toward each other, surrounding a central mass of yolk; the result of this is the formation of the median part of the digestive

system. Both ends of the system and also the Malpighian tubes are derived from the ectoderm during the differentiation of the primitive stomodaeum and proctodaeum. From the mesoderm the muscular system, the fatbody, germ cells and the circulatory system are derived. The ectoderm forms a part of the digestive system as already mentioned in connection with the development of stomodaeum and proctodaeum; it also forms the nervous system on the ventral side, the tracheal system laterally, and the skin together with its complicated glandular system (silk glands and salivary glands).

B. MESODERM FORMATION IN THE INSECT

The writer has promised above to discuss this problem and it must now be taken under consideration. Although many important observations have been contributed to the embryology of the insect, scientific knowledge on the formation of the mesoderm has not made particular advance, and it has become a topic of discussion. The studies on *Anurida maritima* (Collembola) by CLAYPOLE (1898), on *Isotoma cinerea* (Collembola) by PHILOPTSCHENKO (1912), on *Campodea staphylinus* (Eutotrophi) by UZEL (1897) and on *Lepisma saccharina* (Thysanura) by HEYMONS (1897) contributed much to this problem in the apterygote insects, and they coincide unexceptionally well in results, confirming a multipolar immigration of the mesoderm cells from the blastoderm. In the case of the pterygote insects, many authors came to varying conclusions in the different orders or species, and it seems to be hard to evolve a general theory. Some authors observed a quite different formation of the mesoderm in the lower pterygote insects (Orthoptera, Isoptera) from that in the higher Metabola (Coleoptera, Diptera, Lepidoptera, Hymenoptera). However from a precise examination of the studies of our predecessors and also from the result of the observations described in the present work, the writer could find a universal law of the mesoderm development in the Pterygota.

The writer has already clearly pointed out the mesoderm formation by means of the invagination which takes place throughout the entire embryonic plate but varies as to intensity in the different parts; it shows a typical invagination at the anterior portion, while posteriorly it become unclear to become at the post-thoracic part a slurred invagination and at the posterior-most portion only an inward growth of the floor. Similar facts can be seen by the examination of the other reports illustrative of different points of view. Moreover, it appears to the

writer that the diverse results with the different materials for the studies comes only from the differences of the illustration, that is, he believes that the mesoderm, at least in the cases of the pterygote insects, is formed by means of an invagination, though it is effected by more or less modification according to the different orders or species.

As to Orthoptera BRUCE (1886) described in his study (on *Stagnomantis carolina* ?) the formation of the mesentoderm¹⁾ by a median ingrowth of the germ band similar to that seen in the posterior part of the tusser. GRABER's (1888) figures and description of mesentoderm formation in *Stenobothrus variabilis*, show that there is a distinct median longitudinal infolding of the ventral plate cells or a true invagination (His fig. 11). In his study on *Blatta germanica*, CHOLODKOVSKY (1891) gives an account of the germ layers in harmony with what is known generally in the process of development in the higher insects (Coleoptera); WHEELER (1889, 1893) describes in the same insect, the formation of it by a slurred invagination, that is, the invagination closes in such a way that no tubular form results as seen in the thoracic region of the tusser. WHEELER also found the same type of development in the study on *Xiphidium* (1889, 1893). These scholars' observations, all affirm mesoderm formation by invagination. On the other hand, neither KOROTNEFF (1885) in his study of *Gryllotalpa*, nor AYERS (1884) in his study of *Oecanthus*, succeeded in finding an invagination. But AYERS seems to miss the earliest stages in the development of the germ band, as the youngest germ band shown by him (His figs. 1,18) lies near the posterior end of the egg with its tail pointed towards the micropylar pole. According to AYERS "A tract of the blastoderm along the median line of the ventral (concave) side, lying nearest the deep or primitively head end of the egg, becomes thickened into a germinal band, which is the first trace of the body of the embryo". According to the descriptions by the other authors, e.g., WHEELER (1893), it is clear that this is not the first trace of the embryo, and it does not arise from the concave side of the egg. For example, the germ band of *Oecanthus*, first makes its appearance as a thickening of the blastoderm on the convex surface, and soon moves around the egg pole to a position on the concave side of the egg. It seems true that AYERS could not see the stages preceding the arrival of the germ band

1) The formation of the mesentoderm was wrongly observed by him as the writer proves later.

to the dorsal concave surface at which the invagination occurs and ends. The error of KOROTNEFF's observation is pointed out by the study of NUSBAUM and FULINSKI (1909) with the same material.

In addition to the above studies, those by HEYMONS (1894, 1895) and NUSBAUM and FULINSKI (1906) on *Blatta germanica*, and that by LEUZINGER, WIESMANN and LEHMANN (1926) on *Carausius morosus* may be taken as representative works upon the mesoderm formation of the Orthopteran egg. In all cases, mesoderm is formed from the cells which are budded from the ventral plate into the yolk, that is, by the ingrowth of the cells at the median part of the embryo. This is the same process as seen at the posterior part of the tusser.

In respect to the relation of mesoderm to the endoderm formation in the Orthoptera, there are two main views which are sharply opposite to each other. On *Blatta germanica*, WHEELER (1893) concluded as follows: "The view is now pretty generally held that in the Insecta both mesoderm and endoderm arise from a median longitudinal furrow (the former layer occurs almost throughout the entire length, and the latter is limited only in the oral and anal regions of the germ band)". However, HEYMONS (1894) says that the layer generally known as the mesentoderm is in reality only mesoderm, the endoderm appearing relatively late and arising from the ectoderm of the stomodaeal and proctodaeal invaginations. The present author's observation resembles in general that of HEYMONS, and we agree with each other insofar as to consider that the mesoderm formation of insects occurs by means of an aggregation of cells arising from the ectoderm by invagination, but the writer differs from HEYMONS in the interpretation of the invaginated groove. He thinks it correct to interpret HEYMON's observation as follows; the area of the endoderm formation exists at both the ends of invagination but it remains latent during the mesoderm formation, and as the stomodaeum and proctodaeum appear precedent to its activity the area of the endoderm formation is brought attached to the bottoms of the developing fore-gut and hind-gut. From the above, it may be seen that there is no objection to our view which asserts that the mesoderm formation of the Orthoptera is brought about by invagination in the most incomplete form.

The Coleoptera show more complete invagination than that of the Orthoptera. WHEELER (1889, 1893) remarked that the gastrula of *Doryphora descemlineata* shows an invagination ranging through the whole length of the germ band (1893, his fig. 65), but it is much deeper

at the posterior part than at the anterior where a tendency appears to obliterate (1889, his text-fig. 1). The Coleoptera are rather opposite to the Orthoptera in the intensity of invagination. In 1890, GRABER observed a true invagination in *Lina tremulae*, and he figures more clearly. Moreover, in the work on *Hydrophilus piceus* and *Meloe scabriusculus*, he showed the tube formation after the closure of the primitive groove as is also seen at the anterior part of the tusser. HEIDER (1885, 1889) on *Hydrophilus piceas*, LECAILLON (1889, 1897) on *Donacia*, DEGENER (1900) on *Dyticus* and *Hydrophilus*, SALING (1907) on *Tenebrio molitor*, HIRSCHLER (1909) on *Donacia crassipes* and *Gasteroides viridula* and MANSOUR (1927) on *Calandra oryzae* are of substantially the same view insofar as the origin of the mesoderm is concerned, observing a longitudinal invagination of the middle part of the germ band to form a tube, of which the invaginated part breaks down into an irregular layer of cells between the ectoderm and the yolk to form the mesoderm. The study of coleopterous embryo shows a complete agreement with the present writer's view of the mesoderm formation by invagination.

In regard to the mesoderm formation of the Hymenoptera and Diptera, there is no need to discuss, as all authors without exception have observed the invagination of the middle region of the germ band to form the mesoderm. The term mesentoderm which is occasionally used in these orders for the invaginated tissue seems to the writer to be incorrect. As to this problem the writer will discuss in the section on endoderm formation. The Diptera comes above the Hymenoptera in the completeness of the invagination, that is to say, in the former the middle portion of the germ band invaginates to form a tube, from the wall of which mesoderm is formed, while in the latter the lateral plates grow inwards to enclose the slightly invaginated middle plate without the resultant tube.

Finally to consider the works on the Lepidoptera which is respect to this problem is a more interesting than any other. The study of GRABER (1890) on *Pieris* and *Bombyx* is the first one describing invagination in the Lepidoptera. In the studies made by SCHWARTZE (1899) on *Lasiocampa fasciatella* and by TOYAMA (1902) on *Bombyx mori*, observations of an almost similar process to that in our case were made; they observed the process of mesoderm differentiation varying at the different regions of the embryo. The above cited authors differ from the present writer's opinion in the following points; the inward

growth of cells after TOYAMA (His figs. 5, 6) at the prethoracic part should in reality be considered a cell mass resultant from an incomplete invagination, and so the mesoderm is not formed through different processes other than the invagination at all regions. By means of excellent work on *Pieris* EASTHAM (1927, 1930) showed that the different processes in separate regions are in actual fact separate processes which the germ band undergoes in sequence. In the tusser, however, a similar complete deep invagination at the posterior region as that at the anterior part could not be observed. In the writer's case the invagination is complete only at the anterior part of the primitive groove, while in EASTHAM's case it is accomplished throughout the entire length at succeeding times.

It is of interest to note that the feature of the invagination of the Lepidoptera represents the various phylogenic degrees of the invagination of the other insect orders. The most incomplete invagination at the posterior part showing the only ingrowth of cells, compares with that found in the lower insects, Orthoptera and Isoptera (*Eutermes* studied by KNOWER in 1900). The slurred invagination at the post-thoracic region is similar to the abbreviate invagination found in the Coleoptera. The typical invagination at the anterior part of the tusser is the same as that of the higher insects, Hymenoptera and Diptera.

C. ENDODERM FORMATION IN THE INSECT

The endoderm formation has been a long-studying problem in insect embryology, no definite conclusion having been found. The writer has mentioned above that in many studies on the endoderm formation of the insects there are distinguishable five different views in the rough. The oldest view asserting the origin of the endoderm from the yolk cells, is a result of investigation based on an imperfect technique. The second view, holding the opinion that endoderm comes from the migrate cells into the yolk from the blastoderm, and the third view, showing a continuous existence of the median stripe of endoderm anlage appearing with the mesodermic cells in the invagination, are not acceptable in the presence of the fact of the bipolar origin of the mid-gut. The fourth one, describing the origin of endoderm from both the ends of the invaginated lower layer or mesentoderm cannot illustrate the process of the appearance of the endoderm rudiment and that of the mesoderm formation which takes place at different stages. The fifth view explains

that the endoderm arises independent of the formation of the mesoderm at both ends of the germ band. In short, the first appearance of the endoderm differs according to the insect's species or orders, but it originally comes from double anterior and posterior origins in all insects. The explanation of the endoderm formation and a criticism of the works by a number of other authors on the different insects will be made again in the conclusion.

1. *Apterygote Insect*

The studies on *Anurida maritima* (Collembola) by CLAYPOLE (1898), on *Isotoma cinerea* (Collembola) by PHILIPTSCHENKO (1912), on *Campodea staphilinus* (Eutotrophi) by UZEL (1897) and on *Lepisma saccharina* (Thysanura) by HEYMONS (1897) contribute to scientific knowledge on this subject in the apterygote insects. According to their descriptions the Collembola insects make a very primitive development while Eutotrophi and Thysanura show transition to the type of development of the higher pterygote insect. Both *Anurida* and *Isotoma* show holoblastic cleavage. The subsequent process brings about a change between the two species. In *Anurida* an inner layer results from a process of multipolar immigration differentiating into the mesoderm and at the same time the cells left in the yolk give rise to the endoderm as described by CLAYPOLE (1898); in *Isotoma* the endoderm is derived from the cells passing inwards from the blastoderm at the time of the formation of the mesoblast as observed by PHILIPTSCHENKO (1912) who insisted on the mesentoderm formation. The writer can accept neither CLAYPOLE's theory from the fact that the yolk cells are degenerating ones, nor PHILIPTSCHENKO's theory which is just like it. The endoderm in Collembola to which *Isotoma* belongs, is probably differentiated independently of the occurrence of the mesoderm. It can be accepted at least in the above case that the mesoderm and endoderm develop at the same time. Eutotrophi and Thysanura, higher forms of Apterygota, follow a closely similar development to that of the pterygote insects. In both *Campodea* and *Lepisma*, according to UZEL (1897) and HEYMONS (1897), the cleavage is not holoblastic, and the blastoderm and ventral plate are formed as in the Pterygota. Here the mesoderm formation by the immigration of the cells from the embryonic rudiment possibly occurs, but their announced findings on the endoderm formation from the yolk cells probably come from erroneous observation.

2. *Pterygote Insect*

The endoderm formation of the Pterygota has been studied quite frequently, and it is not easy to make a brief historical review. So only a few important works will be mentioned in relation to this subject, of course setting aside the studies which affirm the yolk cell theory based on an unsuitable technique.

(Odonata) TSCHUROFF (1903) described the development of *Ephitheca bimaculata* and *Libellula quadrimaculata* embryos. Her work is the only one and it is very important, investigating the endoderm formation in the species of Odonata. She stated that the median section of the mid-gut is derived from the yolk cells and that both terminal portions are arised from the cells proliferated from the bottoms of the stomodaeum and proctodaeum. She concludes that this may be an intermediate condition between the Apterygota and Pterygota. Her illustration comes perhaps from HEYMON's theory of the endoderm formation which asserted the yolk cell origin in the Apterygota and the ectodermal origin in the Pterygota.

(Blattariae) Next comes *Blatta germanica*, studied by HEYMONS (1894, 1895) and NUSBAUM and FULINSKI (1906). According to HEYMONS, the definite mesenteron is derived from cells proliferated from the blind ends of the stomodaeum and proctodaeum. His works are quoted by almost every investigator who holds the theory of ectodermal origin. NUSBAUM and FULINSKI, on the other hand, present figures to show that the endoderm rudiment appears before the development of the invaginations of the stomodaeum and proctodaeum (Their figs. 6-9). Nevertheless in the case of the related species, *Gryllotalpa vulgaris* (Saltatoria), they figured the proliferation of the endoderm cells from the lateral bases of the rather deep invaginated stomodaeum (Their text-fig. 9). According to LEUZINGER, WIESMANN and LEHMANN (1926), in the case of *Carausius morosus* (Phasmida), also a kindred species, the mesenteron rudiments develop from the blind ends of the stomodaeal and proctodaeal invaginations. From the study on *Forficula* (Dermaptera) of the same group, HEYMONS (1895) was convinced again of the ectodermal origin of the endoderm. Supposing that HEYMONS worked on the specimen in which the invaginations of the stomodaeum and proctodaeum precede the development of the endoderm, all the above insect group (Blattariae, Saltatoria, Phasmida, Dermaptera) belong to the same category in respect to the endoderm development.

(Coleoptera) Workers such as GRABER (1891) in *Hydrophilus piceus* and *Meloe scabriusculus*, LECAILLON (1897, 1898) in *Donacia*, DEEGENER (1900) in *Dyticus* and *Hydrophilus*, SALING (1907) in *Tenebrio molitor* and MANSOUR (1927) in *Calandra oryzae* obtained the similar results to HEYMON's theory in which it is shown that there is no true endoderm in the insects and that the mid-gut originates from the proliferated ectodermal cells out of the blind ends of the stomodaeum and proctodaeum. On the other hand, the following studies affirmed that the proper endoderm rudiments arise at the anterior and posterior ends of the embryo; in *Hydrophilus piceus* by HEIDER (1885, 1889), in *Chrysomela (Doryphora) descemlineata* by WHEELER (1889) and in *Casteroidea viridula* and *Donacia crassipes* by HIRSCHLER (1909). Further and especially, HIRSCHLER in *Donacia crassipes* observed the anterior and posterior rudiments of endoderm developing from the germ band before the appearance of the invaginations of stomodaeum and proctodaeum. However NUSBAUM (1888) is incorrect as he states in his work on *Meloe proscarabaeus*, that the proper endoderm and the splanchnic layer of the mesoderm contribute to the lining of the mid-gut.

(Hymenoptera) In the studies on hymenopteran development, there is found the clearest account showing that the mid-gut is endodermal. For example NELSON (1911, 1915) described in the work on *Apis* that at the time of differentiation of the mesoderm from the germ band, an area in which the cell proliferation occurs actively, develops at the anterior part of the embryo, and the cells proliferated come to lie in the region where the invagination of the stomodaeum appears, becoming the anterior endoderm rudiment. The posterior endoderm rudiment results from a similar process at the posterior part of the embryo. The excellent study of *Chalicodoma* by CARRIÈRE and BÜRGER (1897),¹⁾ presents a minor modification of NELSON's view. According to them, the invaginating region of the stomodaeum or proctodaeum carries inwards the mid-gut rudiment forming area, and consequently, it appears that the endoderm seems to be formed from the blind end of the stomodaeum or proctodaeum. In addition, there are the studies on *Apis* by BÜTSCHLI (1870) and DICKEL (1904) and on *Camponotus ligniperda* and *Formica* by STRINDBERG (1913) which have contributed much to scientific knowledge on this subject in Hymenoptera. However the

1) The original paper was not available and so the paper by J. NUSBAUM and N. FULINSKI (1909) is referred to.

writer can not agree with them insofar as their belief in the formation of the mesenteron by free cells proliferated from the ectoderm (BÜTSCHLI, 1870) is concerned. He is also against DICKEL (1904) on the point that the endoderm originates secondarily from the division of the yolk cells, and against STRINDBERG (1913) on the point that the cells wander inwards from all parts of the germ band to become the endodermal ones. There is no connection whatever between these and the endoderm, as has been tediously repeated before.

As to Megaloptera, Neuroptera, Panorptera and Trichoptera, there is no value in discussing the accounts. Those on *Chrysopa* (Neuroptera) by TICHOMIROVA (1890) and on *Neophylax* (Trichoptera) by PATTEN (1884) are meagre studies insofar as the endoderm origin is concerned. They are all wrong showing the origin of the mid-gut from the yolk cells.

(Lepidoptera) Concerning the origin of the mid-gut of Lepidoptera there are two main views which are sharply opposed to each other; the one supported by STRINDBERG (1915) on *Bombyx mori* and EASTHAM (1927, 1930) on *Pieris rapae*, admits the existence of the proper endoderm, and the other supported by SCHWARTZE (1899) on *Lasiocampa fasciatella* and TOYAMA (1902) on *Bombyx mori*. A minor modification of the second view is found in the work of HIRSCHLER (1905) on *Catocala nupta*, who observed that in addition to the entodermal cells the median section of the mesoderm contributes to the formation of the mid-gut. The present work of the writer coincides clearly with the first.

(Diptera) The most important embryological work dealing with this subject is that of NOACK (1901) on *Calliphora erythrocephala*, *Lucilia illustris* and *Lucilia regina*, who stated that the endoderm destined to form the midgut is differentiated before the development of the ectodermal invaginations for the fore-gut and hind-gut. BÜTSCHLI (1888) and KOWALEVSKY (1886) who studied on *Musca* (Schmeissfliege), described that the endoderm rudiment is formed by the cells proliferated from the anterior border of the gastrulation furrow (the invagination for the mesoderm) in a similar manner as in the case of NOACK. In the development of *Pyrrhocoris apterus* (Heteroptera) studied by KARAWAIEW (1893) an intermediate type may be seen between NOACK's (1901) observation on Diptera and that of HEYMONS (1895) on Dermaptera (*Forficula*). At first, the mid-gut rudiments develop from both ends of the germ band, and then the invaginations of the stomodaeum and proctodaeum appear in the place where the rudiments have

developed.

So far as the writer is aware, there has been no embryological study on the insect group including Protura, Ephemera, Perlariae, Embiidea, Diproglossata, Thysanoptera, Zoraptera, Corrodentia, Allophaga, Siphunculata, Strepsiptera, Megaloptera, Panorptera, Suctoria and Homoptera.

Having gotten a historical view of the views upon endoderm development in the different orders of insects, an attention may be given to a generalisation of the problem as a whole.

From the above considerations, it has become quite clear that there exists the proper endoderm anlage in all kinds of insects so far investigated. The study of TSCHUROFF (1903) is to be considered exceptional, in which she explains forcibly that *Odonata* occupies an intermediate position between the apterygote and pterygote insects as to the endoderm formation. Otherwise the above stated fact is to be accepted as universal among Apterygota and Pterygota. The theory of the origin of the mesenteron from the mesentoderm held by PHILIPTSCHENKO (1912), is only valid in the case in which the endoderm formation occurs at the same time with that of the mesoderm. None the less in this case each of the germ layers should be independent. In this respect the studies of NOACK (1901), BÜTSCHLI (1888) and KOWALEVSKY (1886), who observed the proliferation of the endoderm cells from the anterior and posterior borders of the gastrulation furrow (the invagination for the mesoderm), are very important having demonstrated the clear fact of the independent formation of the mesoderm and endoderm. The observations of NELSON (1911), CARRIÈRE and BÜRGER (1897) and KARAWAIEW (1893) show that the appearance of endoderm precedes the invaginations of the stomodaeum and proctodaeum. Moreover NUSBAUM and FULINSKI (1906) and HIRSCHLER (1909) observed that the endoderm rudiment develops further before any indication of the invaginations of the fore-gut and hind-gut. On the other hand, NUSBAUM and FULINSKI (1909), HEIDER (1885, 1889), WHEELER (1889), STRINDBERG (1915) and EASTHAM (1927, 1930) and the present writer proved the appearance of the endoderm rudiment after the remarkable progress of the ectodermal invaginations of the fore-gut and hind-gut. The fore-gut and hind-gut at the stage of the formation of the endoderm rudiment in *Carausius*, as studied by LEUZINGER, WIESMANN and LEHMANN (1926), are invaginated more deeply than in the above cases. From the above, the studies of HEYMONS (1895, 1903), GRABER (1891),

LECAILLON (1897, 1898), DEGENER (1900), SALING (1909), MANSOUR (1927), SCHWARTZE (1899) and TOYAMA (1902) holding the view of ectodermal origin of the mesenteron may be interpreted as cases in which the area for the mid-gut rudiment remains latent until the stomodaeum and proctodaeum are well developed.

One of the most interesting problems afforded by the embryological studies on insects is the evolutionary relationship, though there are some orders which have not yet been studied embryologically. As to the primitive embryological features, the holoblastic cleavage, the formation of the mesoderm by means of multipolar immigration, the lacking of either or both of the embryonic envelopes etc. are taken generally as lower phylogenetic phenomena.

According to HANDLIRSCH (1925),¹⁾ from the systematical point of view the Thysanura and Eutrophi are admitted to be the most primitive of Apterygota. But from the embryological studies the Collembola should take the lowest rank as the works on *Lepisma* belonging to Thysanura by HEYMONS (1897) and on *Campodea* belonging to Eutrophi by UZEL (1897) show that these insects have developmental features somewhat closely related with those of pterygote insects rather than with those of Collembola, the cleavage being not total, and blastoderm and ventral plate or embryonic rudiment being like those in the Pterygota. Of the pterygote insects, a group of Saltatoria, Dermaptera, Blattariae and Isoptera are certainly to be placed lowest in the phylogenetic order, their mesoderm being formed by means of the inward growth as seen in Apterygota. In the case of insects systematically higher than Coleoptera, the mesoderm formation is restricted just to the median line showing the typical invagination.

Hymenoptera, Lepidoptera, Diptera and Heteroptera have been studied embryologically to a degree sufficient to warrant the formulation of a general view concerning their evolution. In the endoderm formation of these four orders there is no noticeable feature showing the evolutionary line. But a sequence of order can be obtained in the developmental time of the endoderm in relation to the formation of the mesoderm or stomodaeum and proctodaeum. Thus of the four, Lepidoptera is the first in which the formation of endoderm in comparison with the development of the stomodaeum and proctodaeum occurs

1) A. HANDLIRSCH:—"Handbuch der Entomologie", 1925, Jena (The part dealing with classification).

latest; at so advanced stage on the invagination of the stomodaeum and proctodaeum, that the endoderm appears as if it were of ectodermal origin (SCHWARTZE (1899), TOYAMA (1902), STRINDBERG (1915) and NUSBAUM (1927, 1930)). *Chalicodoma* in Hymenoptera as studied by CARRIÈRE and BÜRGER (1897) shows a formation like that in the case of Lepidoptera, but in *Apis*, the endoderm formation precedes the invaginations of the stomodaeum and proctodaeum (NELSON, 1911, 1915). The same process as in the latter are observed in Diptera without exception (NOACK (1901), BÜTSCHLI (1888) and KOWALEVSKY (1886)). *Pyrrhocoris* (Heteroptera) as studied by KARAWAIEW (1893) takes an intermediate position between Lepidoptera and Diptera in this relation. Though no exact evolutionary order can be observed in the development of the endoderm, it is rather interesting to note the fact that the stage of the formation of the endoderm rudiments differs according to the ordinal position of the different insects.

Conclusion

From the consideration of the embryological studies carried out by others and also of the writer's own findings, the following predicative conclusions have been reached:—

(I) The free-cell formation theory after WEISMANN (1863) at the time of the segmentation of the insect egg is not applicable to all kinds of insects. The cell, according to LEYDIG's definition (1857),¹⁾ is a mass of protoplasm (in recent terminology the cytoplasm) containing a nucleus. On the other hand, recently WILSON (1925)²⁾ added to this, based on SCHULTZE's statement (1861), that both nucleus and cytoplasm arise by division of the corresponding elements of the pre-existing cell. Now from the above definition of the cell, in the special cases as seen in a few lower insects such as apterygote Collembola which assume the holoblastic cleavage as in *Isotoma* studied by PHILIPTSCHENKO (1912) and *Anurida* studied by CLAYPOLE (1898), the resultant products of the segmentation are cell aggregations containing an enormous amount of deutoplasm but in the cases of the higher insects, the products of the segmentation are not considered to be a cell mass in the strict sense. The cleavage products make the syncytium condition of nuclei

1) LEYDIG:—Lehrbuch der Histologie. 1857.

2) E. B. WILSON:—The cell in development and heredity. 1925.

(II) The studies asserting the amitotic division of the segmentation nucleus as observed by STRINDBERG (1915) in *Eutermes* and by SCHWARTZE (1899) in *Lasiocampa* may be mistaken because based on technique of observation. The present writer could observe the mitotic division of the segmentation nucleus very commonly. However after the completion of the blastoderm he failed, though his attention was directed particularly to this regard, to observe the yolk cells in the mitotic figure but he found the vitellophags only in amitosis taking the lobe-form. On this point the writer agrees completely with the excellent observation of JOHANNSEN (1929) on *Diacrisia*.

(III) The region of the blastoderm in which the differentiation first appears differs according to the different species of insects. This seems to have no significance in the insect development. There is a faint tendency for the mode of the blastoderm differentiation throughout the species belonging to the same order to be generally similar. The results of the studies on the Coleopteran insects, i.e., on *Hydrophilus* by HEIDER (1885, 1889), on *Doryphora* by WHEELER (1889), on *Tenebrio* by SALING (1907), on *Lina*, *Clythra*, *Gastrophysa* and *Agelastica* by LECAILLON (1897), on *Donacia* by HIRSCHLER (1909) etc. show similarly the advance of the blastoderm formation from the ventral side of the middle part toward the lateral and polar regions. There is a single exception observed by NUSBAUM (1882) in *Meloe*, in which the blastoderm appears at first at the hind pole. Such similarity of the development in a definite order simply may be ascribed the morphological resemblance of the eggs. Generally in the rather long shaped eggs there appears some particular center of the blastoderm differentiation from which the development extends to the surrounding parts, in most eggs the ventral surface being covered at first by the blastoderm, while in the spherical eggs the blastoderm is formed simultaneously on the entire surface.

(IV) That the want of the embryonic envelopes shows the primitive condition may be proved true from the fact that the higher insects are the possessors of complete envelopes while just the embryo of some lower apterygote insects is deprived of them. Perhaps the development of the amnion by means of invagination as studied in *Eutermes* by KNOWER (1900) shows an intermediate type between the so-called amniote and anamnian insects. At present the writer has no available data to shed light upon the problem.

(V) As to the yolk cells, vitellophags, the present writer can

accept neither the view holding their direct participation in the formation of the blood cells nor that considering them as the element of the endoderm. The blood source theory of the vitellophags held by DOHRN (1876) on *Bombyx*, by AYERS (1884) on *Oecanthus*, by WILL (1888) on *Aphis* and by CHORODKOWSKY (1891) on *Phyllodromia* is perhaps due to the confusion of the yolk cells with the true blood cells from the mesoderm because of the similar appearance of the two. Again the bipolar origin of the endoderm disproves the endoderm formation from the yolk cells. They take no part whatever directly in the formation of the embryonic body, but serve as a kind of nutritive cells which have the fate of degeneration.

(VI) The present writer has pointed out that the mesoderm formation by means of the immigration of cells from the ectoderm, blastoderm in the strict sense, is universal in all apterygote insects, and also that the formation by means of ectodermal invagination is in this case universal process in the pterygote insects. However the invagination of the lowest pterygote insects is so incomplete that it appears instead as a mere inward growth of the elements of the primitive groove. There is almost a continuous grade of development between the perfect invagination of the Pterygota and the type of cell immigration as seen in the Apterygota. Therefore the process in the case of the Apterygota is nothing but a modified invagination. In other words it seems highly probable that the greater completeness in the invagination proves the higher specialized development. From this standpoint of embryology, Orthoptera and Isoptera stand in a more primitive position than the Coleoptera does, and Hymenoptera and Diptera are the most specialized, while Lepidoptera occupies a position between Coleoptera and Hymenoptera in evolution. The invagination appears in the development of the Lepidopterous insects not with equal intensity throughout the entire length but it shows grades according to the locality in the blastoderm and so the mesoderm seems at the first sight to be formed in a different manner at different regions.

(VII) Though the formation of endoderm (mid-gut) is explained erroneously sometimes by the mesentoderm theory in case it appears simultaneously with the mesoderm invagination or sometimes by the ectodermal theory in case it comes forth with the invagination of the stomodaeum or proctodaeum, the writer is convinced of the existence of the proper origin of the endoderm in all insects by the consideration of all existing embryological facts.

(VIII) HENSON's presentation (1932) on the origin of the Malpighian tubes of the insect in the study on *Pieris brassicae*, asserting that the Malpighian tubes of the insect homologous with the organs which are known as the Malpighian tubes in Crustacea and Arachnida are not necessarily ectodermal and are indeed more likely to be endodermal in origin, can not be accepted. The present writer's studies show that the Malpighian tubes are without any doubt an ectodermal derivation from the proctodaeum.

(IX) The revolution of the Lepidopterous embryo in the egg during the development, at least in the case of the tusser, is brought about by the displacement of the embryo toward the opposite side from its original position resultant from the yolk consumption accompanying the growth of the body. This change of position is effected chiefly by the act of the posterior half of the embryo while the anterior half seems almost indifferent in this phenomenon.

Explanation of the Plates

Lettering

a	posterior end of ectoderm
abl ¹⁻¹⁰	first to tenth abdominal legs
abs ¹⁻¹⁰	first to tenth abdominal segments
aent	endoderm originated from anterior part
ame	umbilical cord
am	amnion
amf	folds of amnion
ap	anterior blastopore
astg ¹⁻⁸	first to eighth abdominal stigmata
at	antenna
b	boundary of primitive groove
bl	blastoderm
br	brain
ca	coelomic cavity
cal	caudal leg
call	caudal lobe
cy	cytoplasm
dw	dorsal body wall of embryo
ect	ectoderm
fg	fore-gut
ga	ganglion

gc	ganglion-cell
hc	hair-cell
hg	hind-gut
ism	mesoderm of intersomitic part
lb	labrum
ly	liquefied yolk
malt	Malpighian tube
md	mandible
mes	mesoderm
mg	mid-gut
mgw	wall of mid-gut
msm	mesodermic somite
mst	main stigmatal trunk
mus	muscle
mx ^{1, 2}	first and second maxillae
n	nucleus
n ¹⁻¹⁰	first to tenth constrictions for body segment
nc	nerve cord
neb	neuroblast
nf	neural furrow
pb	bottom of primitive groove
pent	endoderm originated from posterior part
pg	primitive groove
pl	procephalic lobe
pr	primitive ridge
prt	proctodaeum
s ¹⁻¹⁶	first to sixteenth body segments
sag	salivary gland
se	serosa
sega	suboesophageal ganglion
sg	silk-gland
sn	segmentation nucleus
st	stomodaeum
stg	stigma
thga ¹⁻³	first to third thoracic ganglia
thl ¹⁻³	first to third thoracic legs
ths ¹⁻³	first to third thoracic segments
thstg ¹⁻³	first to third thoracic stigmata
us	unsegmented part of body
vp	ventral plae
y	yolk
yc	yolk cell

Plate I

- Fig. 1. Longitudinal section through the center of an egg about 36 hours old in the segmentation stage, in which the nuclei (n) take a spherical arrangement in the homogeneous yolk (y). $\times 60$.
- Fig. 2. Longitudinal section of an egg about 50 hours old at the blastoderm stage; there is a uniform layer of cells enclosing a mass of yolk (y) in the center which commences to divide from the peripheral part into a number of distinct spheres. $\times 60$.
- Fig. 3. Cross section of an egg in which the ventral plate (vp) is differentiated from the blastoderm (se), showing the growth of the amnion. $\times 60$.
- Figs. 4a, b. Two transverse sections of an egg 130 hours old. Fig. a, a section through the procephalic lobe (vp) of the embryo, showing the formation of the primitive groove (pg) by the invagination of the median part which consists of large spherical cells. Fig. b, a section through the posterior part of the same embryo; there is no invagination but the median part (pg) destined to invaginate in the future is thinner than the lateral (vp). $\times 60$.
- Fig. 5. A sagittal section of an egg at the same stage as fig. 4, showing the primitive groove (pg) and the constriction of the body segment (n^1). $\times 60$.

Plate II

- Fig. 6. A polynuclear yolk cell. $\times 500$.
- Figs. 7a-d. Three cross sections (Figs. a-c) at different levels from a serial section of an egg about 140 hours old. The cells at the bottom of the primitive groove (pg) are round in form and larger than those of the sides. Fig. d, a diagrammatic figure of the ventral plate, showing the primitive groove in surface view; the meshed part is the floor of the groove. Figs. a and b, $\times 80$, Fig. c, $\times 30$.
- Figs. 8a, b. The longitudinal sections of an egg in the same stage as Fig. 7. 8a, a section through the white part of fig. 7d, which consists of the columnar cells, while 8b is a section through the primitive groove or the meshed region of fig. 7d. Its cells are round in shape. $\times 60$.

Plate III

- Fig. 9. The median longitudinal section through the anterior blastopore (ap) of an embryo 160 hours old. The closure of the primitive groove occurred at the anterior part, and consequently there are two kinds of tissue, ectoderm (ect) and mesoderm (mes), while the primitive groove at the posterior half, where it consists of one tissue (pb), is still open. $\times 60$.
- Figs. 10a-j. The cross serial sections of an egg in the same stage as fig. 9, showing the manner of the closure of the primitive groove, that is, the formation of the mesoderm. Fig. 10a, a section through the anterior blastopore (ap). The cells at the bottom of the blastopore are different in form from the other cells. Fig. 10b, eight sections posterior to fig. a, in which the primitive groove (ap) has almost closed and the mesoderm (mes) shows a mass adhering to the underside of the ectoderm (pl). Fig. 10c, a section through the second body segment. The primitive groove has completely closed over the mesoderm

(mes) which assumes a plate form. At the third body segment (Fig. 10d) the primitive groove (pg) appears again widened and deepened posteriorly, and at the sixth body segment (Fig. 10f) it attains the greatest depth. Then the groove suddenly widens and at the same time gradually becomes shallow to be brought up at last to the general level of the ventral plate (Figs. 10g-i, pg). The groove bifurcates at the eighth body segment (Fig. 10g, pg). $\times 60$.

Plate IV

- Figs. 11a-e. The serial sections of an egg at a little further advanced stage than that of fig. 10. The procephalic lobes (pl) are completely formed and the anterior blastopore becomes narrower than that of fig. 10a. At the thoracic part the closure of the primitive groove is completed (Fig. 11b), and consequently there are two layers of tissue. At the fifth body segment (s^5) the primitive groove appears again (Fig. 11c), and at the seventh body segment (s^7) it attains its greatest depth (Fig. 11d). It is brought up to the general level of the ventral plate at the eleventh body segment (Fig. 11e, s^{11}). Figs. a, b, c and e, $\times 80$, Fig. d, $\times 30$.
- Fig. 12. A median longitudinal section of an embryo at the stage of the above figure, showing a minute blastopore (ap) remaining at the anterior end. At the anterior half the primitive groove is closed completely, and the mesoderm (mes) adheres to the underside of the ectoderm (ect), and at the posterior half, there is no differentiation of the mesoderm. $\times 30$.
- Fig. 13. Diagrammatic figure of the ventral plate, in which the primitive groove has closed partially. The meshed part is the future mesoderm, the spotted part shows the part differentiated into the ectoderm and mesoderm, and the white part is the ectoderm.

Plate V

- Fig. 14. Longitudinal section of an embryo about 200 hours old, which has grown greatly in body length so that the posterior body part bends to the opposite side of the original position in the egg. The body segments are formed and the stomodaeal (st) and proctodaeal (prt) depressions have come into view. $\times 60$.
- Figs. 15a-h. Serial cross sections of an embryo in the same stage as fig. 14. Fig. a, a section through the proctodaeum (prt) which is a depression of the ectoderm (ect) at the posterior end of the body (Fig. 15a, prt). Fig. c, eleven sections anterior to fig. b, showing two cell-masses of the endoderm (ent) originated at the posterior part and the splitting of the mesoderm (mes) into two masses, which were formerly one mass in fig. 10c or 11b. At the ninth abdominal segment (Fig. 15d), there appears the neural furrow (nf) on the ectoderm, and on the other hand, the mesoderm (mes) differentiates into two, splanchnopleure and somatopleure, consequently a coelomic cavity (ca) forms between them. Fig. 15e, a section through the intersomitic part of the fifth abdominal segment (abs^5), in which the poor mesoderm (mes) adheres to the inner side of the ectoderm and the rudiment of the anterior endoderm (aent) appears as a triangular cell mass succeeding anteriorly to the stomodaeal bottom (Fig. 15h, ent and st). $\times 60$.

Plate VI

- Figs. 16a, b. Fig. a, a longitudinal section of the proctodaeum (prt), appearing as a faint depression of the posterior end of the embryo; this condition is shown plainly in the cross section as seen in fig. b. $\times 80$.
- Fig. 17. The proctodaeum (prt) of an embryo about 200 hours old which is sectioned longitudinally; its blind end consists of a thick ectoderm which possesses an outgrowth of the Malpighian tube (malt). The posterior endoderm (pent) adheres to its inner side. $\times 80$.
- Figs. 18a-c. Three serial sections through the proctodaeum in nearly the same condition at that in fig. 17, showing the development of the Malpighian tube (malt) as the outgrowth of the wall of the proctodaeum (prt). $\times 80$.
- Figs. 19a-c. Three serial cross sections of the proctodaeum (prt) which have developed a little further than the above. The tubular condition of the Malpighian tube (malt) is shown. $\times 80$.
- Fig. 20. Cross section through the end of the embryo about 10 days old. It shows the origin of the Malpighian tube (malt) as the outgrowth of the wall of the proctodaeum (prt). $\times 80$.
- Fig. 21. Part of a cross section of the second abdominal segment of an embryo about 200 hours old, showing the neuroblast. $\times 400$.

Plate VII

- Fig. 22. Cross section through the portion of the insertion of the Malpighian tube (malt) into the proctodaeum (prt) of the embryo about one week old. It shows the direct connection of the wall of the tube with that of the gut. $\times 80$.
- Fig. 23. Longitudinal section through the cephalic part, showing a faint stomodaeal depression (st). $\times 80$.
- Figs. 24a-c. Three serial cross sections of the cephalic part of an embryo about 200 hours old, in which the stomodaeum (st) appears as a ventral depression and the antenna (Figs. a and b, at) and mandible (Fig. c, md) are formed as an outgrowth of the body wall. $\times 80$.
- Fig. 25. Cross section through the fifth body segment of an embryo about 170 hours old, showing the plate-like existence of the mesoderm (mes). $\times 80$.
- Fig. 26. Cross section of the corresponding segment with that in the above figure of an embryo about 200 hours old, in which the mesoderm (mes) splits into two lateral bands and the dorso-ventral splitting again occurs in each band, making the coelomic cavity (ca). $\times 80$.

Plate VIII

- Fig. 27. Slightly oblique longitudinal section of an embryo about one week old, in which the ganglia (aga^{6,7}) and stigmata (astg²) are formed completely. $\times 60$.
- Figs. 28a-e. Serial cross sections of an embryo in the same stage as the embryo in fig. 27. Fig. a, a section through the mouth parts (md, mx^{1,2}) and fig. b through the first thoracic segment (thl¹). The muscle originated from the mesoderm enters into the constitution of the wall of the stomodaeum (st). Fig. b shows the fusing of the first two thoracic ganglia (thga¹) and the existence of the anterior end of the endoderm (ent) at the dorsal side of the stomodaeum (st). Fig. c, an intersomitic part (left side of the figure) of the

second abdominal segment (abs²), in which the second abdominal stigma (astg²) grows as a depression and the ventral transverse trachea has extended ventrally along the body wall as a horn. The endoderm (ent) appears as two bands at both lateral sides. Fig. d is a section of the somitic part (msm) of the fifth abdominal segment (abs⁵), showing the joint of the Malpighian tube (malt) with the proctodaeum (prt) and the posterior situation of the endoderm (ent). In fig. e there appears the sixth abdominal segment (abs⁶). $\times 60$.

Plate IX

Fig. 29. Longitudinal section through a little lateral part of an embryo about 350 hours old in which the dorsal body wall has so formed that the so-called umbilical cord (ame) has been limited to the thoracic dorsal part. The band of the endoderm (ent) completely connects the bottoms of the fore-gut with the hind-gut (st and prt). $\times 60$.

Figs. 30a-i. Serial cross sections showing the relation of the various organs of the embryo in the same stage as in the above figure. The body length is diminished in length, but the dorsal surface of the embryo is still directed towards the center of the egg. Compare with the next figure. $\times 60$.

Plate X

Figs. 31a-f. Serial cross sections of an embryo about 20 days old. The revolution of the embryo has completed and the body length is greatly increased, and now the body begins to bend ventrally and the dorsal wall of the embryo comes directly to the under side of the serosa. $\times 60$.

Plate XI

Figs. 32a, b. Fig. a, a median longitudinal section of an embryo about 250 hours old. All body segments are formed and the embryo consists of two kinds of tissues, the ectoderm and mesoderm (mes). The depressions of the stomodaeum (st) and proctodaeum (prt) are grown, and moreover, the endoderm (ent) comes into view as a mass of cells at the end of the stomodaeum and proctodaeum. Fig. b, a proctodaeal part (prt) which is magnified in order to indicate the posterior origin of the endoderm (pent). Fig. a, $\times 60$, Fig. b, $\times 350$.

Figs. 33a, b. Fig. a, a cross section of the second abdominal segment of an egg about 200 hours old, in which the coelomic cavity (ca) is formed and the second abdominal appendage (abl²) protrudes ventro-laterally as an ectodermal pouch. Fig. b, a magnified figure of a part of fig. a, showing three neuroblasts (neb). Fig. a, $\times 60$, Fig. b, $\times 350$.

Fig. 34. Longitudinal section of the proctodaeum (prt) having a pouch which is the rudiment of the Malpighian tube (malt). $\times 60$.

Fig. 35. A cross section through the portion of the entrance of the Malpighian tube (malt) into the proctodaeum (prt) in an embryo about one week old. $\times 60$.

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Fig. 1

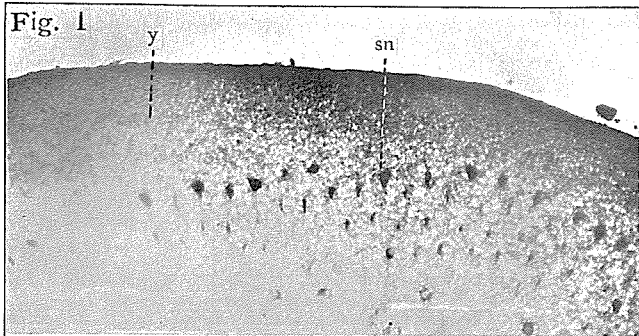


Fig. 2

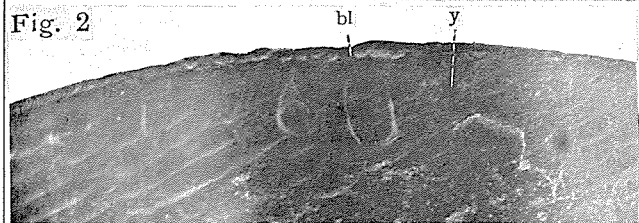


Fig. 3

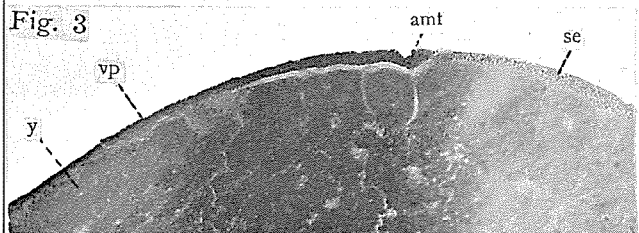


Fig. 4a

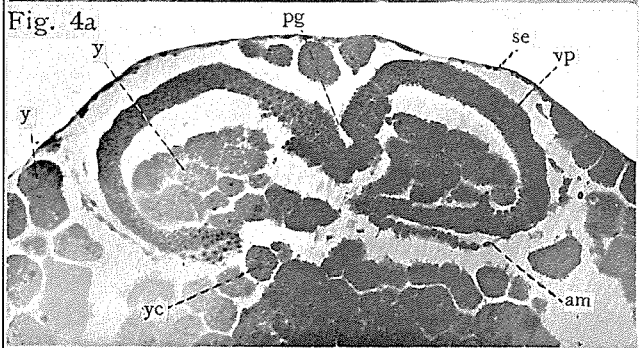


Fig. 4b

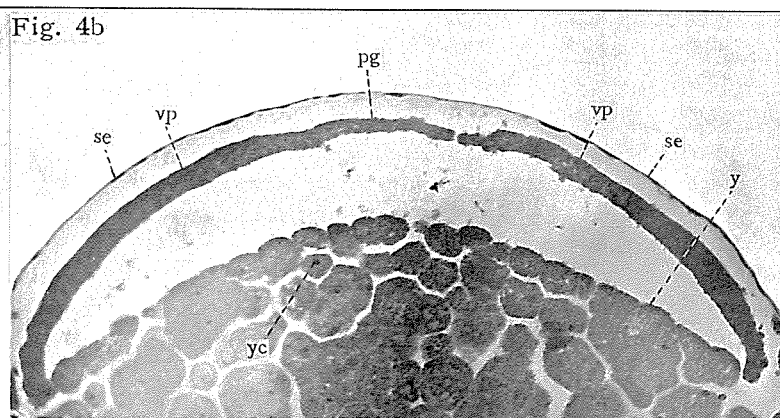
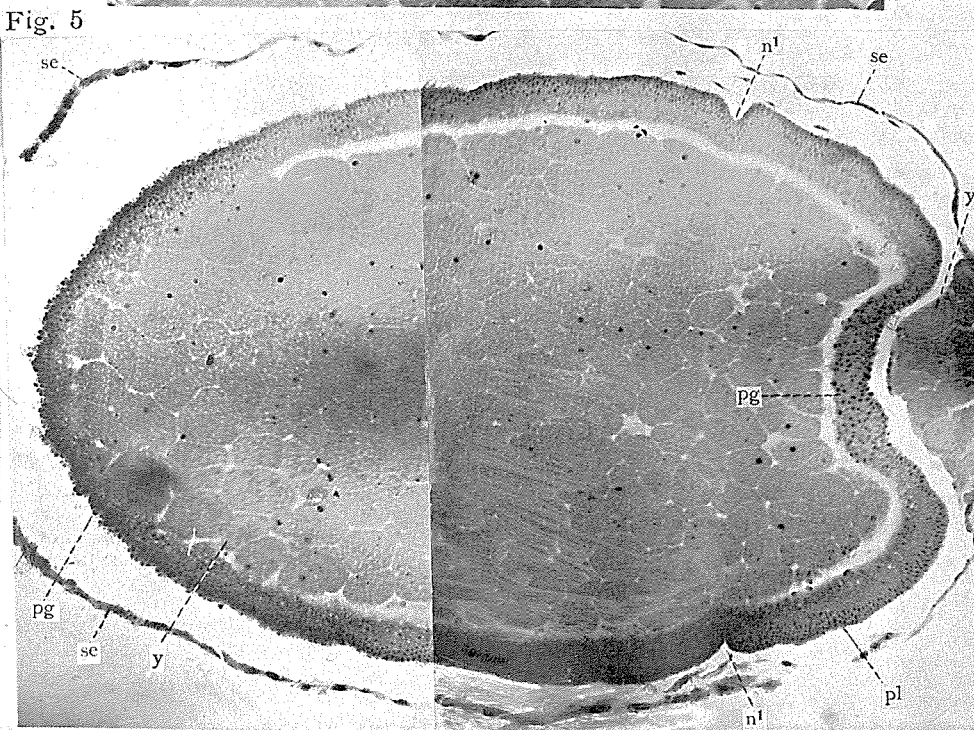
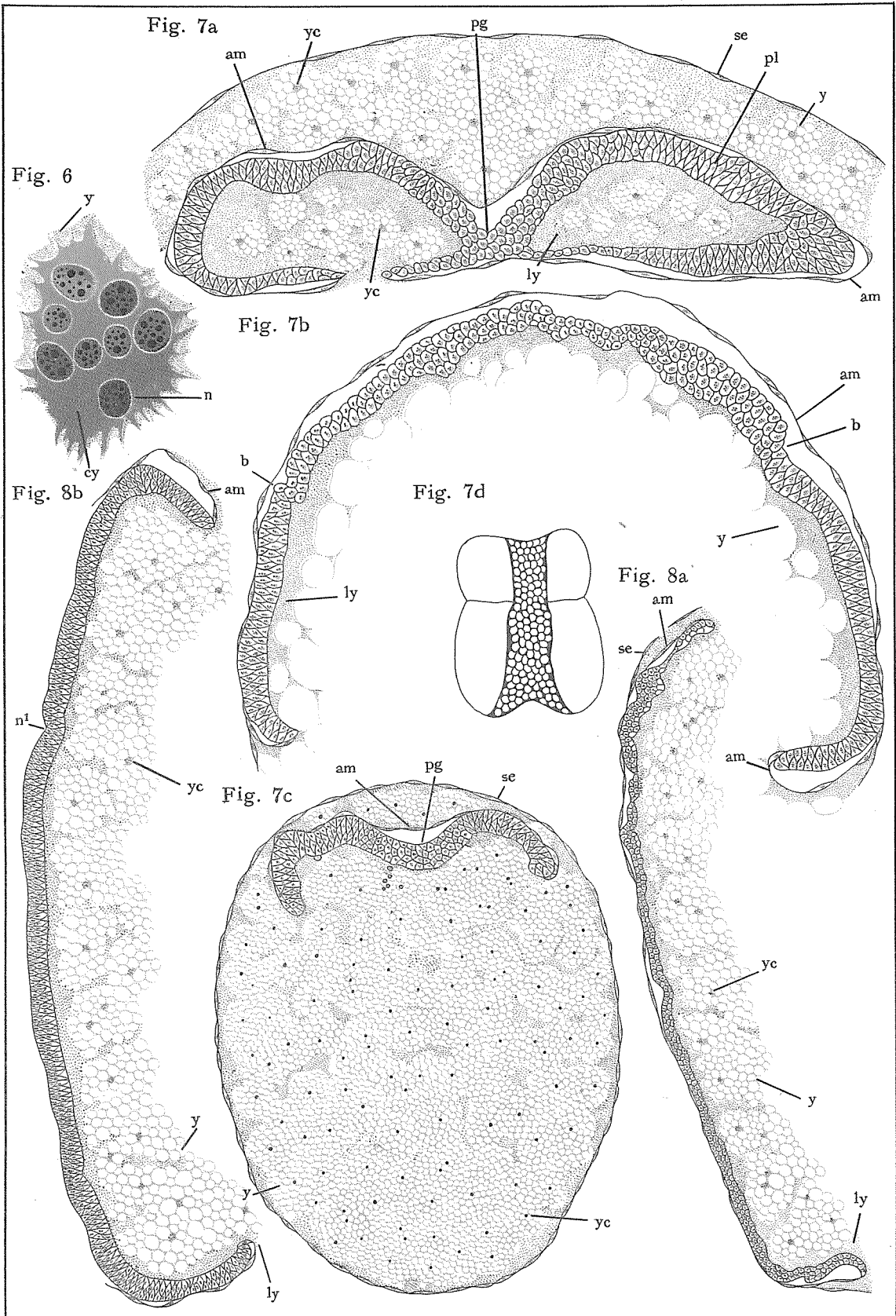


Fig. 5





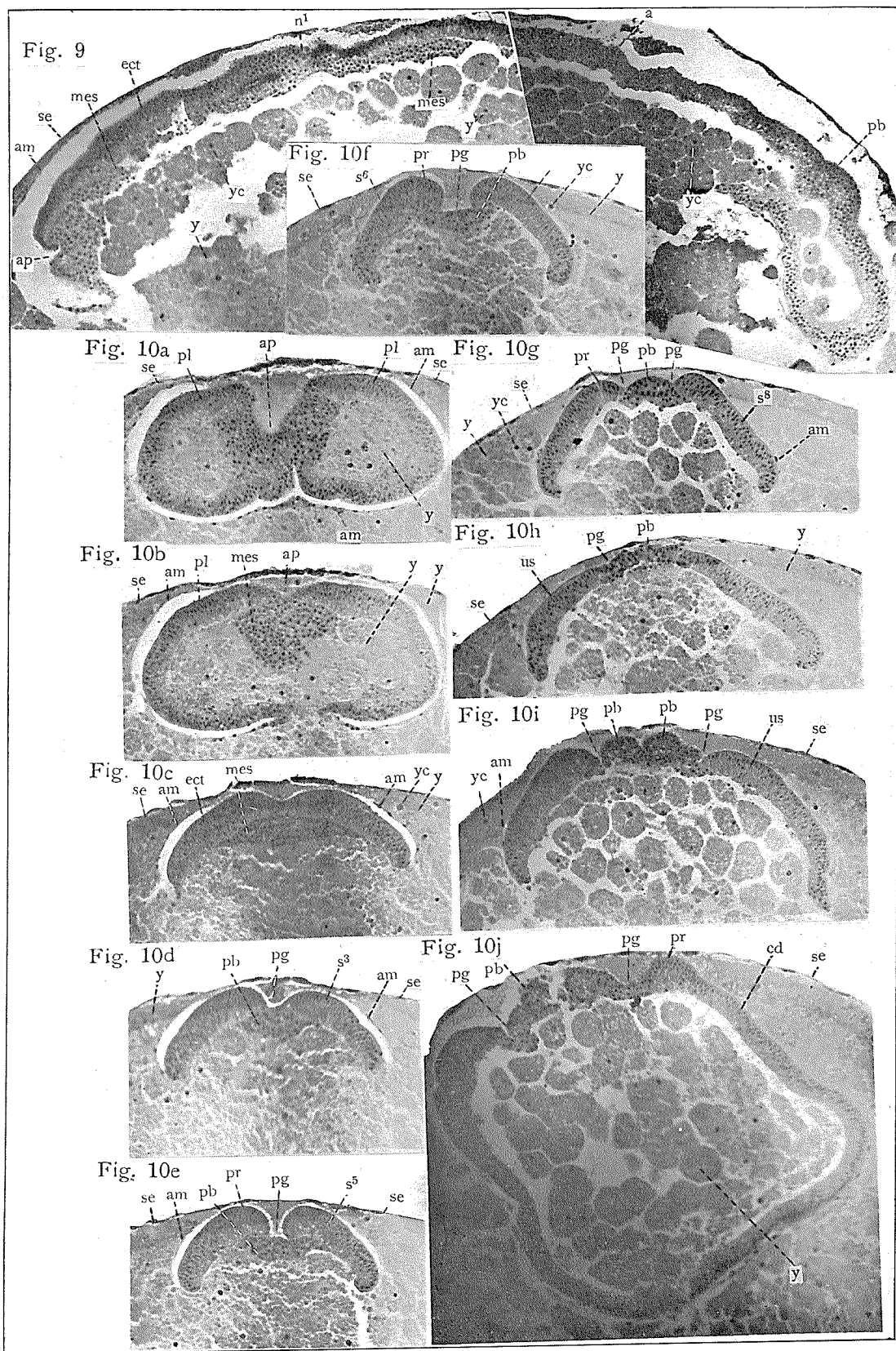


Fig. 11a

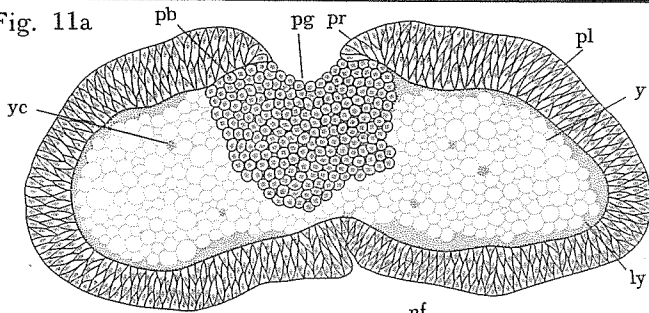


Fig. 12

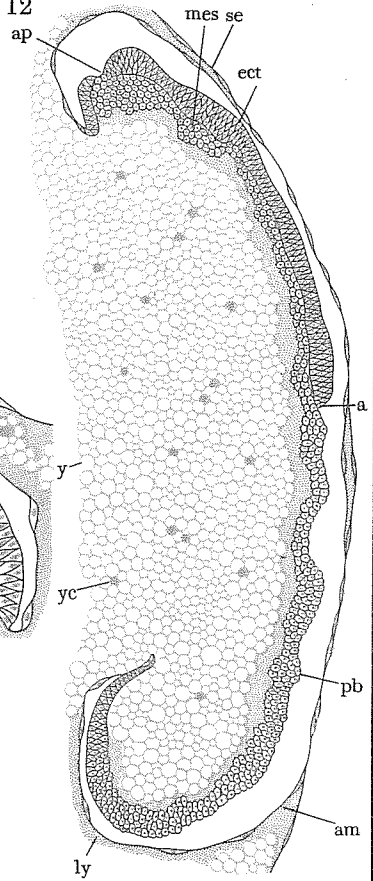


Fig. 11b

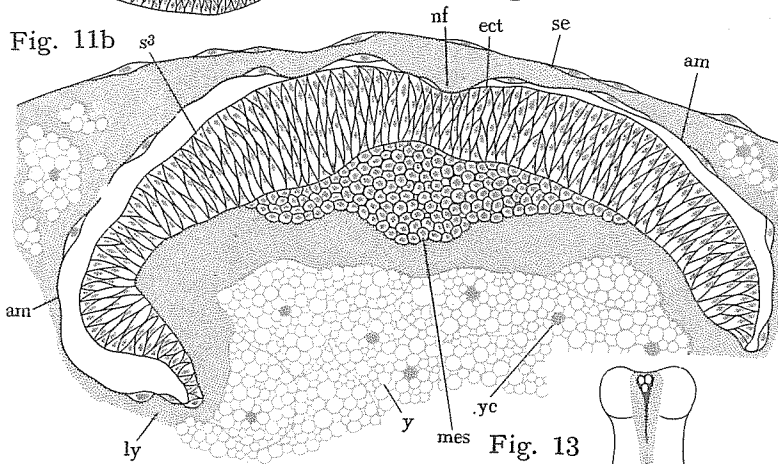


Fig. 13

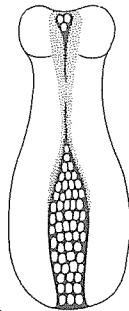


Fig. 11d

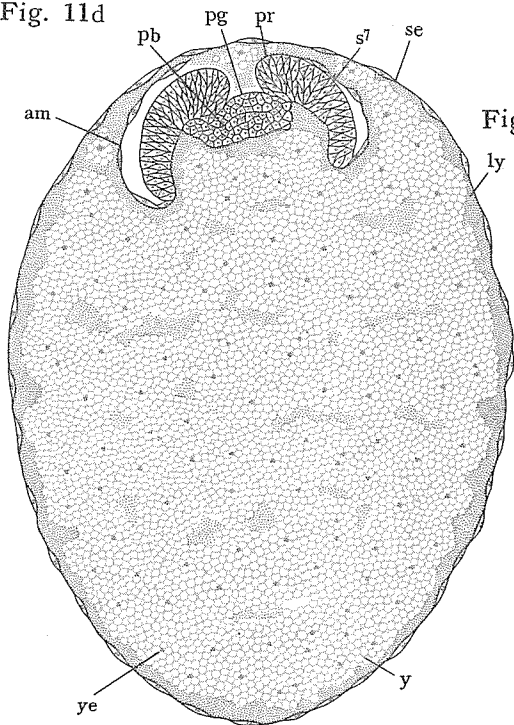


Fig. 11c

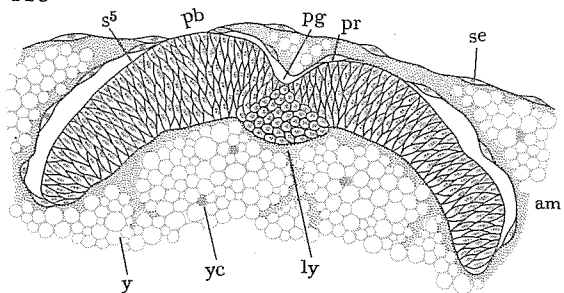
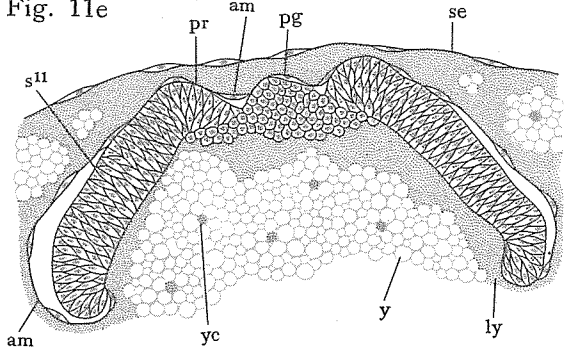
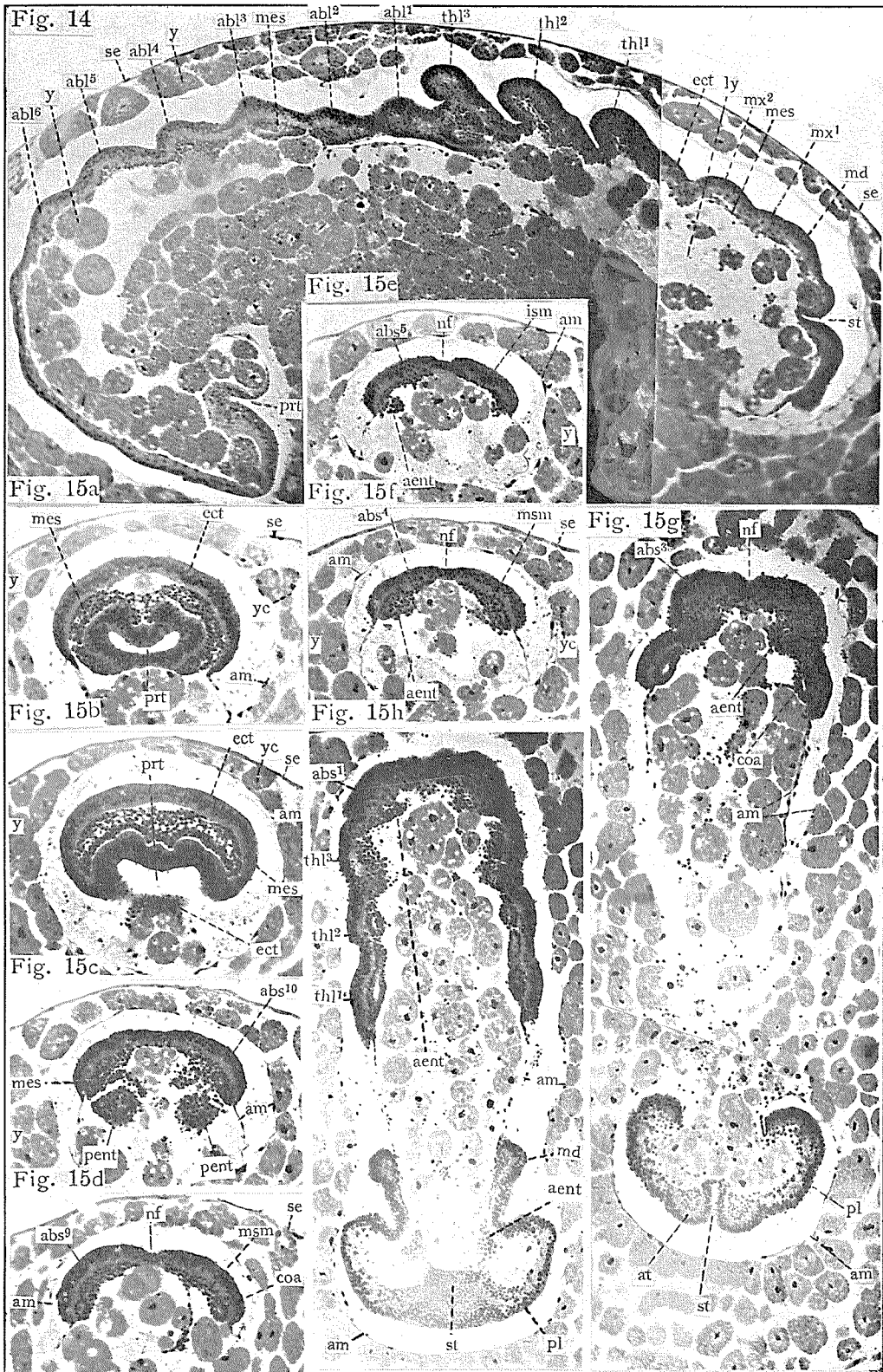


Fig. 11e





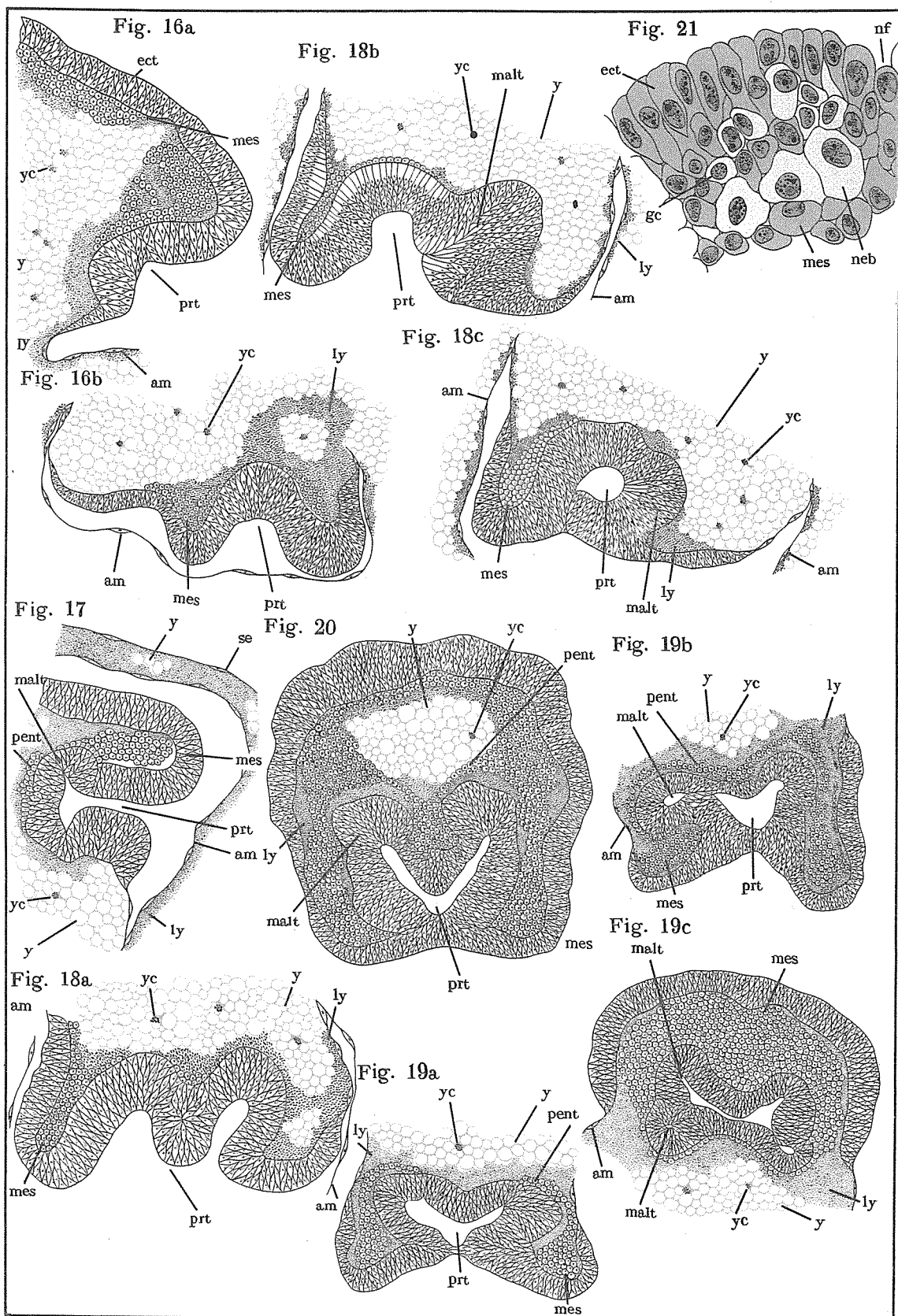


Fig. 26

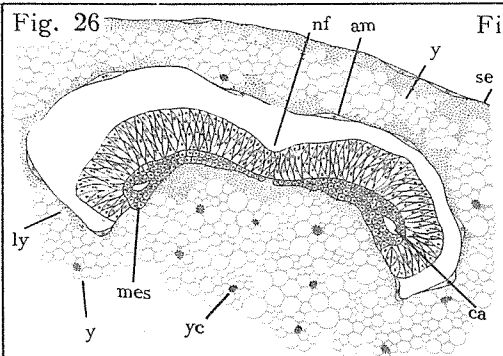


Fig. 24a

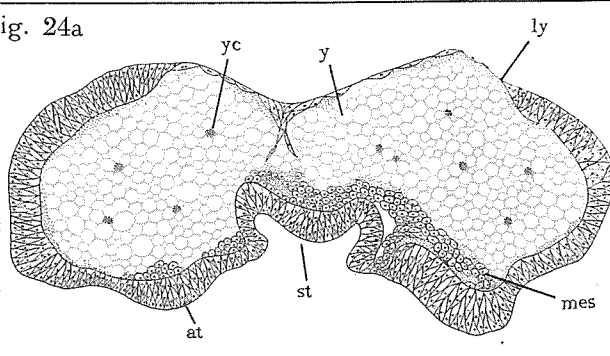


Fig. 23

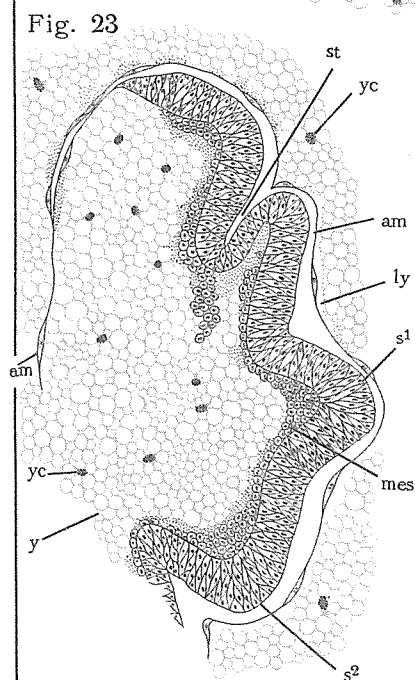


Fig. 24b

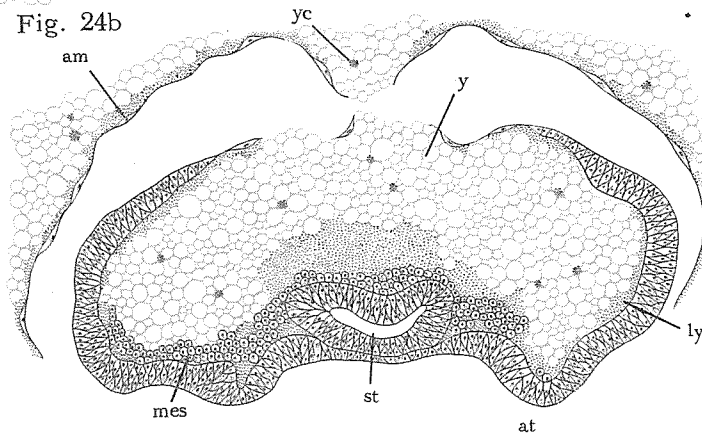


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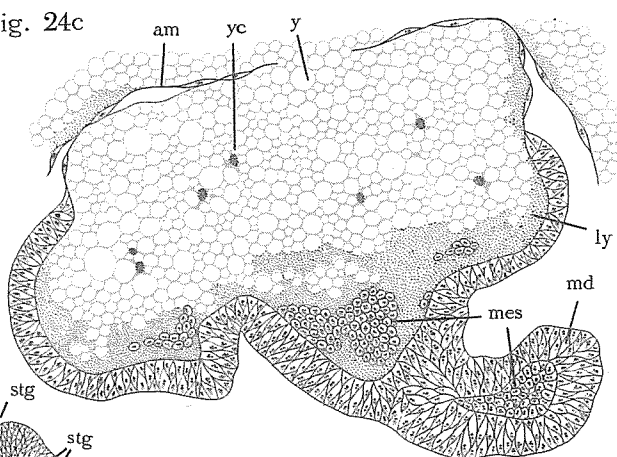


Fig. 22

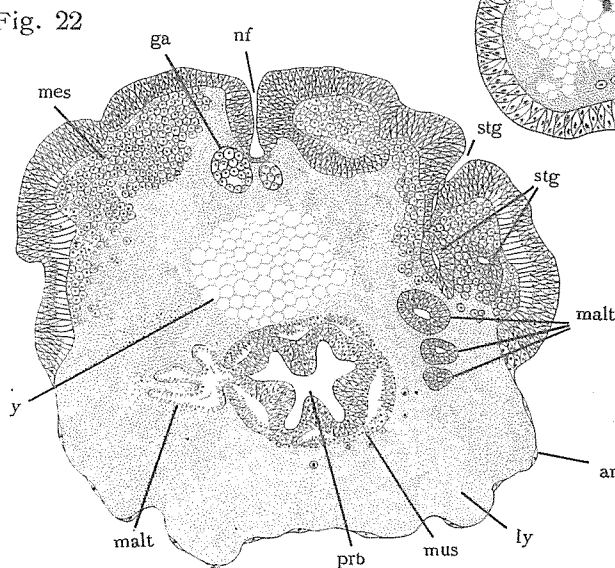


Fig. 25

